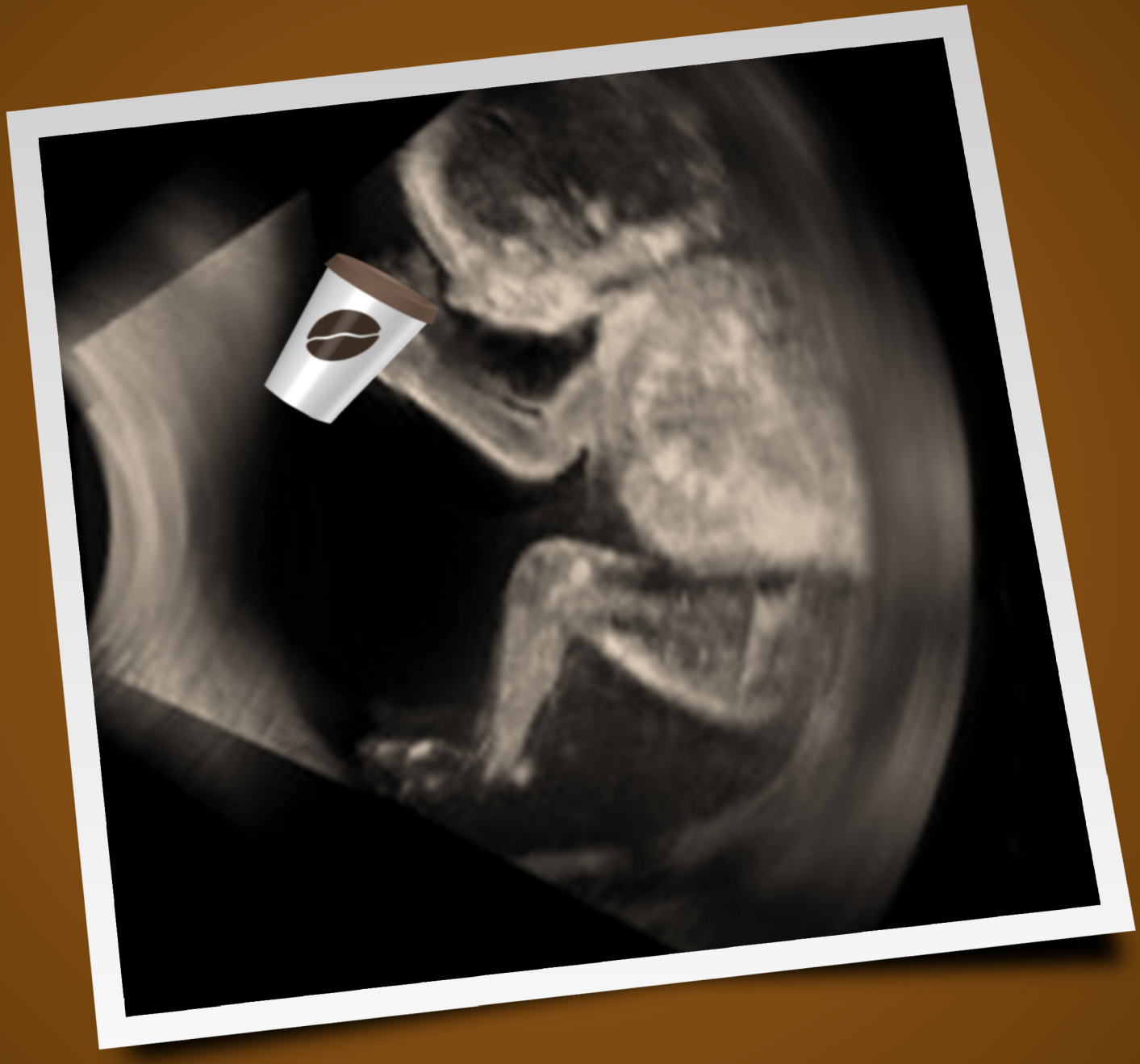
The Science Journal

LANDER COLLEGE OF ARTS & SCIENCES
A DIVISION OF TOURO COLLEGE IN FLATBUSH

Where Knowledge and Values Meet



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LANDER COLLEGE OF ARTS & SCIENCES
A DIVISION OF TOURO COLLEGE IN FLATBUSH
Where Knowledge and Values Meet

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The cover illustration, created by Professor Antony O'Hara of the Digital Multimedia Design Program, refers to the paper on The Effects of Maternal Caffeine Intake on a Fetus.

The Effects of Drug Abuse on Oral Health

Betzalel Krasnow

Betzalel Krasnow graduated in January 2018 as a Valedictorian of his class with a B.S. degree in Biology and will be attending Touro College of Dental Medicine.

Abstract

Drug abuse, currently a national epidemic affecting millions of Americans, causes numerous health issues including increased dental disease. There are several factors which can contribute to an increase in caries and missing teeth. One of the most discussed mechanisms is xerostomia. Drug abusers tend to have large sugar intakes which exacerbates the problems caused by xerostomia. Drug abusers are also at risk for oral infections associated with dental decay due to their altered saliva composition. Unfortunately, once the symptoms are present there is little that can be done to correct them and the goal of the treating dentist should be increased dental hygiene and ongoing prevention.

Introduction

Substance abuse and misuse is a national public health crisis. In 2015, more than 27 million people in the United States reported current illicit drug use or misuse of prescription drugs. In addition, The National Survey on Drug Use and Health reported that over 66 million people misuse alcohol in a month. Alcohol and drug misuse and related disorders are taking an enormous toll on individuals, families, and society as a whole as a result of the increased costs of healthcare associated with these conditions.

Methods

The articles mentioned in this paper were collected and compiled using research available to the public through Google Scholar, PubMed, and EBSCO host. Some of the articles may be under restricted access to academic circles and was made available to the author through the TouroLib system. Sources were evaluated for usefulness based on publication date and original publication source.

Commonly abused drugs

In addition to the many psychological and medical challenges presented by drug abuse, there are also extensive oral health problems which drug abuse causes. To further understand the nature of the oral health issues, it is first necessary to understand what is considered a drug and the major classes of drugs which people tend to abuse. Drugs can be defined as an exogenous chemical not necessary for normal cellular functioning that significantly alters the function of certain cells in the body, even when taken in relatively low doses. Endogenous neurotransmitters are not drugs, synthetic chemicals which mimic the effects of these endogenous chemicals, however, are considered a drug. Another stated qualification for a drug is that it is effective even in small quantities, this is an important part of the definition, as large quantities of any substance will alter the normal function of cells (Carlson, 2013).

People abuse drugs for the euphoric feelings and their ability to cause "mental detachment". The reason why people do not abuse antibiotics is because they do not induce these feelings. The drugs which people abuse can be separated into two distinct categories, central depressants and stimulants, defined by their effects on neurotransmitters. In the world of illicit drugs, these substances are often referred to by a "street name". It is important for a clinician to be familiar with not only the pharmaceutical name of a drug but with the street name as well, since many patients may not know the pharmaceutical name and may resort to using street names (Rome, 2001).

Stimulants, or "Uppers", is a class of drugs that mimic the effects of the sympathetic nervous system. The endogenous agonists of the sympathetic nervous system are the catecholamines; epinephrine, norepinephrine, and dopamine. The catecholamines function as both hormones and neurotransmitters. The pharmacokinetics of these drugs can affect a multitude of mechanisms, direct activation of postsynaptic receptors, the breakdown and reuptake of neurotransmitters, and stimulating production of catecholamines. Stimulants include, caffeine, ephedrine, nicotine, and cocaine.

One of the most commonly abused stimulants is methamphetamine. Methamphetamine, also known as, meth, speed, crank, tweek, chalk, amongst other names, is a white crystalline powder. Methamphetamine can be snorted, smoked, or injected. The popularity of methamphetamine is in large part because of its ease to obtain. Methamphetamine can be synthesized at home from commonly available ingredients (Lineberry and Bostwick, 2006). The FDA approves methamphetamine hydrochloride, sold under the name Desoxyn, to treat attention deficit disorder with hyperactivity as well as exogenous obesity, however the FDA advises that the inherent risk of methamphetamine must be strongly considered when prescribing it. The physical effects of methamphetamine are systemic. They include, loss of appetite, hyperactivity, tachycardia, bradycardia, rapid breathing, dry mouth, excessive sweating, and bruxism. Methamphetamine is taken recreationally to produce euphoria as well as an aphrodisiac. This article will discuss the effects of methamphetamine on oral health. Methamphetamine causes periodontal disease as well as rampant caries (cavities), the effect of methamphetamine on oral health is so unique that it has been referred to as Meth Mouth (Rommel, et. al, 2015).

The second class of drugs is depressants, or, "downers". Depressants, are the opposite of stimulants, they lower neurotransmission levels and depress arousal and stimulation in the brain. The pharmacokinetics of depressants are slightly more complicated. Neurons communicate through excitatory synapses, one neuron excites another which excites another neuron, and so on. If this excitatory process would happen uncontrollably, the neurons in the brain would be firing constantly, resulting in a seizure. To prevent this from happening, there is a class of neurons which have inhibitory effects. These neurons

secrete GABA (gamma-aminobutyric acid) which has a postsynaptic inhibitory effect. Depressants activate the various GABA receptors which results in an overall inhibitory effect. The most commonly prescribed class of depressants are benzodiazepines. Benzodiazepines are drugs which are used to reduce anxiety, promote sleep, and reduce seizures. Valium (diazepam) and Librium (chlordiazepoxide) are two common benzodiazepines. Other common depressants include barbiturates, cannabis, and alcohol (Carlson, 2013).

Opioids, the current medical terminology which refers to both endogenous and exogenous opioid, are drug which have the properties of both a depressant and a stimulant. Opioids create a state of euphoria, much like a stimulant, but its physical expression is similar to that of a depressant, i.e. slowed breathing, sedation, and hypothermia (NAABT.org). What is particularly scary about opioids is their tendency to cause respiratory depression. Even a small dose can be lethal, when taken together with Xanax (alprazolam), or other commonly prescribed drugs, which also causes respiratory depression.

With the exception of alcohol, drug abuse was limited to a small population who tended to be socially disadvantaged. Today, drug abuse is evenly distributed across all social strata (Friedlander and Mills, 1985). The stereotypical drug abuser is one of low socioeconomic status, and frequently neglects both general and oral hygiene. With the increase of abusers who do not fit this image, the issue of drug abuse, and its effect on oral health, has now come to the forefront.

Drug Abuse and Dental Decay

The correlation between drug abuse and severe dental decay has long been known. A study conducted in Iran was composed of 5,900 people; 2,662 were men (45.1%), between the ages of 15 and 75. One thousand and eleven (17.1%) of those people used opioids. The participants in the study underwent a face to face interview with a trained practitioner, during which information pertaining to the frequency of their drug use was obtained. In accordance with the Diagnostic and Statistical Manual of Mental Disorders (DMS-IV2), individuals who reported taking opioids at least three times a week were considered addicted. After the interview a dentist performed a thorough oral exam. Dental decay was recorded according to the DMFT index (decayed, missing, and filled teeth) established by the World Health Organization. Dental plaque and gum disease were also noted using the plaque index and the community periodontal index (CPI).

For people addicted to opioids, participants had a mean number of 9.07 teeth missing, compared to a mean of 6.42 for non-addicted users. There was a mean of 9.50 decayed teeth for addicted opioid users compared to 8.95 decayed teeth for participants not addicted to opioid use. Similar results for the number of filled teeth as well; 6.36 fillings for addicted participants compared to a mean of 3.89 for non-addicted participants. Overall the DMFT index for addicted users had a score of 17.10 compared to 13.10 for non-addicted users (Mohammadi, et al. 2017).

The authors say that the data proves a clear increase in the number of missing, decayed, and filled teeth, in opioid users. There are however some shortcomings with the study. Although other studies consider self-reporting of drug use a reliable indicator of actual drug use, there are of course people who are not totally honest with their drug use habits. More challenging, however, is the difference in age among the participants in the trial. The mean age of the addicted individuals was 56.01 while the mean age of non-addicted individuals was 48.19. This difference is statistically significant ($p=0.00$) considering the large sample size. Some of the discrepancy in the DMFT index between the addicted and non-addicted individuals can be associated with the age difference as aging is an influence in tooth loss. The study would have been more reliable had the mean age of the two groups been the same or at least statistically insignificant.

A study of 571 methamphetamine users in Los Angeles County, California was conducted and also shows a correlation between methamphetamine use and dental decay. This study focused exclusively on methamphetamine users and compared their results to a study done by National Health and Nutrition Examination Survey (NHANES). NHANES does a survey every two years, with participants selected to be nationally representative without being selected based on risk factors or pre-existing conditions. This study was conducted to answer two questions, do methamphetamine users have a higher rate of dental disease compared to non-users, and are different teeth affected between methamphetamine users and non-users.

Participants in the study were selected based on stratified sampling protocol of heavy, mild, and moderate, methamphetamine users. Intraoral exams were conducted by dentists who were trained by the national examiner in the NHANES study. All protocols of the study adhered to NHANES regulations so that the two studies can have maximum comparability. Although many variables were tested, the main one focused on was the DMFT index. As opposed to the Iran study which relied on self-reporting of drug use alone, this study utilized urine testing to confirm drug use. Data was also collected on sociodemographic and behavioral variables; gender, age, ethnicity, education, history of smoking, frequency of basic oral hygiene, and soda consumption.

Whereas the Iran study compared opioid users and non-users with little regard for sociodemographic information and the differences it may make, this study divided the participants into five propensity score groups, with each group having comparable sociodemographic backgrounds as the participants in the NHANES study. Multiple statistical analysis tests; t tests, chi-square tests, and Fisher tests, were used to confirm the accuracy in comparing the data from the Los Angeles County study to that of the NHANES study.

Of the 571 participants in the study, 19 of them were

completely edentulous (missing teeth). A shocking number considering that mean age of the participants was only 44. Compared to the NHANES study, methamphetamine users were forty percent less likely to have all their teeth. Methamphetamine users were also found to have approximately four times more caries and were twice as likely for the caries to be untreated. Close to twenty percent more methamphetamine users had decayed, missing, or filled teeth compared to the NHANES participants. Methamphetamine users were also more likely to have missing teeth when compared to the demographically similar NHANES participants (Shetty, et.al. 2016).

Similar studies from other locales also point to an increase in both the number of missing teeth and the number of carries in patients addicted to drugs. A study in Queensland, Australia studied several drugs and their role in dental decay (Reece, 2007). Another study, based in Munich, Germany also determined that there is a correlation between methamphetamine and rampant caries (Rommel, 2015).

Xerostomia

The most common complaint methamphetamine users express is that of "dry mouth" (Shafer, 2005). One study has 72% of substance abusers reporting suffering from an excessively dry mouth (Rommel, et. al., 2016). Dry mouth or xerostomia, is a fairly common complaint with estimates ranging between 0.9% and 64.8% of the population suffering from a form of dry mouth. There is a lack of data on the prevalence of xerostomia leading to such a wide range (Navazesh and Kumar, 2009). Xerostomia is usually associated with salivary gland hypofunction.

Although saliva is ninety-eight percent water, the other two percent contains many important substances. Included in those substances are the electrolytes, sodium, potassium, calcium, chloride, bicarbonate, and phosphate. Saliva also contains important enzymes needed for digestion, amylase, lingual lipase, and kallikrein. Another important component of saliva is its antimicrobial enzymes; lysozyme, lactoperoxidase, lactoferrin, and immunoglobulin A.

The importance of the electrolytes in saliva is their ability to regulate and maintain the pH of the mouth. Ideally, the pH of the mouth should be between 6.2 and 7.4. Anymore acidic and the acid can dissolve the hard minerals which make up the teeth. The ions present in saliva act as a buffer, keeping the pH within that important range.

Although there have been some studies which did not find a correlation between decreased saliva production and drug abuse (Busfield, 1961), more recently there are others that have reported a correlation (Heng, et al. 2008). The mean saliva production of a person is 1-2 ml/min. A stimulated salivation of less than 0.7 ml/min is considered low. In one study, the average methamphetamine user had an average saliva flow rate of only 0.36 ml/min (Rommel, et. al., 2016).

Saliva production is regulated by both the sympathetic and parasympathetic nervous systems. When norepinephrine binds to the alpha-adrenergic receptor it causes an increase in calcium levels which results in increased saliva production. Methamphetamine seems to activate the alpha-2-receptor which is a salivary inhibitor in the brain (Saini, 2005).

Xerostomia is considered to be an adverse drug event of properly prescribed opioid medication (Chapman, et. al., 2010). Although a concrete mechanism between opioid use and xerostomia has not been found, there are several working theories as to what may cause these symptoms. One theory is based upon the proven correlation between opioid use and decreased pancreas function. Perhaps there is a similar effect on the salivary glands which also are exocrine glands. Another theory is that after opioid use, there is a clear change in color, from red to very pale, of the oral mucosa. This change in color suggests local vasoconstriction of the capillaries and small arterioles in the mouth. This decreased blood flow can also inhibit saliva production (Odeh, et al. 1992).

Additionally, drug abusers also tend to go long periods with inadequate food and drink. This leads to a generalized dehydration, resulting in decreased saliva production especially when coupled with the hypermetabolic effects of illicit drugs (Goodchild, et al. 2007) Another theory points to the concomitant use of antidepressants and other drugs which can also cause xerostomia (Darke and Ross, 2000).

Sugar Intake

Regardless of any proven cause of drug abuse induced xerostomia, the mere fact that drug abusers consider their mouth to be dry, leads to another problem. Drug abusers tend to crave sugar and drink large amounts of non-diet soda. Mountain Dew is a commonly reported favorite drink of methamphetamine users and contains 31 grams of sugar, the equivalent of eight sugar cubes, in a single eight ounce serving. When considering that that is a single serving, and one can have many cups over the course of one day, that is an abnormally high level of sugar consumption (Goodchild, et al. 2007). One case report mentions a light user of methamphetamine, who would drink 1.5 liters of soda a day, that is a staggering one hundred and ninety six grams of sugar consumed from drinks alone (Wang, et al. 2012). As part of the Los Angeles County survey of 541 methamphetamine users, researchers obtained the number of non-diet soda drinks they had per day. The results of the study when compared to national averages, show a direct correlation between non-diet soda consumption and methamphetamine use. Although for years there was anecdotal evidence to this, this was the first survey to confirm it from a scientific and statistical standpoint (Murphy, 2016).

Opioid users have an additional factor which increases their sugar levels. There are three main opioid receptors in the brain;

mu, kappa, and delta (Titsas and Ferguson 2002). Preclinical animal studies suggest that action of mu and kappa agonists at the nucleus accumbens shell, hypothalamus, and paraventricular nucleus is associated with a development of a preference for sweetened food. Furthermore, eating sugar results in a down regulation of enkephalin MRNA production which then results in an increase in mu receptor agonism (Mysels and Sullivan, 2010). This increase in sugar consumption, especially when coupled with hyposalivation, leads to rampant caries and subsequent tooth loss.

Methamphetamine abuse leads to hyperactivity and excessive neuromuscular activity which causes bruxism (Rommel, et. al., 2016). Bruxism is excessive jaw clenching and teeth grinding. Opioid users also suffer from bruxism as there is an increase in neurosis which results in jaw clenching and teeth grinding (Titsas and Ferguson, 2002). Although bruxism is relatively common in non-drug dependent adults as well, the degree of grinding and clenching seems to be much greater in the addicted population. Bruxism shows to be particularly damaging to drug abusers due to the already weakened enamel from sugar consumption, poor oral hygiene, and hyposalivation.

Oral Infections

Another problem which drug abusers face is oral candidiasis. Opioids have been found to have an inhibitory effect on the phagocytosis of Candida by macrophages (Titsas and Ferguson, 2002). Saliva also plays a role in preventing oral candidiasis as it unifies the innate immune defense against Candida Albicans and prevents its proliferation. Saliva also contains the immunoglobulin IgA which aggregates the Candida Albicans cells and then destroys them by swallowing the aggregate (Salvatori, et al. 2016). Candida Albicans has also been found to cause pulpal inflammation, resulting in tooth loss, when the microorganism reaches the pulp through dental caries (Baumgartner et. al. 2000).

It has been known for a long time that Streptococcus mutans is a pathogenic organism which causes enamel loss and caries (Loesche, 1986). Recent clinical studies have shown large numbers of Candida albicans along with Streptococcus mutans in plaque obtained from carious lesions. Scientists were surprised to find this as no other co-colonization between this bacteria and yeast were previously known (Carvalho et al. 2006). Recent research shows that co-culture of Candida albicans and Streptococcus mutans with sucrose resulted in production of the S. mutans exoenzyme (GtfB) that bound to mannans and β-1,3 glucans found on the fungal outer cell wall, allowing them to survive the innate immune factors present in the mouth. In the same study, it was shown that coinfection in rats with both C. albicans and S. mutans increased the severity and number of smooth-surface caries lesions by 2-fold in the presence of sucrose (Falsetta et al. 2014). It is no surprise then that drug abusers who have an increased presence of both Candida albicans and Streptococcus mutans as well as a large sugar intake are at risk for severe caries.

One theory for the increase of dental disease in drug abusers was the caustic nature of inhaled and smoked drugs (McGrath and Chan, 2005). The Los Angeles County study, however, found that the dental consequences of methamphetamine abuse were more pronounced in users who injected the drug (Shetty, 2016). With injection of the drug there should be little or no decay if the issue was the caustic nature of the smoke. Heavy drug abusers tend to inject the drug as it provides for a quicker and stronger "high." The poor health of heavy drug abusers and the increase of the associated side effects are probably responsible for the findings of the Los Angeles County study.

Treatment Concerns

Drug abusers will usually only visit the dentist after they stop abusing the drug and they are suffering from severe pain. It is therefore important that dentists be aware of previous drug abuse to avoid prescribing a medication which may cause relapse. Most users will not admit that they are or were users for fear of being judged. Therefore dentists must be trained to recognize the main signs and symptoms of drug abuse (De-Carolis, et al., 2015). Dentists should also take notice of skin lesions which can indicate intravenous drug use. The practitioner may decide to take the patient's blood pressure as a way of disguising their search for needle tracks (Saini, et al. 2013). Dentists should not make use of pre-written prescription forms as it can potentially be used to obtain drugs which were not prescribed (Smit and Naidoo, 2015).

When treating patients who have a history of drug abuse, providing adequate pain relief can be challenging. Most abusers have a tolerance to several pain medications as a result of drug abuse, and other medications may cause relapse (Saini, 2013). Local anesthetic containing epinephrine (commonly used as a vasoconstrictor in dental surgery) must be avoided as there are known drug interactions between epinephrine and commonly abused drugs. The importance of this must be stressed to the patient as it may result in cardiac dysrhythmias, cerebrovascular injury, and even myocardial infarctions (Smit and Naidoo, 2015). Dentists may recommend non-steroidal analgesics, for example, ibuprofen and naproxen. Acetaminophen can be used as well (Saini, et al. 2013).

Conclusion

Drug abuse is considered an epidemic and there is a large effort underway to inform the public of the dangers involved. Drug abuse has serious detrimental effects on the body. Oral health is one of the most noticeable side effects, as drug abusers are commonly missing multiple teeth. Dentists must know how to recognize the signs of drug abuse and can be on the forefront of fighting this epidemic.

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Why Doesn't a Mother Reject a Genetically Different Fetus?

Devora Krumholtz

Devora Krumholz graduated in January 2018 with a B.S. degree in Biology

Abstract

Many basic multicellular organisms possess some form of immune response to protect themselves against the invasion of foreign objects. It was not until British scientist, Peter Medawar proposed a fundamental question that changed the way researchers studied the maternofetal relationship. A fetus, being genetically different from its mother should be rejected by the maternal immune system, however, it is not. Researchers have since discovered and developed several mechanisms that aim to explain how the maternal immune system prevents fetal rejection. The formation of a mechanical barrier, general and local suppression of the maternal immune system, and a shift in cytokine concentration during pregnancy have been suggested as reasons Forthcoming discoveries in the field of reproductive immunology may further one's understanding of immune regulation during pregnancy, in addition to other applications, such as, the immune responses regarding organ transplants. Although the proposed mechanisms mentioned above helped improve the understanding of how fetal rejection is avoided, many scientists concur that additional research is required to adequately explain the prevention of fetal rejection.

Abbreviations:

Ab- Antibody
Ag- Antigen
APC- Antigen presenting cell
MHC- Major Histocompatibility Complex
IDO- Indoleamine 2,3-dioxy-genase
Ig- Immunoglobulin
IL-2- Interleukin 2
IFN-γ- Interferon Gamma
TNF-β- Tumor Necrosis Factor Beta

Introduction and Background

The National Vital Statistics Reports presented data from a study conducted regarding the total number of births registered in the United States, in 2015. The results showed that there were 3,978,497 births recorded, only 1% less than the total number of births registered in 2014 (Martin, et al. 2017). Many women, worldwide, have successfully given birth to babies, which interests researchers in the field of immunology. During a lecture in England, in 1953, a British immunologist named Peter Medawar asked, "how does the pregnant mother contrive to nourish within itself, for many weeks or months, a fetus that is an antigenically foreign body (Betz, 2010)?" Since then, researchers have been asking the same question (Anonymous, 1999)?

Methods

The research discussed in this paper was compiled from various published articles, taken from Touro's database, including Proquest Science, EBSCO, and PubMed to research why a mother does not reject a genetically different fetus growing inside of her.

Discussion

A. The immune system targets foreign objects

The human body's immune system is a complex network of biological structures intricately designed to protect the body against foreign objects. Foreign objects may be anything the body does not recognize as self, which may include, bacteria, viruses, parasites, and tumors. When a foreign object enters the body, there is a cascade of events carried out by the body's immune system. First, the body must identify the foreign object as "non-self", destroy the pathogen, and lastly, create memory cells to ensure that the foreign object does not attack again (Gilley, et all. 2009).

1. Recognizing "non- self" Objects

One of the main roles of the immune system is to recognize a pathogen and induce a response to eradicate it. Immune system cells such as white blood cells or leukocytes aid in carrying out the tasks. In order to initiate any immune response, an antigen (Ag) must be recognized by special receptors. T-Lymphocytes, killer T cells and helper T cells, play a crucial role in the recognition of Ag. T-cells recognize foreign Ag by the presentation of peptides by the major histocompatibility complex (MHC). The two classes of MHC are MHC I and MHC II. Major histocompatibility complex class I generally presents an intracellular viral Ag to cells that contain CD8+ proteins. These Ag will be destroyed by Cytotoxic T-Lymphocytes, while MHC Class II generally presents extracellular bacterial Ag to cells that contain CD4+ proteins. These Ag are recognized by Helper T-Lymphocytes and will be destroyed with the help of Ab. (Joyce, 2001). CD8+ and CD4+ are cell surface molecules that bind to their respective MHC on an antigen-presenting cell (APC). One of the most common cells that act as an APC are macrophages. (Miceli, Parnes, 1991).

2. Destroying a Pathogen

After the body recognized a pathogen as "non-self," the immune system will try to rid the body of the foreign object. There are two main types of pathogens that will elicit two different responses. The first type is when bacteria enter the human body; helper T cells will release certain cytokines and chemokines. These are certain chemicals that act as chemoattractants for other leukocytes to aid in an inflammatory response. Secondly, will produce Plasma cells, a derivative of lymphocytes, make antibodies (Ab) that will help destroy the pathogen. Antibodies are a Y shape structure, consisting of glycoproteins called immunoglobulins (Ig). There are 5 main isotypes of Ig with different roles, which include: IgG, IgE, IgM, IgA, IgD. Each Ab will bind to a

specific antigen, comparable to the interaction with a lock and a key (Author Unknown, 2016).

In order to help T cells produce Ab, B- lymphocytes will produce specific Ab to help destroy pathogens. Antibodies produced by B cells will circulate the body via the bloodstream and can bind to foreign objects. After a B-cell recognizes a pathogen, the cell matures into a plasma cell, which produces large quantities of Ab. Antibodies have 3 main functions in the immune system. The first role is neutralization, which is when an Ab binds to the surface of the pathogen, thus denying any entry into the normal body cells. The second role is to activate other defense cells that are in the body that can elicit an inflammatory response, for example, phagocytes. The third role is to activate a complement system, which attracts defense cells to the infection site and destroys the pathogen (Author Unknown, 2016).

The second type of pathogen that will cause an immune response is infection with a virus. In this case, activated Cytotoxic T- cells are able to directly destroy the pathogen when it displays pathogen peptides on the MHC Class 1. Cytotoxic- T cells, also called Killer T cells release cytotoxins, such as perforin, which creates a hole in the wall of the infected cell. This in turn kills the cell along with the pathogen due to the loss of fluid (Author Unknown, 2016)

B. Fetal Cells and a Mother's Immune System

In order to better explain the interaction between fetal cells and the mother's immune system, one must understand what happens after a sperm fertilizes an egg. Once an egg is fertilized, it rapidly divides to form the blastocyst, a hollow ball of cells comprised of two portions. One is the inner cell mass that will become the embryo, which will develop into a fetus about eight weeks after conception. The second is the outer layer of the blastocyst, which will become the trophoblast. The trophoblast will ultimately occupy the lining of the uterus, thus facilitating embryonic implantation (Urman, Balaban 2001).

C. Immune System During Pregnancy

Based on the roles of the human's immune system, one could assume that a mother's immune system would try to eliminate a fetus. When a mother conceives a child, genetic material from both the mother and the father are incorporated into the fetus, however the mother's mother's side of the placenta is genetically different from the fetus. Therefore, the fetus inside the womb would be considered "non-self" (Lightner, et al, 2008). Peter B. Medawar suggested the "immunological paradox of pregnancy" (Mor, 2007). He proposed that since the fetus is considered "semiforeign," there must be a conflict between the fetus and the mother's immune system. For over 60 years since Medawar posed this phenomenon, there have been many attempts to explain why a mother's immune system does not reject a genetically different fetus.

Accepted Hypotheses

a. Mechanical Barrier

The first hypothesis explains that the fetal tissue is unrecognizable as "nonself" by the mother's immune cells due to a mechanical barrier (Mor, 2007). The uterus of a pregnant mother has a mechanical barrier, consisting of synctiotrophoblasts that envelop the fetus. This trophoblast-immune interaction includes three stages (Fig. 1). During the first stage of attraction, the trophoblast cells secrete chemoattractants that will signal immune cells to migrate to the implantation site. The implantation site refers to the area of the uterus in which the trophoblasts invade. After attraction, the trophoblasts produce cytokines that regulate the differentiation of immune cells. This stage is called recruitment and/or education. Upon completion of these two steps, the response can take place. In this stage, the immune cells from stage two respond to different signals (Swain, 2013). After completion of all three stages, the mechanical barrier is formed. This in turn prevents the movement of activated T cells from the periphery to the implantation site and enables antigens that are inside the barrier to be undetected by the mother's immune system.

b. Suppressed Immune System During Pregnancy

Research done by David Munn and his colleagues at the Medical College of Georgia in Augusta suggests a different hypothesis. They discovered that macrophages, an important immune cell involved in antigen presentation, can disable killer T-cells. This in turn will prevent the T cells from attacking any object that is recognized as non-self (Anonymous, 1999). In order for this to occur, the synctiotrophoblasts in the placenta produces an enzyme known as indoleamine 2,3-dioxy-genase (IDO). The function of IDO is to destroy tryptophan, an amino acid required by T cells to destroy a foreign object. In 1990, Andrew Mellor, a colleague of Munn, concluded that IDO inhibits a mother's T cell response towards a genetically different fetus. On the contrary, if a mother fails to produce IDO, it would cause the mother to miscarry (Gura, 1998).

Munn and his colleagues conducted experiments in to prove their hypothesis. They used two groups of pregnant mice; one group had been bred to genetically identical fathers of the same strain while the second group was bred to fathers from a genetically different strain (Gura, 1998). The experimenters then embedded time release-capsules consisting of either 1-methyl-tryptophan, which is an IDO inhibitor, or a control substance underneath the skin of the pregnant mice. Results showed that only the mice carrying genetically different fetuses that had been given the inhibitor rejected their fetuses (Anonymous, 1999) (Gura 1998). Interestingly, the embryos developed normally until inflammatory cells migrated to the implantation site and caused hemorrhaging around the embryo. Munn proposed "the mother is rejecting the placenta and eventually the embryo chokes off and dies" (Gura, 1998). From the data collected,

Munn and his colleagues concluded that after implantation, an embryo starts making connections with the mother's blood supply. Sequentially, synctiotrophoblasts will start producing IDO, destroying tryptophan and suppressing the maternal immune system (Gura, 1998). However, other researchers have reservations about this hypothesis. In Pregnancy Reconceived, Mor argues that if the maternal immune system is suppressed, it would be nearly impossible for a mother and its fetus to survive. Exposure to any pathogen would be fatal (Mor, 2007).

c. Local Active Suppression in Decidua

In addition to the general suppression of a mother's immune system, researchers have found that an important role in the maternofetal interaction is the local active suppression by cells in the decidua (Chaouat, 1990). The decidua is a mucous membrane lining of the uterus during pregnancy. This lining allows for nutrition and gas exchange before the placenta is functional (Mizugishi, et al. 2007). Cells located in the decidua may inhibit the production of lymphocytes thus leading to the inhibition of the production and expression of receptors for interleukin 2 (IL-2). IL-2 is a cytokine signaling molecule produced by activated T cells and is crucial for the rejection of foreign objects in the body. This type of suppressor cell is trophoblast independent. However, there is another type of cell that suppresses the role of IL-2 in the maternal immune system and these suppressor cells in the decidua are trophoblast dependent. ¬ Both types of suppressor cells have an effect on the production or action of IL-2.

Therefore, local active suppression aids in the prevention of foreign objects attacking the mother's immune system and fetal rejection (Chaouat, 1990.)

d. Cytokine-shift Hypothesis

This hypothesis suggests that during pregnancy, the balance of cytokines within the mother's body shifts. This cytokine shift causes immunological changes in the maternal immune response (Mor, 2007). Cytokines, which are important in cell signaling, are released by Th1 and Th2 helper cells. However, each subgroup facilitates a different immune response. Th1 cells secrete IL-2, Interferon gamma (IFN-γ), and Tumor necrosis factor, Beta (TNF-β) causing a cell mediated immune response, while Th2 cells secrete IL-4, 5, 6, 10, and 13. These are mainly involved in antibody production (Rincón et.al, 1997). Additionally, IL secreted by Th2 cells simulate a humoral immunity and aids in the inhibition of the production of TNF-β and IFN-γ (Kidd 2003). The production of IFN-γ, TNF-β and IL-2 are believed to be damaging to pregnancy. In an experiment studying pregnant mice, these cytokines were injected into the mice and caused fetal loss (Koch and Platt, 2003). Previous research suggests that there is a shift towards a higher production of cytokines released by Th2 during pregnancy and a diminished production of Th1 cytokines (Hoshimoto,et al. 2000).

When a foreign object enters the body of a women who is not pregnant, Th1 cells will secrete pro inflammatory cytokines that will signal for a cell mediated response to occur. However, according to the cytokine-shift hypothesis, the balance of Th1 and Th2 will go towards the secretion of cytokines by Th2, resulting in a suppressed inflammatory response (Mor, 2007).) Many studies have been conducted in an effort to better understand the shift of Th1 to Th2 cytokine secretion during pregnancy. In an experiment conducted by Hoshimoto, et. al. (2009), they gathered thirty female subjects. Group A consisted of ten women who were non-pregnant women with regular menstrual cycles and the other 20 healthy and pregnant women. The pregnant women were separated into two groups, B and C according to weeks of gestation. Group B consisted of 10 pregnant women who were in their first trimester and group C consisted of 10 women in their third trimester. The experimenters took samples of peripheral venous blood and separated the plasma at -80°C until ready for analysis. The results of the experiment showed the correlation between the plasma levels of sCD26 and sCD30 and pregnancy. sCD26 and sCD30 are molecules on the surface of activated Th1 and Th2 cells, respectively. Therefore, plasma levels of sCD26 and sCD30 correlate to Th1 and Th2 responses, respectively.

sCD26 concentrations among group A were significantly higher than group B. Furthermore, the concentration of group B was significantly higher than group C. This indicates that the highest concentration of Th1 cells were among those who were not pregnant, and the lowest concentration was among the pregnant women in their third trimester. Concentrations of sCD30 among groups A and B did not significantly vary, however, compared to the concentration level of group C, they were significantly higher. The researchers concluded that there was a significant decrease in sCD26 levels among pregnant mothers possibly due to the shift from Th1 cytokine secretion. However, sCD30 levels did not differ between non-pregnant women and women in their first trimester, but decreased among women in their third trimester. This decrease may be explained by the increase in water retention as pregnancy progresses. Furthermore, the researchers advise for additional investigation in order to validate the cytokine-shift hypothesis.

Many researchers agree that cytokines play a crucial role during pregnancy. As explained in the research done by Hoshimoto et.al, Koch and Platt mutually agree that a Th2 response is necessary for the fetus to survive in the womb. Results from an experiment with mice showed that there was a 20-50% rate of fetal loss due to a lack of Th2 cytokine production. Furthermore, they applied this idea to humans and suggest that irregularities with Th2 cytokine response may lead to miscarriages. The mechanism that causes the shift between Th1 and Th2 cytokine response in pregnancy is unknown. However, Koch and Platt propose two plausible possibilities. Firstly, there

is a prevention of Th1 cytokine secretion that allows the Th2 response to take over or secondly; there is a specific Th2 response, which inhibits the Th1 response (Koch, Platt, 2003).

Conclusion

This paper attempted to explain the reasons why a mother does not reject her genetically different fetus. Although it was not until the 1950's when Professor Medawar first posed this question, the wealth of knowledge on this topic is rapidly expanding. However, despite the few flaws with the hypotheses, additional research is being studied to resolve the issue.

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Possible Causes of Preeclampsia and Potential Treatments

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Abstract

Preeclampsia is a common complication of pregnancy characterized by hypertension and proteinuria. Its symptoms are well-defined, but the pathophysiology is not fully understood. This paper analyzes several of the most credible causes of this syndrome and attempts to relate these to the known risk factors. Current preeclampsia treatments are examined, and special focus is given to novel experimental treatments which offer hope of ending preeclampsia and eclampsia.

Key Words:
Preeclampsia
Eclampsia
Risk Factors
VEGF
sFlt1
Gene Therapy

Acronyms Used:
sFlt1–Soluble fms-like Tyrosine Kinase 1
HELLP–Hemolysis, Elevated Liver Enzymes, and Low Platelet count
VEGF–Vascular Endothelial Growth Factor
PlGF–Placental Growth Factor
BMI–Body Mass Index
CNS–Central Nervous System
NICU–Neonatal Intensive Care Unit
PE–Preeclampsia
APLN–Apelin

Introduction

Preeclampsia is a relatively common complication of pregnancy. Occurring in 7% of all pregnancies in the US, it is defined as an increase in blood pressure combined with proteinuria. More severe cases of preeclampsia have the potential to advance into eclampsia, which is preeclampsia with the addition of seizures. Preeclampsia and eclampsia have the ability to cause lasting harm or death to both mother and fetus, but the cause of these diseases is largely unclear (Papadakis & McPhee, 2017).

Risk factors for preeclampsia are known. Hypertension, kidney disease, family history, diabetes, and obesity are all linked to a higher incidence of preeclampsia. Preeclampsia is commonly called a disease of the first pregnancy, though women who have had preeclampsia before are also at greater risk of developing it in subsequent pregnancy. Strangely enough, cigarette smoking seems to prevent preeclampsia. There are other known risk factors as well.

Some of the pathophysiology of preeclampsia is understood. It is believed that preeclampsia develops from poor placentation and the release of soluble fms-like tyrosine kinase 1 (sFlt1) into the mother's bloodstream. However, the increase in sFlt1 levels is often not distinct enough to use as a screening tool. Additionally, the original cause of the increase is unknown.

Treatment of preeclampsia is largely based on symptoms, with the goal being the continuation of the pregnancy for as long as possible. Almost all symptoms disappear within 48 hours after delivery, so it is in the mother's best interest to give birth. Conversely, the fetus needs more time in utero to develop. The physician must balance the conflicting needs of the two patients by managing the mother's symptoms. Further understanding of the underlying causes of preeclampsia can result in more effective treatment and better outcomes for preeclamptic women and their children.

Methods

Peer-reviewed journal articles were obtained using Touro College's database and Google Scholar. The articles were critically read, analyzed, and compared. Special attention was given to retrospective studies and reviews due to the dearth of original studies in their references. This can be attributed to the fact that pregnant women tend to be apprehensive about joining clinical studies.

Discussion

Symptoms of Preeclampsia

Preeclampsia is a complication of pregnancy involving hypertension and proteinuria. It cannot be prevented. Instead, pregnant women are screened for symptoms and treated accordingly. These symptoms usually present in the third trimester, but can occur from 20 weeks' gestation. It is diagnosed when a patient presents with blood pressure of 140/90 or greater, and more than 0.3 g of proteinuria in 24 hours. Some women also have edema. Severe preeclampsia involves higher blood pressure as well as thrombocytopenia, headache, and blurred vision (Papadakis & McPhee, 2017).

Severe preeclampsia is also characterized by hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome. This diagnosis was first made in 1982 and is currently found in 0.2-0.8% of pregnancies. HELLP syndrome is rarely seen in non-preeclamptic patients, though preeclampsia is about ten times as common as HELLP. Like preeclampsia, HELLP is a syndrome, a collection of symptoms whose one underlying cause is uncertain. The hemolysis aspect is a result of damaged endothelial cells in small blood vessels. Fibrin strands in the blood vessels cause red blood cells to become fragmented, often causing anemia. Microangiopathy also slows hepatic portal blood flow, damaging the liver. The same microangiopathy also exposes tissue factor on blood vessel walls which activates coagulation pathways. These blood clots lower platelet counts and can lead to further complications such as uncontrolled bleeding or placental abruption (Abildgaard & Heimdal, 2013).

If preeclampsia continues, it can develop into eclampsia, which

is defined as all of the symptoms of preeclampsia with the addition of seizures. Eclampsia is easily prevented by administration of magnesium, so it is usually only seen in patients lacking prenatal care, or in developing countries where access to this anticonvulsant is unavailable. Hyperexcitability and extreme reflex responses are typical signs that eclampsia may be developing (Papadakis & McPhee, 2017; Duley, 2009).

Preeclampsia is primarily a disease of the mother, so the fetus is not always affected. This is especially true in milder cases of preeclampsia, where the pregnancy is allowed to continue until term. However, this is not to say that the fetus is never endangered. Fetal growth restriction is a complication often correlated with preeclampsia. Because of the mother's angiopathy, blood supply to the fetus can be reduced. This does not allow the fetus to receive adequate nutrition, causing a low birth weight (Duley, 2009).

More seriously, preeclampsia often forces an early delivery because of the mother's symptoms. Preterm delivery, in this case meaning before 34 weeks' gestation, is associated with various complications. There is the immediate problem of insufficient surfactant maturity in the lungs, causing the baby to go into respiratory distress upon birth. Low birth weight often causes complications later in life, including cardiovascular problems, diabetes, and obesity. Additionally, increased risk of cerebral palsy has been associated with children of preeclamptic mothers. Although preeclampsia primarily affects the mother, the fetus is not spared of all effects (Papadakis & McPhee, 2017; Duley, 2009).

Causes of Preeclampsia

The causes of preeclampsia are poorly understood. Because preeclampsia is a syndrome, a collection of symptoms which may or may not come from one underlying cause, the pathogenesis can be different for different patients. Most people agree that preeclampsia's cause lies in the placenta due to the fact that the disease disappears upon delivery. It is hypothesized that poor placentation is a cause of preeclampsia. In normal pregnancies, cytotrophoblasts from the placenta invade the mother's myometrium and increase blood supply to the fetus. In preeclamptic placentas, insufficient invasion and remodeling of the mother's arteries occur. The placenta is therefore hypoxic, and parts of it break down and release debris into maternal circulation. This debris, containing trophoblast cells and keratin fragments, causes the inflammatory response of preeclampsia, microangiopathy and hypertension (Redman & Sargent, 2005).

Normal placentas release vascular endothelial growth factor (VEGF) and placental growth factor (PlGF). Both are angiogenic factors which allow the placenta to build an adequate vascular network for the fetus's development. When VEGF or PlGF binds to a receptor on placental cell surfaces, increased vascularity is shown. A soluble form of this receptor, soluble fms-like tyrosine kinase-1 (sFlt1), increases in the third trimester. This receptor floats freely in the placenta and maternal serum. When sFlt1 binds to VEGF or PlGF, they are unable to create blood vessels, proving sFlt1 to be antiangiogenic, and possibly the cause of the poor placentation described above. This factor is further increased in preeclampsia, and is found to increase even before clinical signs of the syndrome are evident. It is believed that excess sFlt1 can cause preeclampsia; what is unknown is how this excess develops in the first place and what can be done to prevent it (Redman & Sargent, 2005; Levine et al., 2004).

It is believed that decreased free VEGF and PlGF in the maternal blood supply leads to the endothelial dysfunction of preeclampsia. In the kidneys, the lack of free VEGF causes glomerular endotheliosis; constantly circulating VEGF is necessary for renal function. The swelling of kidney cells then does not allow for proper filtration of the blood, causing proteinuria. Exactly how or if sFlt1 causes hypertension is unknown.

As would be expected, women with lower VEGF and PlGF levels and higher sFlt1 levels tend to have more severe symptoms of preeclampsia and eclampsia. Additionally, when sFlt1 is administered to nonpregnant rats, they develop preeclamptic symptoms. VEGF inhibitors or VEGF gene knockouts cause hypertension and proteinuria in pregnant and nonpregnant rats. Cancer patients taking VEGF-inhibitors to limit blood supply to a tumor will also often exhibit hypertension and proteinuria, further proving the role of sFlt1 in preeclampsia (Levine et al., 2004).

Preeclampsia is widely considered a disease of the nulliparous woman. In a Norwegian study examining data from over 700,000 births between 1967 and 1998, preeclampsia was found in 3.9% of first pregnancies (Skjærven et al., 2002). This is compared to a 1.7% incidence for second pregnancies, and 1.8% for third. The incongruous increase for third pregnancies is quite small and probably a statistical artifact. It is unknown exactly why preeclampsia is much more common in first pregnancies. What is known is that if a mother was preeclamptic in her first pregnancy, she is at risk for preeclampsia in later pregnancies as well. Somehow, nulliparity seems to cause preeclampsia. This Norwegian study claims that preeclampsia is a result of the mother being exposed to her partner's foreign antigens and responding inflammatorily, causing preeclampsia. In later pregnancies by the same man, preeclampsia is less likely because the partner's antigens are no longer unrecognized by her body. Giving weight to this theory, the more time that elapsed between pregnancies, the more likely the mother was to develop preeclampsia, even if she had not previously been preeclamptic. As time passed, the immune system's ability to recognize a partner's antigens decreased. Women whose second pregnancies were by different partners appear to have the same risk of preeclampsia as nulliparous women. This supports the idea that preeclampsia is partially caused by foreign antigens; a new partner supplies new antigens for which the mother is unprepared. However, since women who have changed partners often have a larger birth interval, it is very uncertain if the change in partner

actually increases the risk for preeclampsia. Interestingly, previous miscarriage seems to reduce the risk of preeclampsia in a later, successful pregnancy. This can also be connected to the idea of interbirth interval, as many women who miscarry attempt to get pregnant again very soon thereafter. Nulliparity is known to be a risk factor for preeclampsia; the reason for this is not totally clear (Skjærven et al., 2002; Sibai et al., 1995).

Risk Factors

There are many risk factors for preeclampsia, including chronic hypertension and kidney dysfunction, obesity, diabetes, previous preeclamptic pregnancies, family history of the mother or father, birth interval, age of the mother, as well as other preexisting conditions. This paper will analyze some of the more credible risk factors and attempt to explain the connection between them and the disease.

Nulliparity is the most known risk factor for preeclampsia. Whether this is due to an immune response or some other mechanism is largely unknown. What is known is that nulliparous women are more than three times more likely to develop preeclampsia than women who have previously had normotensive pregnancies (Duckitt & Harrington, 2005).

Women with chronic hypertension unrelated to their pregnancy are known to have a higher risk of developing preeclampsia and eclampsia. In one population-based study, 12.1% of preeclamptic women were found to have had hypertension before they became pregnant (Duckitt & Harrington, 2005). This is quite logical; high blood pressure before becoming pregnant leads to even higher blood pressure while pregnant. Clinically, blood pressure is used to diagnose preeclampsia. Preeclampsia is defined as systolic blood pressure of 140 mmHg or greater and/or diastolic blood pressure of 90 mmHg or greater, and at least 300 mg of proteinuria over 24 hours. Blood pressure can also be used to screen for preeclampsia. Elevated blood pressure is normal in pregnancy, but too high an elevation can be a sign that preeclampsia will develop. In one study of nearly 3000 women, the mean systolic blood pressure at 20 weeks gestation was 105.4 for healthy pregnancies, and 110.4 for those who later developed preeclampsia. This is a substantial difference, but clinically it would be difficult to gain any benefit from this statistic. Diastolic and mean blood pressure were not as useful for prediction. Not surprisingly, women who displayed significantly elevated blood pressure at 20 weeks were more likely to develop preeclampsia earlier than those whose blood pressure was not as elevated. This is a more useful diagnostic tool. Systolic blood pressure of 110 as opposed to 105 is hardly grounds to begin watching more closely for preeclampsia. Systolic blood pressure of 125 or 130 is (Sibai et al., 1995).

As with hypertension, kidney dysfunction before pregnancy is likely to lead to increased kidney dysfunction while pregnant, i.e. preeclampsia. Less research has been done on kidney dysfunction leading to preeclampsia, though. In one study of 69 pregnancies, 6.7% of women with recurring urinary tract infections developed preeclampsia, and only 2.6% of the control group did. In other words, 2 of the 39 women with renal disease developed preeclampsia, and only 1 healthy woman did. This is hardly enough evidence on which to build a treatment plan. Logically, the connection between renal disease and preeclampsia seems just as strong as that between hypertension and preeclampsia. However, not nearly as much research was done on this juxtaposition, perhaps because of the relative rarity of renal disease in women of childbearing age (Duckitt & Harrington, 2005).

Non-gestational diabetes greatly increases the risk of preeclampsia. A meta-analysis of multiple studies with a total count of 56,968 women revealed that the risk of developing preeclampsia is 3.56% greater for women who have diabetes before pregnancy. These studies are often connected to studies linking preeclampsia and obesity, which also approximately quadruples the risk of preeclampsia. Because women with diabetes and high BMI often have other health problems, it is difficult to assess exactly which of their characteristics make them more likely to develop preeclampsia (Sibai et al., 1995; Duckitt & Harrington, 2005). In a Saudi Arabian study of the effects of obesity and gestational diabetes on pregnancy, it was found that approximately 7% of women with obesity and/or gestational diabetes develop preeclampsia, compared to only 0.5% of women with neither. The combination of the two seems to increase the risk (Wahabi et al., 2014).

The connection between obesity and preeclampsia has been linked to inflammation. Obesity is inflammatory, as is preeclampsia, so it stands to reason that one inflammatory state can cause the development of an inflammatory disease. Additionally, preeclampsia often develops from hypoxic conditions in the placenta. Obesity can encourage this. Obesity is often linked with hyperglycemia, causing hemoglobin to pick up glucose and lose its affinity for oxygen. This does not allow enough oxygen to reach the placenta, leading to an inflammatory response, the release of cytokines, and subsequent endothelial dysfunction of preeclampsia (Redman & Sargent, 2005; Schmatz et al., 2010).

Previously having preeclampsia puts a woman up to seven times at risk for developing the disease in subsequent pregnancies when compared to women who have never had preeclampsia. It is assumed that whatever caused her to be susceptible in the first place will also cause later incidences of preeclampsia. A family history also puts a woman more at risk, though the mother-in-law's pregnancies do not seem to have much of an effect (Sibai et al., 1995; Duckitt & Harrington, 2005).

Treatment

Treatment options for preeclampsia are largely based on symptoms. The best treatment is delivery, but immediate delivery is

not always an option. Other treatments therefore are based on prolonging the pregnancy for as long as possible, keeping the mother's health in mind (Papadakis & McPhee, 2017).

When systolic blood pressure rises above 160 mmHg, or diastolic above 110, antihypertensive drugs are administered to bring blood pressure back down to 140/90, the threshold for preeclampsia's diagnosis. In severe preeclampsia, steps are taken to ensure that the patient will not develop eclampsia. If the mother begins to experience muscle spasms or hyperexcitability, showing that her CNS is beginning to be affected, magnesium sulfate is administered as a relaxant. Magnesium is also given to patients with eclampsia. Obviously, these women are monitored for toxicity. Calcium has been suggested as an aid in preeclampsia, but no real benefits have been proven (Papadakis & McPhee, 2017; Levine et al., 2004).

Delivery is the best option for the mother, and it is the treatment of choice from 36 weeks onward. Before that point, clinicians must decide if the fetus is ready for life outside the womb. If the fetus's lungs are not mature enough for birth, corticosteroids are administered to the mother for 48 hours, followed by induced delivery or cesarean. The steroids allow the fetus's lungs to mature more quickly. If the mother's symptoms are severe, such as in eclampsia, waiting for fetal lung maturation is not necessarily an option. Delivery must then be induced to the detriment of the fetus (Papadakis & McPhee, 2017).

Preeclampsia is a major cause of preterm delivery. Early delivery is often necessary for the mother's sake; the child suffers. Premature birth can result in respiratory distress, admittance to the NICU, low birth weight, jaundice, seizures, as well as other complications in infancy and later in life (Papadakis & McPhee, 2017). Fetal mortality is a large concern; preeclampsia is correlated with 25% of stillbirths and neonatal deaths in developing countries (Duley, 2009).

Both aspirin and nicotine appear to reduce the risk of preeclampsia. Aspirin has antihypertensive properties, so its role in decreasing the incidence of preeclampsia makes sense. In one study of healthy, nulliparous women, 4.6% of those given aspirin developed preeclampsia, compared to 6.9% of those given placebo. However, the aspirin did increase the risk of abruptio placentae, which makes the idea of treating all pregnant women with aspirin less appealing (Sibai et al., 1993). It is recommended by some for women at high risk for preeclampsia (Sibai et al., 1995).

Cigarette smoking is known to decrease the risk of preeclampsia, but doctors are hardly likely to begin advising smoking during pregnancy. One explanation for this phenomenon is that nicotine appears to play a role in reducing the amount of sFlt1 in the mother's bloodstream. This ameliorates preeclampsia's antiangiogenic effects and does not allow inflammation to cause the mother to develop hypertension and proteinuria. Even if smokers develop preeclampsia, it is usually not very severe. In a clinical trial testing aspirin's effect, 5.9% of nonsmokers developed preeclampsia, compared to 2.7% of those who quit during pregnancy, and 3.7% of those who smoked throughout. The many negative effects of cigarette smoking on both mother and child, though, far outweigh the small shielding from preeclampsia's effects (Sibai et al., 1993; Jeyabalan et al., 2008).

The current treatments for preeclampsia are less than ideal, but experimental treatments may give us some better options. It is important to note that designing preclinical and clinical trials for these treatments can be quite difficult. Animal models are often less than ideal as these animals may have very different placentas than humans. Many of these trials involve animals that do not ever develop preeclampsia. Scientists instead create situations to mimic preeclampsia's symptoms in vivo. Moving on to a clinical trial in humans will only occur after extensive testing. Nobody wants a repeat of the thalidomide disaster, so advancement in pregnancy treatment moves slowly (Sibley, 2017).

In a 2016 study of 28 rats, apelin was used to successfully ameliorate symptoms of preeclampsia. Apelin is a peptide found in the cardiovascular system. Among other effects, it reduces blood pressure in atherosclerosis and encourages angiogenesis. In this experiment, half of the rats served as control (N group), and half of these were given apelin (N+APLN group), an angiogenic factor naturally present in mammalian placentas. Preeclamptic placentas usually have lower apelin than normal. The acceptable rat model of preeclampsia was used on half the rats (PE and PE+APLN); uterine arteries were clamped to prevent adequate blood flow to the placenta, leading to the release of cytokines and debris causing hypertension and proteinuria. Half of these rats were then treated with apelin. The rats given apelin had lower blood pressure and proteinuria than the preeclamptic control, but not as low as the healthy rats. Apelin also increased fetal survival rate and birth weight. One hundred percent of N and N+APLN embryos survived, 25% of PE survived, and 50% of PE+APLN survived. Considering that fetal survival rate for humans with preeclampsia is much better than 25%, it is reasonable to believe that apelin would cause even better survival rates in humans. The effect on preeclampsia's maternal symptoms are also likely to carry over. However, as apelin was only tested on 8 preeclamptic rats, and because rat placentas differ greatly from human ones, much more research must be done before apelin is the drug of choice for preeclampsia (Sibley, 2017; Wang et al., 2017).

Researchers have also recommended mediating the effect of sFlt1 on the mother's body by somehow increasing the levels of VEGF in the placenta. One method of doing so is by injecting adenovirus vectors for VEGF into the placenta. This was done in sheep and guinea pig models, as well as in human placentas in vitro. The increase in VEGF levels mediates the inactivation of VEGF by sFlt1, resulting in an approximately normal quantity of angiogenic factors. This appears to create normal uterine blood flow and cure preeclampsia. The virus vector also does

not appear to cross the placenta and cause other complications. This treatment is extremely promising and is moving towards clinical trial in Europe (Sibley, 2017).

Conclusion

Preeclampsia is a major obstetrical complication still common in the developed world. Its causes are not fully understood, but are largely believed to develop from hypoxic conditions of the placenta. The placenta releases inflammatory factors, causing hypertension and proteinuria for the mother, as well as other side effects. Fetal effects include growth restriction and the negative effects related to premature birth. Risk factors are well-known yet poorly understood, and prophylactic treatments such as aspirin are available. Treatment for preeclampsia and eclampsia now mainly revolve around symptoms, but experiments are underway which will hopefully lead to a greater understanding and more effective treatment for this syndrome.

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Pathogenic Mechanisms of Takotsubo Cardiomyopathy or Broken Heart Syndrome

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Abstract

Takotsubo Cardiomyopathy (TTC) is a temporary heart-wall motion abnormality with the clinical presentation of a myocardial infarction. Found predominantly in postmenopausal women, TTC most often appears with apical ballooning and mid-ventricle hypokinesis. Often induced by an emotional or physical stress, TTC is reversible and excluded as a diagnosis in patients with acute plaque rupture and obstructive coronary disease. The transient nature and positive prognosis of this cardiomyopathy leaves a dilemma as to what precipitates it. This paper explores the theories of the pathogenesis of TTC including coronary artery spasm, microvascular dysfunction, and catecholamine excess. A thorough analysis of the pathogenesis was conducted using online databases. The coronary artery spasm theory involves an occlusion of a blood vessel caused by a sudden vasoconstriction of a coronary artery. This condition was confirmed in some patients with TTC using provocative testing, but failure to induce a coronary artery spasm in many patients led to its dismissal as a primary pathogenic mechanism. It is however a significant occurrence in patients with TTC and cannot be dismissed entirely. The microvascular dysfunction theory is challenged in the limited and underdeveloped methods of testing for its presence. However, using the corrected Thrombolysis in Myocardial Infarction frame count method to evaluate the flow of contrast in coronary arteries, researchers were able to indicate diffuse impaired coronary microcirculation in the myocardium. The theory involving catecholamines is based on the catecholamine surge that many patients experience with emotional or physical stressors. The stressor leads to excitation of the postsynaptic sympathetic neuron and the adrenal medulla, stimulating an influx of norepinephrine and epinephrine and the resulting hypokinesis of the apical portion of the left ventricle. Further research focused on this theory discovered the protective nature of estrogen against the catecholamine surge, explaining the prevalence of TTC in post-menopausal women. Genetic research perpetuates this theory by presenting predisposed genetic factors that prevent TTC. Analysis of the three theories found the catecholamine theory to be the most probable mechanism behind TTC, but further research is necessary to confirm TTC pathogenesis.

Introduction

Background

The cardiovascular system encompasses the extensive network that supplies the body with the nutrients and oxygen it needs to function. At the center of this complex system, the heart serves as an anatomical pump to push the blood out of its chambers and throughout the body. The sinoatrial node stimulates the cardiac muscle to contract periodically, and its normal rhythm can be measured on an electrocardiogram (ECG). In the presence of cardiac dysfunction, this test can indicate specific abnormalities in the sinus rhythm. The left ventricle is the heart's main pumping chamber, and a weakening or abnormality in its function can produce severe repercussions. There are multiple causes of left ventricular dysfunction. In the case of Takotsubo cardiomyopathy (TTC), sources like coronary artery spasm, microvascular dysfunction, and catecholamine excess are suspected to contribute to its pathogenesis (Komamura, et al. 2014). These theories are evaluated and debated in many recent studies that seek to discover the likely origin and development of TTC.

The syndrome was first described in 1990 by a Japanese cardiologist which prompted its name, Takotsubo cardiomyopathy (Sato, et al. 1990). Translated from Japanese to "octopus trap", takotsubo describes the shape of the left ventricle during systole in many patients suffering from TTC. Resembling its namesake's rounded bottom and narrow neck, TTC most commonly appears with apical ballooning and mid-ventricle hypokinesis. TTC is also known as stress-induced cardiomyopathy, ampulla cardiomyopathy, transient left ventricular apical ballooning, and broken heart syndrome. The latter title indicates the likely presence of an emotional or physical trigger that precipitates the condition. Researchers found stressors such as the sudden death of a loved one, anxiety over a family member's congenital disorders, and vigorous excitation to be responsible for the onset of TTC in some of their subjects (Tsuchihashi, et al. 2001). There are instances where no physical or emotional stressor is indicated and the trigger is unknown (Gianni, et al. 2006).

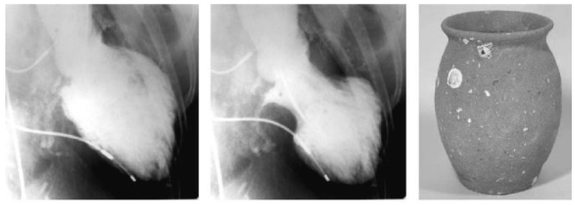

Figure 1. Left ventriculogram exhibiting Takotsubo. A: end-diastolic phase; B: end-systolic phase. Here, the apex shows akinesis with visible hypercontraction of the basal portion. C: Takotsubo, an octopus trap. (Akashi, et al. 2008)

Epidemiology

This specific cardiomyopathy is a relatively new diagnosis with the number of published cases growing in the past 20 years. The condition occurs predominantly in postmenopausal women which lends to one of the pathogenic theories of TTC regarding lack of estrogen and its effects on catecholamine levels in the body (Komamura, et al. 2014). A study found a 6.3-fold higher incidence of the condition in women than in men, differing

from the usual male dominance in coronary artery diseases (Tsuchihashi, et al. 2001). A systematic review of 14 studies found that 88.8% of TTC patients were women, leaving significant room for a pathogenic mechanism that can explain the specific epidemiology of TTC.

Clinical Manifestations

Takotsubo cardiomyopathy's most common symptomatic presentation includes severe chest pain and dyspnea, resembling the symptoms of an acute myocardial infarction (Komamura, et al. 2014). Where myocardial infarctions are generally preceded by atherosclerosis, plaque buildup in arteries, TTC is excluded as a diagnosis in patients that present with artery blockage or a history of obstructive coronary artery disease. A study found 90% of its subjects without preceding conditions admitted to chest pain or discomfort with the onset of TTC (Tsuchihashi, et al. 2001). Serum level indication using cardiac biomarkers found creatine kinase and troponin T to be elevated in many cases, indicating a heart muscle abnormality. Creatine kinase expressed in cardiac muscle is used to assay damage to the heart in various cardiac conditions. In TTC, there is a slight elevation from the normal 22-198 IU/l (Tsuchihashi, et al. 2001). Troponin T, a cardiac protein released when the heart muscle is damaged, is found to be similarly elevated (Gianni, et al. 2006). In 86.5% of patients with TTC, left ventricular ejection fraction was reduced to 40.7±11.2% from its normal 55% or higher. Studies also note increased left ventricular end-diastolic pressure in 93% of patients with this condition (Templin, et al. 2015) (Tsuchihashi, et al. 2001).

Diagnosis

According to Heart Failure Association's diagnostic criteria, patients need to fulfill the following specifications for their condition to be classified as TTC. The first is the presence of a wall motion abnormality involving the dyskinesis, hypokinesis or akenesis of the left ventricle mid segments. This includes the ballooning of the apex as it fails to contract. Echocardiograms or cardiac ventriculography can be used to identify this cardiomyopathy (Abe, et al. 2003). In addition, TTC is indicated only in the absence of obstructive coronary disease and acute plaque rupture and can be determined using angiographic images of the coronary arteries. This exclusion differentiates TTC from a myocardial infarction, a heart attack, which is caused by acute plaque ruptures and presents with similar symptoms. The third criteria for the diagnosis includes the appearance of new, reversible sinus rhythm changes on the patient's ECG. Most commonly, the condition appears with ST-segment elevation and T wave inversion (Gianni, et al. 2006). Another criterion is the significant elevation of serum natriuretic peptide (BNP or NT-proBNP), a blood indicator of heart failure. Bloodwork to assess cardiac enzyme levels like Troponin T is also used in the diagnosis of TTC. Perhaps the most important aspect of diagnosing a patient with TTC is the transient nature of the condition. Essentially, the patient should recover full cardiac function within a week of the acute episode. If a patient does not recover full systolic function within 12 weeks, a different diagnosis should be considered (Komamura, et al. 2014).

The Mayo Clinic's diagnostic criteria includes the absence of pheochromocytoma, a hormone releasing tumor of the adrenal glands. If a patient presented with additional symptoms such as sweating, tachycardia, and headaches, pheochromocytoma was suspected (Reeder, Prasad, 2017). However, the updated conclusive criteria released by the Heart Failure Association, allows for pheochromocytoma as a trigger for TTC in the event that it precipitates a catecholamine storm (Ansari, El-Battrawy, 2017). Mayo Clinic also addresses the exclusion of myocarditis, inflammation of the myocardium, as part of its diagnostic criteria. Myocarditis involves slower recovery than TTC and can be excluded with the absence of scarring and myocardial inflammation on cardiovascular magnetic resonance imaging (MRI). MRI also confirms the reversible wall motion abnormalities and the quantification of the ventricular function in a patient with suspected TTC.

Management

Due to its transient nature, TTC can often be managed by addressing and alleviating the physical or emotional stressors that triggered the onset of the cardiomyopathy. Although there are not any conclusive treatments, treatments generally administered for heart failure such as beta blockers, ACE inhibitors, and diuretics, are often administered as supportive care for TTC patients. A possible treatment specified for older women is estrogen administration. This was found to be beneficial in an animal model of TTC, though clinical trials have not yet been performed. The general prognosis of TTC is positive, but complications have occurred in many patients ranging from cardiogenic shock and left ventricular outflow tract obstruction, to severe systolic dysfunction. Diagnosis and management of these conditions is imperative to reduce the fatalities attributed to TTC (De Backer, et al. 2014).

Methods

The research in this paper was compiled using the online databases, Pubmed and UpToDate, and through Touro's online library system for access to databases Proquest and Ebsco. Keywords Takotsubo cardiomyopathy and Broken heart syndrome were used in the initial research of this paper. Careful analysis of the gathered material prompted further research using sources cited in articles on this topic. Both peer reviewed articles and clinical studies were analyzed to evaluate the hypothesized pathogenic mechanisms of Takotsubo cardiomyopathy.

Discussion

When evaluating the pathogenesis of TTC, researchers are faced with various differentiating origins and pathways that TTC

can develop from. A worldwide investigation of patients with TTC has led to several possible theories of the cardiomyopathy including a coronary artery spasm, catecholamine excess, and microvascular dysfunction. While studies have produced results that support all three theories, there is still a significant amount of research required to determine the definitive pathogenic mechanism of TTC.

Coronary Artery Spasm

The heart is a recipient of its own labor in its utilization of its blood supply from the coronary arteries. In order for the heart muscle to function, the correct amount of blood supply in regular increments must be delivered through these arteries. Many have hypothesized that an abnormality in this cycle is the source of TTC in the form of a coronary artery spasm. A coronary artery spasm is a temporary contracting of the wall of the artery that constricts blood flow and leads to decreased blood supply to the heart muscle. The occlusion or near occlusion of the vessel can cause decreased muscle movement in the heart chambers that depend on the regular blood supply from the coronary arteries.

Studies performed on patients with TTC support this theory. In one case study, a 79-year-old man was admitted to a hospital presenting with three consecutive days of chest pain. Echocardiography showed hyperkineses in the basal wall and akenesis in the apex of the left ventricle. In order to locate the source of the cardiomyopathy, doctors performed provocative testing using ergonovine, a muscle contractant. The diagnostic screening test induced a right coronary artery spasm and resulted in ECG changes with increased ST segment elevation in leads II, III, and aVF. The patient's normal left ventricle wall motion was restored one week later, and he was subsequently diagnosed with TTC with a pathogenesis attributed to a coronary artery spasm (Misumi, et al. 2010).

Although there are many cases of patients with TTC experiencing induced coronary artery spasm from provocative testing using either ergonovine or acetylcholine, there are more that have not. Many patients with TTC did not present with increased susceptibility to ergonovine or acetylcholine in provocative testing, and according to reports, only 30% of patients presented characteristics of a vasospasm with testing (Komamura, et al. 2014). This research leaves a substantial gap in the coronary artery spasm theory (Madias, 2014). Nonetheless, the presence of coronary vasospasm in some TTC patients cannot be ignored and this theory has therefore not been completely dismissed.

Microvascular Dysfunction

Another theory explored as a pathogenic mechanism of TTC is microvascular dysfunction. The primary obstacle in exploring this mechanism is the limited technology available to evaluate microvascular function. One study was performed using the Thrombolysis In Myocardial Infarction (TIMI) frame count technique, a quantitative, continuous variable that assesses flow changes by counting the cineframes it takes for contrast to reach coronary landmarks. Researchers evaluated the flow of the left anterior descending artery, left circumflex artery, and the right coronary artery in order to suggest diffuse impaired coronary microcirculation in the myocardium. In 23 of the 24 patients studied, there was a slowdown in coronary microcirculation noted (Fazio, et al. 2010). A more recent assessment pointed out that the akinesis in the left ventricles of those patients was too large of an area to be attributed to the dysfunctional microvessels' supply (Vitale, et al. 2016). A corrected TIMI frame count was performed and found that the flow was slower in the left anterior descending artery and the researchers suggested this being the source of akinesis in the apex while the base is relatively spared in TTC (Khalid, et al. 2015). However, the combined studies do not find microvascular dysfunction to be the primary source of TTC, but it is likely to play a role in the etiopathogenesis.

Catecholamine Theory

The catecholamine theory is perhaps the most well developed and significant theory of TTC. Many patients are noted to have grossly elevated plasma catecholamine levels, measuring at two to three times greater than in patients with myocardial infarctions and twenty times higher than normal adults (Zeb, et al. 2011). These elevations were noted of both adrenomedullary and sympathoneurally-derived catecholamines. The adrenal medulla releases epinephrine and norepinephrine after stress, which activates preganglionic sympathetic nerves. In addition, the peripheral sympathetic nerves release norepinephrine, both of which contribute to the β-adrenergic pathway. The common emotional or physical stressor in patients is tightly connected with this theory in its influx of catecholamines. Researchers concluded that the stressor induced hyperactivity of the sympathetic nervous system and prompted the release of catecholamines into the patient's blood stream (Tarkin, et al. 2008).

The apex of the left ventricle has higher adrenoceptor density than the base, presenting location specific evidence for the cardiomyopathy. The influx of catecholamines, both epinephrine and norepinephrine, are directed to the β-adrenergic pathway. This pathway is specifically located at the apex of the left ventricle, likely to produce the heart's fight or flight response of hypercontraction of the cardiomyocytes. The β-adrenergic receptors in the cell membranes bind to the catecholamines which ignites this response. The overstimulation of Gs (activator) through β2-coupling due to catecholamines can cause apoptosis of the myocytes. The process therefore switches to Gi (inhibitor) to protect the myocytes from further damage. This causes a decrease in contraction and results in the hypokinesis of the apex of the left ventricle. There is serum evidence

of slight necrosis caused by the excess catecholamine in the minimally elevated troponin levels in patients with TTC. Studies showed that a signaling pathway known to exhibit anti-apoptosis functions, phosphatidyl inositol 3-kinase protien kinase B, presented increased activity. This may be the source of the quick recovery of the myocytes. In addition to this, the transient nature of this condition can be attributed to the inverse switch from Gi to Gs resulting in the expeditious recovery of systolic function in patients (Nef, et al. 2009).

Another theory on the rapid recovery of the myocytes is related to stem cells. A study using in vivo and in vitro cardiomyocytes and cardiac stem cells found that the cardiac stem cells were resistant to neurohumoral overstimulation. Researchers injected male Wistar rats with isoproterenol and noted left ventricle dysfunction in the subjects. The left ventricular function began to improve on day three post isoproterenol stimulation. β-adrenoreceptor hyperactivity from the catecholamine stimulation leads to PKA-mediated hyperphosphorylation of the ryadonine receptor 2, a calcium channel that mediates the release of Ca2+ from the sarcoplasmic reticulum to the cytoplasm. This hyperphosphorylation causes Ca2+ leakage which, in turn, produces myocyte damage. The cardiac stem cells have low levels of β-adrenergic receptors and do not express ryadonine receptor 2. This explains the resistance cardiac stem cells possess to the catecholamine overstimulation and the regeneration of cardiomyocytes that restore normal left ventricle function (Ellison, et al. 2007).

There is research that suggests specifically local release of catecholamines are at play in TTC. This is based on the findings in a study performed on blood samples from both the aortic root and coronary sinus of patients with the condition. Catecholamine concentration was found to be higher in the coronary sinus signaling excessive local catecholamine release from the heart (Kume, et al. 2008).

Besides for emotional stressors, catecholamine excess resulting in TTC is precipitated by other factors like acute brain injury or treatment of respiratory distress. A study done on patients with subarachnoid hemorrhage reports of eight patients developing TTC as a result of a brain aneurysm rupture. Researchers theorize that at the time of the event, patients experience a catecholamine surge which can mediate cardiopulmonary dysfunction (Franco, et al. 2010). A case report presenting a patient with acute asthma exacerbation who was treated with β-2 agonist nebulization and intravenous aminophylline. After fourteen hours of treatment she complained of shortness of breath and pain in her jaw. Testing showed classic TTC with ST segment elevation, T wave inversion, and left ventricular apical akinesia. The treatment was immediately stopped and replaced with ipratropium nebulization and intravenous corticosteroids. After 48 hours the echocardiogram revealed full recovery. Additional studies report that methylxanthines, the structural classification of aminophylline, stimulate the release of catecholamines from the adrenal medulla and of norepinephrine from cardiac β-adrenergic nerve endings (Khwaja, Tai, 2016). Another study reports a similar case with a patient treated with nebulized adrenaline to manage an airway obstruction. Like the previous case study, this patient developed TTC post adrenaline treatment (Keshtkar, et al. 2016). These reports present a medication administration that possibly caused a catecholamine surge suspected to have induced the patients' TTC.

While the catecholamine theory is the most developed and scientifically supported mechanism of TTC, not all patients are found to have elevated catecholamine levels, prompting continued investigation into a definitive pathogenesis of the condition (Tarkin, et al. 2008).

Low Estrogen Levels

The occurrence of TTC specifically in post-menopausal women prompted the investigation of estrogen deficiency as a predisposing factor. One study demonstrated increased estrogen serum levels in rats weakened cardiac changes in response to immobilization stress. P44/p42 mitogen-activated protein kinase was activated by the immobilization stress along with the upregulation of immediate early genes in the myocardium. Immediate early genes are activated in response to cellular stimuli at the transcription level. The study theorizes that estrogen attenuated this process and inhibited the activation of the sympathetic nervous system by decreasing the formulation of immediate early genes in both the brain and heart (Ueyama, 2004). In a study published by this author more recently, it was determined that, in response to immobilization stress, ovariectomized rats that received estradiol did not experience a significant decrease in left ventricular contraction. The ovariectomized exposed to stress sans estradiol treatment did, however, experience percentage contraction reduction (Ueyama, et al. 2003). An evaluation of women with TTC found that majority of the patients were post-menopausal and had not undergone estrogen replacement therapy. Researchers postulated that lack of estrogen replacement therapy may predispose women to TTC (Kuo, et al. 2010). Combined, these studies propose that post-menopausal women, who experience a deficiency of estrogen, lack a protective barrier against the development of TTC.

Genetic Predisposition and the Catecholamine Theory

With analysis, the catecholamine theory appears problematic because not all patients experience this cardiac dysfunction with an emotional or physical stressor that may cause a catecholamine surge. Genetic research introduced new theories that address this dilemma.

The apparent exclusiveness of patients who develop TTC suggests that the general population possesses a molecular mechanism that protects their cardiomyocytes from a catecholamine

surge and prevents necrosis. Bcl2-associated athanogene 3 (BAG3) is a constituent of an autophagy pathway and one that allows for the degradation of intracellular components. A study found that it is expressed in response to various stressors and is therefore theorized to promote stress resistance. The ablation of BAG3 in mice resulted in lethal cardiomyopathy shortly postnatal. The study found that BAG3 single-nucleotide polymorphisms resulted in TTC. It introduced a novel post-transcriptional pathway that, in response to epinephrine treatment, leads to BAG3 expression. Micro-RNAs are fundamental in their role as repressors of messenger RNAs' translation. The study describes miR-371a-5p, a pathway that binds miRNA to 3'-untranslated region (3'-UTR) of the BAG3 gene. Epinephrine induces miR-371a-5p which leads to BAG3 upregulation in cardiomyocytes. However, this protective mechanism is lost in patients who possess a single nucleotide variant involving the 3'-UTR in the BAG3 gene which alters the miR-371a-5p pathway and eliminates its binding. This genetic variant of BAG3 is found in many patients with TTC and could explain the cardiac dysfunction post catecholamine (D'avenia, et al. 2015).

Further research into the catecholamine mechanism produced an underlying theory involving another genetic role in TTC. The study concluded that genetic susceptibility involving β-adrenergic signaling increased the risk of toxicity induced by catecholamines in TTC.

Researchers utilized technology involving the reprogramming of somatic cells into induced pluripotent stem cells (iPSC) to produce this conclusion. Reprogramming somatic cells of these patients allowed for differentiation into cardiomyocytes that could be experimented on.

The study involved healthy donors for controls and patients with TTC. The somatic cells of the Takotsubo patients were reprogrammed and expanded into high quality iPSC clones. Using Wnt modulation and metabolic selection, cells were then differentiated into cardiomyocytes (CMs). After three months, the iPSC-CMs were subjected to isoprenaline or epinephrine in order to replicate the catecholamine stimulation suspected to be experienced in patients with TTC. After analysis, studies showed that the catecholamine excess in the TTC iPSC-CMs was apparent in the increased expression of NR4A1, a cardiac stress-related gene. Compared to the control iPSC-CMs, the TTC iPSC-CMs recorded much greater expression of NR4A1 post subjection to catecholamines. Three weeks after catecholamine administration, there was a reversal in changes to the NR4A1 expression.

The researchers proceeded to investigate β-adrenergic signaling using the TTC iPSC-CMs. They measured cAMP levels and PKA activation by phosphorylation and found increased levels in both compared to the control iPSC-CMs after catecholamine treatment. In addition, extracellular signal-regulated kinase was phosphorylated maximally in the TTC iPSC-CMs after epinephrine or isoprenaline treatment where the controls were significantly reduced in comparison. Increased lipid accumulation was also noted in catecholamine treated TTC iPSC-CMs.

Researchers examined the electrical activity of catecholamine treated iPSC-CMs from patients with TTC and found that more than half were silenced under certain isoprenaline concentrations where only some of the controls were. For those that were not silenced, beating frequency was significantly increased in the TTC iPSC-CMs in comparison to the control subjects. The changes to the electrical activity was reversed following washout of the isoprenaline after 24 hours.

Additionally, the study found that engineered heart muscles using the TTC iPSC-CMs presented an impaired force of contraction. Muscles also presented a higher sensitivity compared to control subjects to isoprenaline-stimulated inotropy, an altering of the force of muscle contractions. Further investigation into the genetic makeup of the patients with TTC found variants in some patients' genes that are key regulators of cardiac function. These findings may contribute to the hypothesis of a predisposition to TTC, specifically involving catecholamine toxicity (Borchert, et al. 2017).

Conclusion

The pathogenesis of TTC is an evolving phenomenon with developing theories. While coronary artery spasm is found in some patients with TTC, the majority failed to experience induced coronary artery spasm with provocative testing. Microvascular dysfunction is a challenge to evaluate, but it has been found to be a contributing factor in many patients with TTC, though it is likely not the primary cause. Of the three theories presented, the catecholamine excess theory, supported by genetic research, and the estrogen deficiency theory is the most well developed pathogenic mechanism. In order to understand this cardiomyopathy in its entirety and to produce an appropriate treatment, more research is required involving integration of the cardiovascular, central neural, autonomic, and endocrine systems in their response to stress, and the genetic predisposition to TTC.

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Is Detection of Preclinical Alzheimer's Disease Possible?

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Abstract

Alzheimer's disease, the most common form of dementia, is a neurodegenerative disease that causes progressive memory loss and severe cognitive impairment with time. As the sixth leading cause of death in the United States, Alzheimer's disease kills more people than breast cancer and prostate cancer combined, and there are over five million Americans suffering from Alzheimer's disease today. A diagnosis of probable Alzheimer's disease is typically given after rigorous neurological and psychological examination, neuroimaging, and cognitive testing, although the diagnosis cannot be confirmed unless a postmortem examination finds specific pathological lesions in the brain. The lack of proper diagnostic technique often leads to patients being diagnosed after years of the disease's progression, at which point there is already significant neurological damage. Biomarkers for Alzheimer's disease are therefore crucial to accurately monitor the disease's progression and eventually determine the effectiveness of certain therapies. There are countless studies being performed to discover the different biomarker possibilities for detecting Alzheimer's disease in its preclinical stage. Constructed on multiple original scientific research articles, this paper focuses on two such approaches toward preclinical Alzheimer's disease detection, specifically focusing on blood-based biomarkers. These detection methods have been developed based on the two pathological characteristics of the disease, beta-amyloid plaques and neurofibrillary tangles in the brain that lead to cerebral atrophy. The ADAM10 protease can be used as an Alzheimer's disease biomarker by having reduced levels in patients with the disease, lending to the hypothesis that its reduction leads to the formation of beta-amyloid plaques. Using plasma-based tests to measure inflammatory-related responses such as cytokines and plasma signal proteins to neurofibrillary tangles is another effective way in detecting preclinical Alzheimer's disease. These biomarker possibilities show promising opportunities to detect Alzheimer's disease before or as soon as symptoms appear, thus enabling patients to receive optimal care and treatment.

Introduction

According to the 2011 Criteria and Guidelines for Alzheimer's disease developed by the National Institute of Health and the Alzheimer's Association, there are three stages of Alzheimer's disease. In the first stage, preclinical Alzheimer's disease, patients exhibit no symptoms of memory loss or changes in behavior, but begin to form progressive pathological characteristics of the disease in the brain, cerebrospinal fluid, and blood. The second stage, mild cognitive impairment, or MCI, due to Alzheimer's disease indicates noticeable changes in behavior and cognition that do not affect everyday life function and activities. The final stage is dementia due to Alzheimer's disease. This is the stage in which a person exhibits distinct memory loss and cognitive decline that severely impairs everyday function (www.alz.org).

Although there are already measurable pathological changes in the brain, cerebrospinal fluid, and blood during the preclinical stage of Alzheimer's disease, the changes are rarely detected or tested for due to the lack of symptoms. While preclinical Alzheimer's is considered the first stage of the disease, the 2011 criteria and guidelines for Alzheimer's disease do not list diagnostic criteria that can be used in diagnosing it, causing the typical Alzheimer's disease patient to be first diagnosed after years of the disease's progression, and only after symptoms appear.

In the past two decades, there has been a huge effort to develop disease-modifying drugs to slow or stop the progression of Alzheimer's disease. These treatments are predicted to be most effective in the preclinical and early mild cognitive impairment stages. However, because most patients partaking in clinical trials are already in advanced stages of the disease, many of these trials have failed. Therefore, specific, easily-identifiable and accessible biomarkers are crucial for diagnosing patients in the preclinical stage so that their fitness for disease-modifying treatments can be determined, and the effectiveness of such treatments can be accurately monitored (www.alz.org). Understanding specific biomarkers would also lend patients more time to plan for their future and would give them opportunity to be involved in decisions involving their home care and legal matters while their cognition is still intact (Gomez Ravetti, Moscato, 2008).

Current detection mechanisms of Alzheimer's disease include brain imaging and cerebrospinal fluid testing. These methods, however, are expensive and invasive, and are not easily accessible to large portions of the population (O'Bryant et al., 2011). Additionally, these methods are inconclusive and are usually only performed once the disease has reached the mild cognitive impairment or dementia stage and has already progressed significantly.

Researchers are therefore in search of conclusive biomarkers that can be used in routine screenings and detection of preclinical Alzheimer's disease (Berg, 2008). Peripheral blood-based biomarkers are ideal for Alzheimer's disease screening, as they can be easily accessed via routine venipuncture, which is cost-effective, noninvasive, and available to majority of the population (O'Bryant et al., 2011). This paper explores several blood-based biomarker possibilities that can be used as routine screenings for preclinical or early-onset Alzheimer's disease.

Alzheimer's Disease Pathology

Although the exact pathological cause of Alzheimer's disease is still unknown, there are two major pathological characteristics that researchers have pinpointed to indicate the disease and its progression. These characteristics are excessive beta-amyloid peptide plaques and phosphorylated tau protein deposition in

the brain. Through different mechanisms, these characteristics both cause neuronal death and eventual atrophy that spreads throughout the brain (Manzine et al., 2013).

In a normal, nonamyloidogenic pathway, amyloid precursor protein, or APP, an enzyme embedded in the cell membrane of neurons, is cleaved by alpha-secretase and releases a fragment called aAPP. aAPP is a large and insoluble molecule, and is believed to have a protective effect on the neural cells it occupies. aAPPs protect the neurons of the hippocampus against excitotoxicity, A beta toxicity, and glucose deprivation (Furukawa et al., 1996), and enhance learning and memory function. In patients with Alzheimer's disease, there is a decrease in APP processing via a-secretases (Manzine et. al., 2013). Instead, APP is cleaved by beta-secretase, followed by gamma-secretase cleavage. Beta-amyloid, the fragment produced by this pathway, is shorter than aAPP, and when beta-amyloid fragments aggregate, they become insoluble, forming beta-amyloid plaques. These plaques interfere with inter-neuronal communication, causing information transfer at synapses to fail, eventually leading to neuronal death (Cummings et al., 1996).

The second pathological characteristic of Alzheimer's disease is neurofibrillary tangles caused by abnormal tau protein deposition in the brain. In normal neurons, the tau protein attaches to microtubules and stabilizes the important internal structures of the cell by acting as a firm, scaffolding-like structure. Nutrients are carried throughout this structure, and the tau protein is essential for normal cell function and nutrient transport. In patients with Alzheimer's disease, however, the tau protein is modified and behaves differently. Tau protein separates from the microtubules in neurons of patients with Alzheimer's disease, causing the structure to fall apart. Strands of tau combine and form tangles inside the neuron, disabling the transport system and eventually destroying the cell (Ingelsson et al., 2004).

The accumulation of both beta amyloid plaque and neurofibrillary tangles leads to cell death. In patients with Alzheimer's disease, the abundance of cell death causes the brain to shrink in size and many important functions, such as memory, to fail. Atrophy begins in the lateral entorhinal cortex, which together with the hippocampus plays an integral role in long-term memory storage. Over time, the brain atrophy spreads to other areas of the cerebral cortex, specifically to the parietal cortex, the region of the brain associated with navigation and spatial orientation (Liu et al., 2013), leading to severe cognitive decline.

Researchers are still unsure of the direct causes of beta-amyloid plaque accumulation and neurofibrillary tangle formation. They have, however, pinpointed these characteristics as the cause of cerebral atrophy and cognitive decline in patients with Alzheimer's disease. It is therefore useful to utilize these known pathological markers of Alzheimer's disease as the basis for biomarker research, as they are present not only in the mild cognitive impairment and severe Alzheimer's disease stages, but begin to form in the preclinical stage, prior to the onset of cognitive symptoms (www.alz.org).

Methods

Research was done by studying original research articles and scientific papers found on the Touro College online library. Specific scientific databases such as Proquest and EBSCO were perused, and additional information was obtained by analyzing articles found.

Discussion:
Current Detection of Alzheimer's Disease Progression

Today, when there is concern for possible Alzheimer's disease, there is no definitive step to reach a diagnosis. Instead, a series of steps must be taken to ultimately lead to a probable diagnosis of the disease. Even then, it is only when neurofibrillary tangles

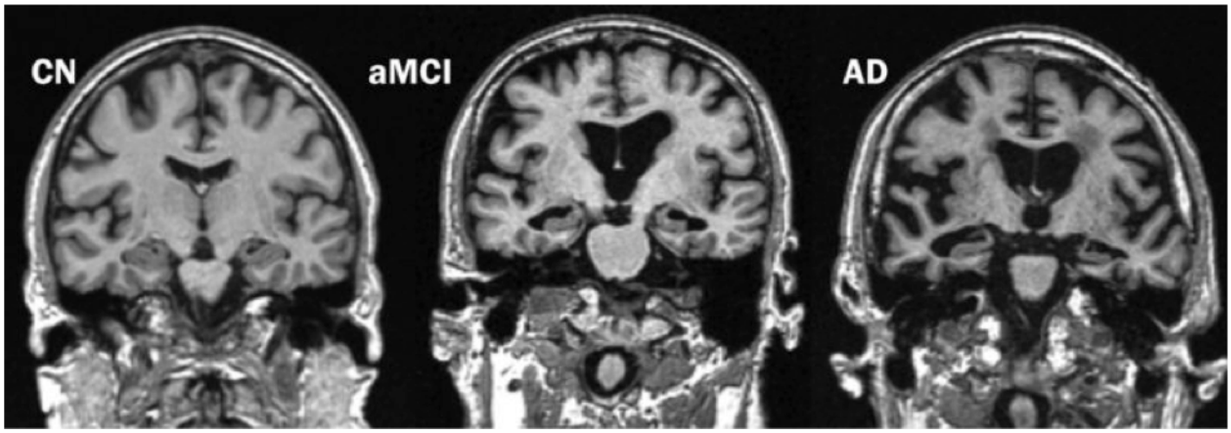

Figure 1: MRI scans of the brains of (i) cognitively normal (CN) patient, (ii) patient with amnesic mild cognitive impairment, and (iii) patient with Alzheimer's disease (Vemuri, Jack, 2010).

(NFTs) in neurons, amyloid plaques, and synapse or neuronal loss and atrophy are found upon postmortem examination of the brain that Alzheimer's disease can be fully confirmed (Berti et al., 2010).

To reach a probable diagnosis of Alzheimer's Disease, a mini mental state exam, or MMSE, is first performed, in which the patient is asked to fulfill certain tasks and answer a variety of questions ranging from recalling the date and time to repeating words or sentences and copying drawings and shapes. A gross score is assigned, and a score ranking of "cognitively impaired" will cause a doctor to send a patient for further testing (O'Bryant et al., 2008).

Blood tests will be done to rule out other causes of dementia, such as abnormal thyroid function or vitamin B-12 levels (Agarwal et al., 2008). The patient may then be referred to a neurologist or sent for brain imaging to detect more specific Alzheimer's disease pathological markers or other causes of cognitive impairment by looking for blood accumulation, brain tumors, strokes, and change in brain size.

While brain imaging such as magnetic resonance imaging (MRI) and positron emission topography (PET) scans are currently used on patients in the mild cognitive impairment or late Alzheimer's dementia stage, there is still much uncertainty regarding the use of such imaging techniques in detecting the disease in its preclinical stage. As seen in figure 1, MRI imaging shows clear cerebral atrophy in patients with mild cognitive impairment and Alzheimer's disease when compared to patients with normal cognition (Vemuri, Jack, 2010). Several studies have shown that MRI detection of atrophy of the medial temporal lobes (MTL), the brain region responsible for language and long-term memory, can be an indication of early AD and predicts conversion to dementia (Rusinek et. al., 2003). Additionally, Fluoro-2-deoxy-D-glucose (FDG) PET scanning has played the leading neuroimaging role in the early detection of Alzheimer's disease by using estimates of cerebral metabolic rate of glucose (CMRglc), an indication of synaptic density and function. Patients with Alzheimer's disease will exhibit CMRglc reductions that are visible on FDG-PET scans, which can even be detected before the onset of Alzheimer's symptoms (Berti et al., 2010). However, FDG-PET and MRI scans are limited as they lack disease specificity; other dementias exhibit reductions in CMRglc, as well, and there can be other causes of brain atrophy detected on an MRI (Wollman, Prohovnik, 2003).

Currently, cerebrospinal fluid (CSF) levels of tau protein and beta-amyloid plaques are the most reliable and widespread protein biomarkers used for Alzheimer's disease detection. This is because cerebrospinal fluid interacts directly with extracellular brain space and can therefore be used as an accurate marker for pathological changes in the brain (Hampel et al., 2012). However, obtaining cerebrospinal fluid requires a lumbar puncture, or "spinal tap", a procedure that is expensive, painful, and time-consuming, and usually needs to be performed in a hospital setting. Testing CSF is therefore not a practical way to screen for preclinical Alzheimer's disease.

Genetic Risk Factors of Alzheimer's Disease

Although environmental factors such as diet, exercise, and education play a role in the development of Alzheimer's disease, twin studies strongly suggest that genetics is the major factor in its development (Gatz et. al., 2006). While many genes have been suggested as genetic risk factors, only one, the ε4 allele of the apolipoprotein E, or APOE, gene has been confirmed to directly correlate to Alzheimer's disease development (Corder et. al., 1993).

APOE, a glycoprotein composed of 299 amino acids, functions as a ligand, combining with lipids to form lipoproteins. APOE is essential for cholesterol homeostasis throughout the body, and is the principal cholesterol carrier in the brain (Puglielli et al. 2003).

Every person inherits one copy of the APOE gene from each parent. The APOE gene has three common single nucleotide polymorphisms (SNPs) leading to three common isoforms of APOE: Apoε2, Apoε3, and Apoε4. The most common form of APOE is ε3, with about 60 percent of the U.S. population inheriting one ε3 gene from each parent. Only about 20 to 30 percent of the U.S. population carries one or two copies of the ε4 gene, and 10 to 20 percent of the population has one or two copies of ε2. Despite these alleles differing in only one or two amino acids, they have drastically different functions and cause stark differences in the genotype (Raber et al. 2004).

Numerous studies have established that the Apoε3 form neither increases or decreases the risk of developing Alzheimer's disease, and that the Apoε2 form may decrease the risk of developing the disease. The Apoε4 allele, however, is a strong genetic risk factor for the development of Alzheimer's disease (Corder et al., 1993). Compared to people who possess no ε4 allele, people who are heterozygous for the ε4 allele exhibit a 2-3-fold increase in developing Alzheimer's disease, and people who are homozygous for the ε4 allele exhibit a 12-fold increase in developing the disease (Bertram et al., 2009).

Yet despite the strong correlation between patients with the Apoε4 allele and the development of Alzheimer's disease, the mechanism of how Apoε4 causes an increase in AB-deposition is still not fully understood (Holtzman et al., 2012). Furthermore, inheriting the ε4 allele does not guarantee that someone will develop the disease. The ε4 allele had a significantly weaker effect in some ethnic groups, such as African Americans and Hispanics (Farrer et al., 1997), suggesting that there are other environmental and genetic factors involved in the onset of Alzheimer's disease, and that the predisposition to developing Alzheimer's disease cannot be determined based on this gene alone.

Inflammatory Responses Associated with Alzheimer's Disease

As with any other disease, upon sensing abnormal neuronal degeneration caused by Alzheimer's disease, the body launches an immune response (Cagnin et al., 2001). When neurofibrillary tangles begin to form from abnormal tau proteins, astrocytes and microglia, usually inactive in brain tissue, are activated as part of the initial response (Leung et al., 2013). When activated, microglia become phagocytes and secrete many inflammatory molecules such as cytokines and chemokines, growth factors, complement molecules and adhesion molecules.

Several inflammatory agents have been specifically associated with amyloid deposits, including IL-1, IL-6, a1-antichymotrypsin (ACT), tumor necrosis factor-a (TNF-a), and transforming growth factor (TGF-b) (Mcgeer et al., 2006). When tested in blood plasma, these inflammatory agents showed a positive correlation with beta-amyloid peptide found in cerebrospinal fluid (Sun et al., 2003).

Another study was done comparing cytokine expression in blood plasma to neuropsychological testing and neuroimaging indicating Alzheimer's disease progression. Twenty-seven cytokines and chemokines (chemical messengers that cells release as an immune response) were tested. The study found that five cytokines and chemokines, IL-6, TNF-a, IL- 1ra, IL-10, and IL-13, were inversely related to ventricular volume, whole brain volume, or entorhinal cortex volume in Alzheimer's disease. That is, the greater the immune response to the disease, the smaller the brain volume, or the further along the disease progression. There was also an increase in the level of two cytokines, IL-10 and IFN- γ, when tracked in patients between visits (Leung et. al., 2013).

Aside from acting as an immune response to the atrophy of brain cells, the inflammatory response itself may also be contributing to brain atrophy. According to the amyloid cascade-inflammatory hypothesis, researchers postulate that Alzheimer's disease is caused by the inflammatory response to beta-amyloid deposits and is later intensified by tau protein tangles. As the disease progresses, so does the inflammatory response, and it may be for this reason that multiple attempts at developing disease-modifying drugs have failed, as they fail to take the inflammatory response into account. In this strain, possible therapies dealing with anti-inflammatory agents may show promise for developing successful disease-modifying drugs (McGeer et al., 2013).

In a highly-publicized study, it was hypothesized that the pathological processes related to Alzheimer's disease in the body would trigger an immune response with specific changes in certain signal proteins in the blood. A longitudinal study was done in which patients were tested at the initial diagnosis of Alzheimer's disease with mild cognitive impairment, and were then followed up with two to six years later. Using an algorithm called PAM (partition around medoids), eighteen of the one hundred and twenty proteins tested were identified as Alzheimer's disease predictors, with a 95% positive agreement and 83% negative agreement with the clinical diagnosis (Ray et al., 2007).

These results showed promising statistics that warranted additional study. The PAM algorithm was then used to predict Alzheimer's disease progression in twenty-two patients with mild cognitive impairment. The prediction of twenty of the twenty-two patients to develop Alzheimer's disease was proven correct when the patients were followed up with two to five years later (Ray et. al., 2007). This highly specific plasma biomarker test can therefore be used to predict Alzheimer's disease years before a clinical diagnosis would typically be made or even before any symptoms appear.

A further study found that of the original nineteen predictor proteins found by Ray et al., just five proteins could be used with 96% accuracy to detect preclinical Alzheimer's disease. Using less proteins caused less errors in prediction, and higher biomarker specificity and accuracy (Gomez Ravetti, Moscato, 2008).

Immune responses to beta-amyloid deposition and neurofibrillary tangles, including activation of microglia and astrocytes to release cytokines and chemokines, can be used as an index of Alzheimer's disease progression (Leung et. al., 2013). Additionally, the use of specific signal proteins can be used to accurately predict development of Alzheimer's disease (Ray et. al., 2007). Treatments aimed at dealing with these inflammatory responses may also prove to be effective although more research is needed to prove that hypothesis (McGeer, McGeer, 2013).

ADAM10 as a Biomarker

Efforts have been made to find biomarkers relating to the pathogenic mechanism of abnormal beta-amyloid aggregation causing the formation of amyloid plaques. The products of the ectodomain shedding process, in which amyloid precursor protein (APP) is cleaved by a-secretase into its soluble counterparts, can be used as detection mechanisms of preclinical Alzheimer's disease by methods similar to those used in measuring the immune response (Pruessmeyer, Ludwig, 2009).

In the amyloidogenic pathway, after APP is cleaved by β-secretase, also called β-site APP-cleaving enzyme 1 (BACE1), it is then cleaved by a γ-secretase, leading to the release of the AB fragment. BACE1 competes with the a-secretase for cleavage of the APP substrate, so that an increase in BACE1 activity causes a decrease in a-secretase activity, and vice versa (Vassar, 2013).

The identity of the a-secretase acting in the amyloid precursor protein cleavage process has never been confirmed. However, in three studies done, three members of the ADAM (A Disintegin And Metalloproteinase) family, ADAM9, ADAM10, and ADAM17, were suggested as possible a-secretases as their overexpression in cells or mice led to an increase in APP cleavage (Koike et al, 1999; Lammich et al, 1999; Slack et al, 2001). To further pinpoint a single protease as the predominant a-secretase, knockout methods were used on the three ADAM metalloproteases.

Only ADAM 10 was found to completely suppress a-secretase cleavage of APP when knocked out. ADAM10, therefore, can be used as a measure of a-secretase cleavage and its reduction can be used as a biomarker to detect preclinical Alzheimer's disease (Kuhn et al., 2013).

In patients with Alzheimer's disease, platelet levels of ADAM10 were reduced. A study was done to determine whether ADAM10 could act as an a-secretase and whether obtaining measurements of ADAM10 from platelets in blood plasma was an accurate measurement of ADAM10 levels in the brain. When compared to levels in cerebral spinal fluid, ADAM10 levels in platelets were similar. This was done because platelets present the highest levels of APP among the peripheral tissues (Colciaghi et al., 2002).

A study was done of two groups of Brazilian elderly people to compare ADAM10 protein levels in participants with Alzheimer's disease versus non-Alzheimer's disease participants. By studying the platelet levels of ADAM10 as the only statistically significant factor to increase probability of Alzheimer's disease, it was found that ADAM10 levels were significantly reduced in patients with Alzheimer's disease versus patients without it. The ADAM10 levels were also stage dependent; the further the disease had progressed, the lower the ADAM10 levels were, indicating a decrease in a-secretase cleavage of APP (Manzine et. al., 2013).

Conclusion

While many ethical concerns can be raised regarding the detrimental psychological effect of a preclinical Alzheimer's disease diagnosis, most patients do not react with a negative emotional response. On the contrary, an Alzheimer's diagnosis often comes as a relief to patients and their families by delivering a cause for a patient's cognitive decline (Carpenter et al., 2008).

Through the implementation of blood-based biomarkers, there is promise for easy detection of preclinical Alzheimer's disease. A combination of testing levels of a-secretases like ADAM10 and specific cytokine and chemokine inflammatory markers can help lead to a definitive preclinical Alzheimer's disease diagnosis. By using a combination of the above testing methods, Alzheimer's disease will be able to be definitively diagnosed before symptoms appear, giving patients time to discuss therapies and make certain decisions on their own. However, these testing methods must still be regarded with caution as many of them have not been tested on a vast enough population to be considered fool-proof. With further and more widespread research on the above-mentioned biomarker possibilities, there is promise for early and even preclinical detection of Alzheimer's disease. Easy and early detection will not only help patients struggling with unknown causes of cognitive decline, but will lend to more reliable research for possible drugs to help cure this prevalent disease.

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By What Mechanism Does Stress Affect Ovulation?

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Abstract

This paper explores the many mechanisms of how stress influences ovulation. For ovulation to occur, there needs to be a series of hormones released in a specific order, and in a specific amount. This paper will discuss the various type of stress a person can experience, and which specific reproductive hormone each different type of stress disrupts. This paper will also bring proof as to how each hormone is disturbed, and by which mechanism it is disrupted. There has not been one main mechanism that has been found thus far, therefore, it is important to take into consideration the various routes that stress can take to disturb ovulation. Understanding the various mechanisms is important in finding a way to treat women with menstrual or ovulatory dysfunction.

Introduction

Stress plays an important role in the daily life of a healthy person Stress can elicit biological and emotional responses which may affect the female's hormones, leading to change in the menstrual cycle, and ovulation. Ovulation is the release of an oocyte from the ovary. For ovulation to occur, a sequence of events must happen. The hypothalamus will secrete gonadotropin releasing hormone (GnRH), which stimulates the adenohypophysis to secrete both follicle stimulating hormone (FSH) and luteinizing hormone (LH). The levels and timing of these secretions is controlled by GnRH, and other factors such as inhibin and activin (Hawkins, Matzuk, 2008). The gonadotropins then stimulate the ovary to produce the steroid hormones estrogen or progesterone, as well as other peptides. Adequate presence of FSH stimulates the follicles in the ovary to grow even more and become competent to develop into an antral follicle. When it is exposed to LH, it produces estrogens from androgenic precursors. The concomitant increase in FSH on the follicular cell results in more binding of FSH and greater amount of estrogen secretion, resulting in a very high estrogen environment. Once high estrogen levels are triggered, a message is sent to the anterior pituitary to induce the LH surge, which leads to ovulation, when it picks a dominant follicle (Hawkins, Matzuk 2008). These mechanisms occur in a normal, healthy functioning female with normal hormone levels. However, when a female is exposed to stress, whether it is physical or mental, her hormones may not be at normal levels, thus her menstrual cycle and her ovulation mechanism may be disrupted. This paper will explore the effects of stress on mechanism in ovulation, and specifically, which hormones it disrupts when a female is exposed to a variety of different types of stress.

Method

The research obtained about the mechanism of how stress affects ovulation was collected from a variety of sources. The majority of the articles were collected using Touro's library to access databases such as Proquest, Pubmed, and many more. Other articles were also found through google scholar. After reviewing and studying many articles on the topic, there was adequate evidence to answer the research question.

The Impact of General Stress on The Reproductive System

There is a direct biological relationship between stress and the reproductive system, where stress activates a part of the body called the HPA Axis- the Hypothalamic-Pituitary-Adrenal axis, which is the central stress response system. When a person is under stress, the HPA axis is activated, and there are increased levels of cortisol and corticotropin-releasing hormone. The cortisol and corticotropin releasing hormone has the ability to suppress the normal levels of the reproductive hormones, which can potentially lead to abnormal ovulation, anovulation and amenorrhea (Biller, et al 1990). This paper will explore exactly which hormones the HPA axis acts upon in order to interfere with ovulation.

When there is a presence of adequate estrogen levels and there is stress-induced activation of the HPA axis, a significantly higher amount of LH is released. They found that the increase in LH was most probably associated with the increase in adrenal progesterone, which should feed back negatively on the gonadotrops in the pituitary to reduce LH that accompanies the release of cortisol in response to stress. It was also discovered that progesterone can stimulate LH secretion by acting directly on the pituitary (Couzinet, et al. 1992). Because of the stimulation of the LH secretion, this may provoke a premature LH surge and thereby interfere with proper follicular maturation and ovulation. Furthermore, elevated LH concentrations at different stages of the menstrual cycle may have conflicting effects on the maturing follicle and on the developing oocyte and may result in early pregnancy loss, due to the egg not implanting properly.

There are many studies and research articles that support the observation of a rise in the LH after stress. It was also found that one of the major causes of decreased fertility is the occurrence of premature LH surges. Although there are many research articles, there are only a few clinical studies in humans that support our observation of a rise in LH after stress. The Puder study suggested that one of the major causes of decreased fertility of unstimulated cycles is the occurrence of premature LH surges (Puder et al. 2000). These premature LH surges, are usually a response to stress. When there is a premature LH surge, the follicle may not be ready, and ovulation will not occur. Injecting endotoxin, a mild inflammatory stress into his subjects revealed that those who received the highest dose of endotoxin exhibited decreased LH levels. Generally, for an LH surge to happen, there needs to be a high estrogen environment They found that, although the subjects may have been in a low estrogen state when the endotoxin was administered, it lead to a significant stimulation of the LH levels. Thus, according to the Puder study, stress can bring on ovulation, although it may not be at the right time of the cycle. Additionally, one of the

potential negative effects of poorly timed increase in LH may be premature luteinization. It could thus be speculated that the main general mechanism by which stress affects ovulation is by causing an increase in LH, or premature LH surges.

The Impact of Stress on The Reproductive System as it is Exposed to Physical Stress

One of the many types of stress that a person can be exposed to is physical stress, such as exercise. Over the past decade, women have become much more physically active. Physical stress affects 6-79% of females, with the severity varying on the different type of athletic activities More specifically, the mechanism by which exercise-associated abnormalities of the reproductive system generally stem from disruption at the hypothalamic level (Warren, Perlroth 2001).. The amenorrheic abnormalities within the general female population and within the population of females that were involved in sports were studied and found that the percentage with amenorrheic dysfunction was substantially higher in females that engaged in athletic activity (Constantini, Warren, 1994). The main mechanism by which stress affects the menstrual cycle and ovulation generally originates in the dysfunction of the hypothalamus and the disturbance of the GnRH pulse generator. However, the specific mechanisms are different based on the different type of athletic discipline. The percentage with irregularities varied with different types of sports or exercises. Additionally, experiments showed that exercise coupled with caloric restrictions severely affected the LH suppression, which negatively affected ovulation. However, the experiment also showed that exercise alone did not affect the LH rhythm (Loucks, 2000). The mechanism by which exercise, and the caloric restrictions affected the menstrual cycle and ovulation, was through the hormone, Leptin. The hormone leptin is a protein product of the obesity gene, and is an independent regulator of metabolic rate, and may be found to be a significant mediator of reproductive function. Multiple studies have shown that menstruation does not occur in women with low leptin levels, and thus they are unable to ovulate. (Laughlin & Yen 1997). However, these studies just prove to us how exercise coupled with unhealthy eating affects the LH rhythm. But how does exercise alone affect ovulation?

The B-endorphins and Catecholamines Effects

Generally, when women exercise, it has been shown that there is an increase in the levels of B-endorphins and catecholamines. When there are increased levels of B-endorphins and catechol estrogens, studies have shown that the body responds with decreased levels of luteinizing hormone. However, Catecholamines have been found to regulate the luteinizing hormone (Russell et al, 1984). When Russell et al studied regularly menstruating active members of a swim club, he found the following results consistent with the above-mentioned mechanisms. They found that the swimmers that were most active, had higher estrogen levels. They had an increase in their catecholamines, and specifically in their levels of norepinephrine, which plays a role in the hypothalamus to regulate hormone release. When there are high levels of norepinephrine, the release of LH was significantly increased. Thus, the increased release of LH in these athletes disrupted many of their menstrual cycles and caused delayed ovulation or anovulation (Russell et al., 1984).

The B-endorphin hypothesis was also confirmed in this study, where they found a rapid rise in plasma B-endorphins and B-lipotropins in competitively training athletes. As it was mentioned previously, B-endorphins cause a decrease in the luteinizing hormone. Additionally, the catecholamine increase that the athletes experienced, inhibited the breakdown of B-endorphins by blocking the enzyme that is needed to break it down. B-endorphin was shown to block ovulation through the mechanism of morphine sulfate. Pang et al 1977 showed that morphine suppresses the preovulatory release of luteinizing hormone, thus it blocked ovulation from occurring. However, they also discovered that naloxone hydrochloride which is the opiate antagonist, reverses the effect. Additionally, this can also be seen by the fact that if women are chronically exposed to heroin or methadone, they had a decrease in their gonadotropin release and the complete absence of the luteal phase, and thus they did not ovulate.

Norepinephrine has a great influence on the LH release hormone in the hypothalamus. As mentioned above, catecholamines actually regulate the Luteinizing Hormone, and assure that it is released at the proper time and with the proper amounts. However, Russell et al. found in their study that the B-endorphins which are released by athletes, actually suppresses norepinephrine and it cannot have its usual influences on the LH release hormone from the hypothalamus. Additionally, in order for the LH hormone to be released from the hypothalamus, it needs to be promoted by naloxone, a synthetic drug, which blocks the B-endorphins. It needs to block the B-endorphins, because as it was mentioned before, the B-endorphins caused a decrease in the LH, and blocked ovulation. Naloxone was discovered to help with menstrual cycle and ovulation regulation, through an experiment with athletes. They found that when a single dose of naloxone was given to runners, they responded with a pulsatile LH surge. Thus, B-endorphin suppression of LH can be overcome by giving naloxone to athletes with excessive beta endorphins (Russell et al. 1984).

Intense Physical Activity and Ovulatory Dysfunction

There was a high correlation that was found between women who experienced intense physical activity and ovulatory dysfunction. It was found, regarding heavy runners, that only 50% of runners ovulated during a test month compared with 83% of controls. Fortunately, it was also shown that the ovulatory

dysfunction was only at the time of the intense physical activity, and once there was a less intense exercise schedule, their regular ovulation schedule returned (Gudmundsdottir et al, 2009). In addition to the blockage of the LH surge by B-endorphin, there is a common mechanism of ovulatory dysfunction which is called Hypogonadotropic Hypogonadism. Hypogonadotropic Hypogonadism is characterized by the failure of the pituitary gland to produce LH and Fsh, which play the key role in ovulation. The most common cause of this is excessive exercise (Fairley, Taylor 2003). Thus, women who exercise excessively are likely to develop this dysfunction, and without the production of LH and FSH, ovulation will not be able to occur. Additionally, women who exercise excessively can develop amenorrhoea because of a physiological reduction in the hypothalamic production of the gonadotropin releasing hormone. GnRH is also an imperative factor in ovulation, and if there is not enough GnRH being produced in the body, normal ovulation will not occur. This is characterized by the fact that the GnRH hormone regulates the timing and secretion of LH and FSH. If there is not enough GnRH being produced, it cannot regulate these hormones, and thus there will be a disturbance in the normal ovulation mechanism.

Moderate Physical Activity and Ovulatory Dysfunction

Furthermore, there was additional evidence found which revealed another mechanism of ovulatory dysfunction in healthy women. The study was done by examining a group of healthy, moderately exercising women. Before this study, it was thought that the effects of exercise at the level of the GnRH pulsator was due to the changes in LH Pulsatility (Loucks et al. 1989). They found that like LH, alterations in FSH secretion can also significantly impact ovarian function by altering folliculogenesis. Usually, there is an elevation of FSH during the luteal-follicular transition, then a decline usually happens after ovulation, during the late follicular and early luteal phases. This study found that the usual rise in FSH is blunted in exercising women that have a luteal phase deficiency. This was the first report of an abnormality in the monthly pattern of FSH excretion in women (DeSouza, Miller et al. 1998). Thus, the above study provides us with an additional mechanism of how ovulation is disrupted through exercise. The rise of FSH during the luteal-follicular transition is very important for the LH surge that comes shortly after. If there is no rise in FSH, there will not be enough LH produced, and ovulation will not occur.

It was also found in the study done by Souza, Miller et al. that there was a progressive suppression of estradiol excretion during the follicular phase. A delay in the estradiol excretion will probably follow a delay in the growth of the follicles, and thus a delay in follicular dominance and ovulation. The data in this study was very much consistent with the data in the previous studies in this paper. It supports the concept that in exercising women, both the luteal phase progesterone excretion and early follicular phase estrogen excretion decrease proportionally (Souza, Miller et al. 1998).

On the contrary to what was found in the study done by Souza, a study done by Jurkowski et al 1981 found opposite results. They studied the effects of exercise on the reproductive hormones, and what exercise had an effect on in the various stages of the menstrual cycle and ovulation. They studied nine women, and they found in all the women that when there was an increase in the intensity of the exercise, it affected the response of both the estradiol and the progesterone. They found that intense exercise increased the estradiol during both the follicular and luteal phases, although Souza found the opposite. In the luteal phase the progesterone also was increased, however, in the follicular phase they were lower. The low levels of estrogen can be a cause of anovulation, and the high levels of estrogen can also affect ovulation; there needs to be the right amounts in order for ovulation to occur.

Sporadic Anovulation in Physical Activity

Although there is adequate evidence and many studies done to prove that physical activity affects ovulation, not all studies seem to have the same results. In a research study done by Ahrens, et al 2014 other evidence was found. They studied 259 healthy premenopausal, regularly menstruating women. Their focus was to see the changes in their hormones as they were physically active as well as to see if they had sporadic anovulation. It was found in their study that the women with higher physical activity did have some sporadic anovulation, but nothing that was statistically significant. However, they did find that women that had a more moderate physical activity routine, instead of an intense physical activity routine, did have a lower risk of anovulation.

This study may not support or agree with all the previous studies, but that does not discredit the studies that have been done in the past. There may have been limitations on this study that led to inaccurate results, and thus there may have been sporadic anovulation that was overlooked. This could have been due to the fact that the study was done based on the hormones present in the women that was used to predict if there was anovulation. If the study was done with ultrasound technology this would have given us more accurate results. Therefore, although this study did not find a mechanism that ovulation was affected by physical activity; there is adequate evidence from previous articles that support the hypothesis that there are mechanisms by which ovulation is affected by physical activity.

The Impact of Stress on The Reproductive System as It Is Exposed to Emotional Stress

There is another type of stress which can critically affect a person's body and hormones, and that is emotional stress. Anxiety

is a type of stress that is characterized by feelings of worry, anxiety or fear, and in many cases, it can strongly interfere and disrupt a person's daily life. Not only does anxiety affect a person on levels which are obvious and can be seen, but it also can affect a person on a biological level. When a person is under stress and has anxiety, their hypothalamus releases Corticotropin Releasing Hormone (CRH) as a response to the stress that they are experiencing.

Many studies, including one by Chen proved that the Corticotropin releasing hormone inhibits GnRH secretion (Chen et al. 1992). Additionally, corticotropin releasing hormone is found in many female reproductive organs; the ovaries, endometrial glands, trophoblasts etc. One mechanism by which stress can affect ovulation was found in an experiment done on women in-vitro. In a study done by Calogero et al, they also found that CRH interfered with reproductive and ovarian function by suppressing the HPG axis to release GNRH and it affected the pulsality of the release of LH. This mechanism was also found and is consistent with the study done by Loucks. However, Calogero also discovered that CRH interfered directly at the gonadal level. Calogero studied many female rats and found that CRH inhibits FSH stimulated estrogen production, by decreasing the sensitivity of the rats to FSH. It was also found that CRH exerts an inhibitory effect on the formation of steroids (Calogero et al. 1996). This finding suggests that ovarian CRH has anti-reproductive actions that might be related to earlier ovarian failure which was observed in women with high anxiety and stress. Through their experiments they hypothesized and proved that this was through CRH inhibiting the production of FSH, thus leading to anovulation. If there is not an adequate release of FSH, the follicles will not be able to grow, there won't be a high estrogen environment and thus there will not be a LH surge, which will lead to anovulation. Thus, CRH may also be the major cause of anovulatory, or ovulatory dysfunction.

Three Levels Where Stress Can Influence Sexual Functions

It is known that stress-related hormones can influence sexual functions at all three levels of the HPG axis; in the brain which will inhibit GnRH secretion, in the pituitary to interfere with GnRH induced LH release, and in the gonads to alter the stimulatory effects of gonadotropins on sex steroid secretion (Rivier, Rivest 1991).

It was hypothesized that when CRF is released during stress, it causes the GnRH hormone to be disrupted because of the short distance between CRF and GnRH secreting neurons. This is believed to be the main mechanism where stress inhibited reproductive functions, because of the direct anatomical connections between CRF axon terminals and dendrites of GnRH secreting neurons (Maclusky, Neranth 1988). This hypothesis was confirmed when the injection of CRF into the brain ventricles of rodents immediately inhibited GnRH secretion. Additionally, they received further confirmation when they injected a CRF antagonist into the ventricle of the rat brain and it reversed the inhibitory action of stress on LH secretion (Rivier, Rivest 1991).

The Effects of Stress on GnRH

The primary mechanism in which CRF inhibits GnRH is yet to be discovered, however; there was another mechanism found where CRF inhibits GnRH. It was found that when CRF was infused into both sides of the medial pre-optic area of the hypothalamus, it significantly decreased the GnRH release and plasma LH levels in female rats. Although there seems to consistently be negative effects of stress on the reproductive functions, the mechanisms will depend on the duration and frequency of the stimulus. For example, prolonged stress will initially have the responses mentioned above, but it will consistently inhibit LH release and then eventually block ovulation all together by peripheral mechanisms like altering the responsiveness of the pituitary and gonadal systems.

Most studies that were done agree that a very common cause of stress induced anovulation is by the mechanism of reduced hypothalamic GnRH input, which is caused by stress. If there is a decline in the GnRH secretion, it will directly reduce the secretion of LH and FSH and could wholly or partially disrupt folliculogenesis (Bourga, Loucks 2001). Bourga and loucks discovered that amenorrheic athletes had less luteal progesterone secretion, fewer LH pulses per day, and higher cortisol levels. Furthermore, they also found that amenorrheic athletes that were anovulatory had the fewest LH pulses in a day and the highest cortisol levels. However, most of the studies done focused on specific stressors and how they affected a women's cycle or ovulation. Not many studies were done on how real life, daily stress has on reproduction.

Daily Stress and its Effects on Reproductive Hormones

A mechanism on how daily stress affects women was provided to us by a study done by Nepomensachy Et Al. 2004. In contrast to our prior evidence, Nepomensachy and his fellow researchers found an alternative mechanism by which stress affects the reproductive hormones. As we have seen in previous research articles, stress is believed to affect reproductive function through a reduction in gonadotropins, which leads to a reduction in gonadal steroids. This also involved the stress activation of the HPA axis triggering the release of corticotropin-releasing hormone. The increase in CRH negatively affects the GnRH pulsatility and the cortisol surge causes a reduction of sensitivity to GnRH, leading to a reduction in the release of Gonadotropins (River, Vale, 1990). Because of the reduction in gonadotropin levels, it altered the maturation of the follicle, which delayed or prevented ovulation, with many other effects on the reproductive

hormones. However, in another study by Xiao et al 2000, they showed a different mechanism. They showed that while many other studies showed that intense levels of inflammatory stress inhibited the secretion of LH in female monkeys, in their studies they found that inflammatory stress can actually promote LH secretion. This was an interesting phenomenon as the monkeys also showed a rise in progesterone, and a rise in LH. Usually, when there is a rise in progesterone, it usually inhibits the secretion of LH (Xiao et al 2000).

In the study done by nepomnaschy, a new mechanism was discovered. They did not discover that the inhibition of progesterone during the luteal phase was triggered by a reduction in the levels of follicular gonadotropins. Rather, their results showed that during the mid-luteal phase, elevated cortisol levels predicted low progestin levels, however; during the follicular phase, higher cortisol levels were associated with higher, not lower, gonadotropin levels. However, whether the mechanism that stress effects by lowering the gonadotropin levels, or increasing the gonadotropin levels, still has detrimental effects on the ovulation and implantation process. Low progesterone levels may cause a degenerative endometrium, and even if normal ovulation does occur, there may be a problem with implantation (Nepomnaschy, et. al.,2004). In order for a normal, healthy pregnancy to occur, there needs to be a normal implantation, and it is critical for the progesterone levels to be balanced.

Although it may seem that different types of stress may have different effects, it has also been shown that people that are more sensitive to stress, will have different responses. In contrast to studies that were done on humans, there was a study done by Herod, Dettmar et al. on monkeys in order to see which specific type of stress produced the most effects on the reproductive dysfunction. In their experiment, they found that the Monkeys with high cortisol levels varied according to the specific physical locations the monkeys were in, and which type of stress they were exposed to. It was found with their monkeys that they did show elevated cortisol in response to mild psychosocial plus metabolic stress. Also, the stress sensitive monkeys showed much higher cortisol levels than non-stressed monkeys. This finding would support the hypothesis that individuals with stress-induced amenorrhea do not have elevated baseline cortisol levels, but they are rather more likely to experience stress and thus have a higher probability of having elevated stress-induced cortisol when studies are performed.

Additionally, we know and have proven that elevated CRH suppresses the hypothalamic-pituitary-gonadal axis. However, the findings in Herod's studies with monkeys do not rule out a role for CRH acting as a neurotransmitter rather than a neuroendocrine hormone in causing sensitivity of the reproductive axis to stress. Increased CRH gene expression may be acting in a non-neuroendocrine manner to regulate other neurotransmitter systems that mediate functions of the reproductive axis, including norepinephrine, dopamine, serotonin, γ-aminobutyric acid, and glutamate. Stress Sensitive monkeys have suppressed physiological release of serotonin, fewer serotonergic cells, and low expression of a number of genes in the serotonin pathway. However, when they were treated with a selective serotonin reuptake inhibitor, it increased the ovarian steroid hormone secretion. Thus, it caused the other functions to start working properly. This study may have given us a new mechanism by which the ovulatory system can be disrupted, by inhibiting neurotransmitter secretion (Herod, et al 2011).

Conclusion

The mechanisms by which stress negatively affects ovulation is an amazing phenomenon with many mechanisms still yet to be discovered. Whether it is because of a physiological stress or physical stress, there have been a various amount of mechanisms that have been discovered, with many of them mentioned in this paper. While many of the mechanisms involved a premature LH surge, an increase in LH, inhibition of GnRH, Inhibition of FSH or excessive FSH, there were a variety of mechanisms that led to each outcome. Of all the mechanisms, there was not one specific one that dominated the others. Different stress caused different biological responses to occur, yet they all led to menstrual or ovulatory dysfunction. This data is extremely important as knowing exactly which mechanism is responsible for the menstrual or ovulatory dysfunction is imperative for assessing proper treatment. A suggestion for future research would be to see if stress affects ovulation subtly without causing amenorrhea.

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The Effects of Maternal Caffeine Intake on a Fetus

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Abstract

The United States has been noted as the country with the greatest caffeine consumption in the world. More than half of all Americans are said to consume caffeine on a daily basis. Caffeine is easily available in a variety of dietary products including coffee, tea, soft drinks, and chocolate. Many pregnant women are unaware of the potential risk that excessive caffeine consumption can have on their unborn child. The purpose of this paper is to explore the ramifications of caffeine intake on a fetus. The studies reviewed propose that heavy maternal caffeine consumption, that of more than 300 mg daily, is associated with increased risk of spontaneous abortion or delivery of an infant of low birth weight. Most researchers agreed that caffeine does cause preterm labor and delivery, nor does it act as a human teratogen.

Acronyms

LBW- Low Birth Weight
SGA- Small for Gestational Age
IUGR-Intrauterine Growth Retardation

Introduction

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring compound. Its pharmacological and physiological effects include stimulation of the central nervous system and cardiac muscle, and relaxation of smooth muscle. Caffeine has been shown to have effects on physical and cognitive performance, as well as mood, memory, and alertness. Caffeine is the most widely used stimulant for the central nervous system. Clinically, caffeine is useful for relaxing the bronchial muscle in asthmatic patients, increasing secretion of gastric acid, and the concentrations of plasma free fatty acids, and glucose (Institute of Medicine, 2001).

Sources of Caffeine

Coffee, tea, and soft drinks are the main sources of caffeine in the diet of the average American adult. Other dietary sources include chocolate and cocoa, sugars and sweets, and flavored dairy products. Tea also contains a significant amount of theophylline (1,3-dimethylxanthine), as cocoa also contains theobromine (3,7-dimethylxanthine), both being derivatives of caffeine that have not been as widely researched (Frary, 2005). Many classes of nonprescription medications including analgesics, cold/allergy products, diuretic products, stimulants, and weight control agents have some caffeine content. All medications have a suggested dose, but consistent usage may lead to the medication becoming a significant source of caffeine consumption.

Absorption, Distribution, and Elimination of Caffeine

Absorption

Caffeine and the other methylxanthines are quickly absorbed in humans. As much as 99 percent is absorbed within the 45 minutes after ingestion. Oral, rectal, and parenteral administration is possible, with the oral route being most common. When consumed in a beverage, the caffeine is quickly absorbed from the gastrointestinal tract and distributed throughout body water. Caffeine in preparations that allow absorption through oral mucosa, such as caffeinated chewing gum, are absorbed even more rapidly. Depending on the source of the caffeine and the individual's metabolism, caffeine's peak plasma levels appear between 15 and 120 minutes after ingestion or administration (Institute of Medicine, 2001).

Distribution

The distribution volume of caffeine within the body is 0.7 L/kg, demonstrating caffeine's hydrophilic quality and ability to distribute freely into intracellular tissue water (Arnaud, 1993). Caffeine can also pass through all biological membranes and freely crosses the blood-brain barrier, demonstrating its lipophilic quality (Institute of Medicine, 2001). In pregnant women, the caffeine can cross the placenta to the fetus, and as early as 7-8 weeks gestation, maternal and fetal plasma can achieve an equilibrium (Goldstein, Warren, 1962). A fetus swallows approximately 500 mL of amniotic fluid daily, and studies suggest that several milligrams of caffeine can be ingested along with the fluids. Additionally, by week 12 of gestation the fetal liver is able to methylate theophylline to caffeine (Brazier, 1981). Both caffeine and theophylline are eliminated in the amniotic fluid, with caffeine's fetal elimination half-life at approximately 150 hours, while theophylline's is 30 hours.

Elimination

The small fraction of caffeine that is excreted unchanged in urine indicates that caffeine metabolism is the rate-limiting factor in its plasma clearance. Its limited appearance in urine is due to caffeine being readily reabsorbed by the renal tubules and filtered by the glomeruli (Arnaud, 1993). Frequent caffeine ingestion has not been shown to affect its absorption or metabolism in healthy humans. The bulk of caffeine metabolism takes place in the liver, catalyzed by hepatic microsomal enzyme systems (Grant et. al., 1987). In the liver, caffeine is metabolized to dimethylxanthines, uric acids, di- and trimethylallantoin, and uracil derivatives. 3-ethyl demethylation to paraxanthine is shown to be the most frequent route for caffeine metabolism in humans (Arnaud, 1987). This is the first step in caffeine metabolism and accounts for approximately 75–80 percent of the process. Being the chief metabolite in humans, paraxanthine is found to have a plasma concentration ten times higher than those of theophylline and theobromine. Eight to ten hours after ingestion, plasma levels of paraxanthine surpass those of caffeine, as caffeine is cleared more rapidly than paraxanthine (Arnaud, 1993). During the second and third trimesters of pregnancy, maternal caffeine elimination rates drop due to changes in progesterone and estrogen levels, with the half-life of caffeine changing from 5.3 hours to 18.1 hours. Maternal clearance of caffeine during pregnancy can be decreased further by other factors, including smoking and long-term use of oral contraceptives, as well as age

and disease. Within a couple of weeks of giving birth, a women's caffeine metabolism rate returns to the same rate as prior to pregnancy (Aldridge et. al., 1981).

Methods

Critical analysis of peer reviewed journal articles and original clinical research papers was used to write this review. The articles and papers were obtained with access to online publications through the Touro College library. Additional references were obtained through Pubmed and Google Scholar.

Effects of Caffeine Consumption on Birth Weight

A low birth weight (LBW) infant is one who weighs less than 2,500 g (5 lbs, 8 oz) at birth. Prematurity due to a shortened gestational period can cause low birth weight, but LBW can also be the consequence of intrauterine growth retardation (IUGR), which results in a small for gestational age (SGA) infant. Intrauterine growth retardation is classified as less than the 10th percentile of birth weight for gestational age in comparison to an external standard of birth weight for gestational age, adjusted for gender and ethnicity, that was developed from all 1999 singleton births in the United States, and updated in 2014 (Talge et. al., 2014). Many studies have shown a strong correlation between caffeine intake during pregnancy and reduced birth weights. For pregnant women who consume more than 300 mg of caffeine daily, a high risk of SGA and IUGR has been found. Intake between 150 mg and 300 mg daily has also been linked to such risks; however, the data is not as consistent.

From 2003-2006, a perspective cohort study following pregnant women between the ages of 18 and 45 with singleton pregnancies was implemented. The caffeine intake of these women was monitored, and the relationship between caffeine and fetal growth was evaluated. At any level of caffeine intake there was an associated risk of fetal growth retardation found, and this risk was maintained throughout pregnancy. They found that after adjustment for smoking and alcohol intake, an average caffeine consumption of more than 100 mg per day was correlated with a reduction in birth weight of 34-59 g in the first trimester, 24-74 g in the second, and 66-89 g in the third. An extra 60-70 g may seem insignificant, but it can make all the difference for an already compromised fetus, and can help avoid perinatal morbidity and mortality. The study did observe a large decline in risk for a daily caffeine intake of less than 30 mg, but this may be due to unmeasured confounding, or simply because lower caffeine intake is probably more common among women who have healthier diets and habits in general (CARE Study Group, 2008).

A second prospective study observed the caffeine intake of 9,921 healthy pregnant women throughout their third trimester. Fifty-three of the women reportedly consumed more than five cups of coffee daily, and they were found to have a 13.2% higher prevalence of fetuses who were SGA (Fuhurhashi et. al., 1985).

The Norwegian Mother and Child Cohort Study conducted by the Norwegian Institute of Public Health followed 59,123 women with uncomplicated singleton pregnancies. At weeks 17, 22, and 30, the women reported their caffeine intakes from different sources. SGA was defined according to ultrasound-based, population-based, and customized growth curves. Based on the three scales, an average of 25 g weight reduction was associated with every additional 100 mg of maternal caffeine intake per day for a baby with an expected birth weight of 3,600 g. Coinciding results for caffeine sources, time of survey, and different definitions of SGA were found for this, substantiating its results. Even caffeine consumption below the recommended maximum such as 200 mg per day, compared to the recommended 300 mg per day, was consistently associated with increased risk for SGA (Sengpiel, 2013).

Gestational age was not linked to caffeine intake in these studies, suggesting that the effect maternal caffeine consumption has on fetal birth weight occurs through IUGR. Possible mechanisms responsible for this effect include caffeine's similar structure to adenine and guanine which may allow it to interfere with cell division and metabolism. Additionally, caffeine has a vasoconstrictive effect on placental intervillous blood flow, which may be a factor to increase the risk of IUGR (Kirkinen, 1983).

During the first trimester of pregnancy, the embryo first starts developing its organs, heartbeat, brain waves, and the rest of its body parts. As this is such a crucial time of development, caffeine intake should be limited especially then. In fact, a greater reduction in risk for IUGR and delivering LBW infants was found among women who reduced their caffeine intake to less than 300 mg within 6 days of their last menstrual period, compared to those who reduced their intake later in pregnancy (Fenster et. al., 1991).

There is a steady negative correlation between LBW infants and daily maternal caffeine intake above 300 mg. In many studies, a daily caffeine consumption between 151 and 300 mg, and occasionally even 150 mg or lower has been associated with risks of low birth weight. Pregnant women should be sure to limit their caffeine intake as much as possible to lessen the probability of reductions in infant birth weight. The seemingly slight decrease in weight can be very harmful for premature infants or infants who are otherwise compromised. Further research is needed to clarify the mechanisms by which caffeine exercises an effect on fetal growth.

Effects of Caffeine Consumption on Preterm Labor and Delivery

Caffeine has not been found to be a strong factor in increasing the risks of preterm labor and delivery. In 1996–2000, 2,291 mothers with singleton pregnancies in Connecticut and Massachusetts were questioned about caffeine consumption and other important confounding factors after their first

prenatal visit. Urine samples were taken to analyze urinary caffeine, cotinine, and creatinine levels. The mothers were followed throughout pregnancy to monitor changes in consumption, and medical records were obtained to confirm pregnancy outcomes. While mean birth weight was found to be reduced by 28 g per 100 mg of daily caffeine intake, mean gestational age was not found to be affected at all (CARE Study Group, 2008).

In the Norwegian Mother and Child Cohort Study, spontaneous preterm delivery was defined as "spontaneous onset of delivery between 22+0 and 36+6 weeks (n = 1,451)". Caffeine from coffee, but not from other sources, was actually associated with prolonged gestation, but no association of increased risk of spontaneous preterm delivery was found with caffeine consumption (Sengpeil et. al., 2013).

Other studies as well found no effect on gestational age, indicating that caffeine influences fetal growth, not gestational age at delivery. Pastor et. al performed a case control study of 408 preterm (less than 37 weeks gestation) infants, and analysis of caffeine intake in the third trimester showed a nonsignificant relationship with preterm delivery (Pastore, Savotz, 1995).

Alternatively, in a population-based study of 7,855 livebirths, increased preterm birth among women who drank caffeinated coffee was found compared with women who drank neither decaffeinated nor caffeinated coffee. Those who consumed only decaffeinated coffee showed no increased odds of SGA birth, LBW, or preterm delivery, while women who consumed caffeinated coffee alone had a higher association with preterm delivery (Eskenzai et. al., 1999). This study has not been replicated, and other analyses did not support it.

Gestational age is difficult to calculate and assess, making this topic more difficult to analyze accurately. Generally, there appears to be no relationship between caffeine consumption during pregnancy and premature labor and delivery in humans.

Effects of Caffeine Consumption on Spontaneous Abortion

Most studies report effects of caffeine on spontaneous abortion, however, there are some who suggest otherwise. In one study, 2,967 pregnant women who delivered at Yale-New Haven hospital between 1988 and 1992 were evaluated for caffeine intake during the first month of pregnancy. After studying the effect of the caffeine on pregnancy outcomes, it was concluded that increased risk of spontaneous abortions was linked to drinking more than 3 cups of tea or coffee daily. The association of risk with tea and coffee intake was shown to be stronger than with caffeine in general, and was primarily correlated with abortions which took place in later trimesters (Dlugosz et. al.,1996).

A prospective cohort study of 3,135 pregnant women found that those who consumed more than 151 mg of caffeine daily were more likely to spontaneously abort in the second or third trimester, in comparison to those who had a daily intake of less than 150 mg of caffeine (Srisuphan, Bracken, 1986).

A study of healthy, pregnant women, all of whom were beyond 24 weeks of gestation revealed that caffeine consumption of more than 600 mg daily was significantly associated with impending abortion, with a higher prevalence of 17% found (Fuhurhashi et. al.,1985).

Another study of 1,324 women demonstrated associations between caffeine intake prior to and during pregnancy with spontaneous abortions in 331 of the 1,324 women. The risk of fetal loss increased for each 100 mg of caffeine ingested daily during pregnancy, as well as smaller increases in risk for each 100 mg of caffeine ingested daily prior to becoming pregnant (Infante-Rivard, 1993).

Dominguez-Rojas et. al conducted a retrospective cohort study of 711 pregnant women, monitoring their caffeine intake, and found caffeine to be a clear risk factor for spontaneous abortion. They determined that the adjusted odds ratio (a measure of association between exposure and outcome) of spontaneous abortion by caffeine consumption was significant for 141-280 mg daily, doubled for 281-420 mg daily, and then almost tripled for intake of greater than 421 mg daily (Dominguez-Rojas et. al., 1994).

Alternatively, different studies found no association between maternal caffeine intake and spontaneous abortions. Four hundred and thirty-one women were enrolled in a multicenter study within 21 days of conception. Throughout pregnancy, they were monitored for caffeine intake, and exposure to other risk factors, and the effects on pregnancy outcome. The investigators found no connection between caffeine intake, neither above or below 30 mg daily, and increased risk of spontaneous abortions (Mills, 1993).

Determining a definite causal connection between caffeine intake and occurrence of spontaneous abortions is difficult, as many of the studies that have been done did not control properly for other factors such as smoking, parity, or alcohol intake. There seems to be a strong association between caffeine consumption and fetal loss, but more research must be done before unambiguous statements can be made.

Effects of Caffeine Consumption on Congenital Malformations

Caffeine can perhaps act as a teratogen due to its chemical structure as a purine, one of the components of DNA. After maternal consumption, caffeine can cross the placenta to the developing embryo. If the molecule were to become incorporated into DNA, there is a possibility that it could induce the production of abnormal proteins (Goldstein, 1962). The literature reviewed showed no significant evidence linking human maternal caffeine intake during pregnancy to major congenital malformations.

In a study performed to analyze information from the Finnish Registry of Congenital Malformation, mothers who had given

birth to infants with the same defects were matched according to place and time of birth. One mother in each pair consumed coffee during pregnancy, while the other did not. To evaluate the hypothesis that coffee consumption during pregnancy is teratogenic, the 706 pairs of mothers of malformed children and their controls were interviewed soon after delivery. The subjects of the study included 112 mothers of children with defects of the central nervous system, 241 mothers of children with orofacial clefts, 210 mothers of children with structural defects of the skeleton, and 143 mothers of children with cardiovascular malformations. The study determined that coffee intake does not appear to increase risk for any of the defects that were studied. Even mothers who consumed more than six cups of coffee per day had no higher risk of giving birth to an infant with congenital malformations. The study also paired these mothers with women who gave birth to non-defective infants, in the same time and place, and who consumed an equivalent amount of caffeine daily during pregnancy. The amount of coffee consumed during pregnancy was similar for the mothers of malformed and non-malformed children, with the broad range of maternal intake being 0-10 cups daily, demonstrating that excessive coffee intake does not increase risk of congenital malformations (Kurppa et. al., 1983).

Mcdonald et al. investigated the relationships between smoking, alcohol intake, and caffeine consumption, and congenital malformations using data from a survey conducted in Montreal from 1982-1984. A weak association between caffeine consumption and heart defects was found, but the evidence was not strong. There was no connection found between caffeine intake and club foot, clefts, neural tube defects, or musculoskeletal renal/urinary, gastrointestinal, or respiratory abnormalities (Mcdonald et al., 1992).

Similarly, a study was performed to determine the possible effects of different chemical and physical factors during pregnancy on the occurrence of cardiovascular malformations, specifically hypoplastic left heart syndrome. Using a standard questionnaire, 573 cases and 1,055 controls were interviewed approximately 3 months after delivery. An increased risk of cardiovascular malformations was not found to be associated with coffee, tea, or cola consumption (Tikkanen, Heinonen, 1994)

One study did show an increased risk for malformations due to caffeine. A retrospective case-control study was executed in which 558 women in England who had delivered an anencephalic stillbirth were matched with 2,232 control women based on maternal age, parity, and date of delivery. Based on a structured questionnaire completed by the cases and controls, it was shown that the women who drank 3 or more cups of tea daily were more likely to give birth an anencephalic stillborn (Fedrick, 1974). However, the results of this study may not be completely accurate, and the authors themselves wrote that caution should be taken when interpreting their results.

Most studies agree that there is no connection between caffeine intake during pregnancy and congenital abnormalities. Any connections that were found have been deemed weak at best.

Conclusion

Maternal caffeine intake during pregnancy should be limited to between 150 mg and 300 mg per day, to mitigate negative effect caffeine has been shown to have on birth weight, risk of IUGR, and risk of spontaneous abortion. More studies must be done to confirm correlation between caffeine and spontaneous abortion, and based on current data, there does not seem to be a significant risk of preterm labor or congenital malformations related to caffeine intake.

Pregnancy is a time when motherly instincts begin to kick in, and women are likely to be receptive to counseling about lifestyle changes. Many women are unaware of the real risk that their caffeine intake can create for their unborn child. Doctors and prenatal counselors should be sure to discuss the matter with soon-to-be mothers so they can make informed decisions when consuming caffeine during pregnancy. In addition, having a health care provider monitor caffeine intake may help establish the degree of risk for use of other drugs or high-risk behaviors during pregnancy.

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A Multi-Domain Approach to Prevention and Reversal of Cognitive Decline

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Abstract

Incidence of dementia has been on the rise over the last few decades and it is projected that more than 130 million people will be affected by dementia worldwide by 2050. The underlying cause remains incompletely determined, and despite numerous clinical trials, no drug to date has proven effective in preventing or reversing symptoms of cognitive decline due to Alzheimer's disease. The amyloid hypothesis as a basis for drug development of Alzheimer's disease has thus far proven to be ineffective, suggesting that perhaps a new approach is required. New studies have shown the efficacy of a multi-domain approach which targets several disease risk factors simultaneously, to achieve a synergistic effect on cognitive impairment. This paper analyzes a multi domain protocol, known as ReCODE protocol, developed to treat and prevent Alzheimer's disease, and provides clinical and experimental research as well as potential mechanisms to support the key elements upon which this protocol is based. Although the results seem promising, more rigorous clinical testing is required to link this approach with prevention and reversal of cognitive decline more definitively.

Acronyms

Alzheimer's disease (AD), amyloid beta (Aβ), β-Amyloid Precursor Protein (APP), Reversal of Cognitive Decline (ReCODE), tumor necrosis factor (TNFα), interleukin-6 (IL-6), interleukin-1β (IL-1β), Apolipoprotein E4 (ApoE4), Positron Emission Tomography (PET), Presenilin 1 (PS1), homocysteine (HC), Beta Hydroxybutyrate (β-HB), ketone bodies (KB), acetoacetate (Ac)

Introduction

Today, nearly 50 million people worldwide are living with dementia (Prince et al., 2016). As one of the leading causes of age-related cognitive decline, Alzheimer's disease (AD) represents a significant health concern for the aging population. The underlying cause remains incompletely determined, and to date no effective treatment has been discovered. Although remarkable progress and scientific breakthroughs in recent decades have led to the development of effective protocols for diseases such as HIV and cancer, treatment therapies for neurodegenerative diseases such as Lewy body dementia and Alzheimer's disease continue to lag significantly behind. Despite billions of dollars funneled into hundreds of clinical trials on various drug modalities, no truly effective etiological treatment or prophylactic medication has been approved by the FDA to date. Of the five drugs that have been approved to treat the cognitive symptoms of Alzheimer's disease, none have been shown to have more than a marginal or sustained effect on symptomatic patients with AD.

The Amyloid Hypothesis

AD onset is associated with a complex neurodegenerative cascade mechanism histologically characterized by parenchymal deposition of amyloid-β (Aβ) and intracellular neurofibrillary tangle formation due to hyperphosphorylation of tau protein (Cunnane et al., 2011). Since the discovery of the Aβ peptide in 1984, the amyloid hypothesis has largely been the paradigm in understanding AD and the basis for researching potential therapeutic treatments. The amyloid hypothesis posits that the formation of senile plaques, composed predominantly of the proteinaceous Aβ peptide, leads to synaptic toxicity and cognitive deficits in AD (Banwait et al., 2008). In recent years, however, the validity of this theory has come into question; for although the neurotoxicity of Aβ is supported by substantial genetic and biochemical data, Aβ-centered therapeutic trials aimed at limiting the production of amyloid or facilitating its removal have failed to prove clinically effective in slowing cognitive decline in AD patients (Pimplikar, 2009). These results suggest that perhaps, rather than being the primary cause of the disease, Aβ is a "downstream response to injury, with both beneficial and injurious properties" (McCaully & Grush 2017). With the entire premise of the current Alzheimer's paradigm under question, it seems that perhaps a new understanding of the role of Aβ is necessary.

Paradigm Shift

Dr. Bredesen and associates address the inconsistencies of the prevalent amyloid cascade theory and clinical outcomes with a new approach, radically different than its monotherapeutic precedents. They have advanced a model in which, "AD results from an imbalance in endogenous plasticity signaling, and in which the β-amyloid precursor protein (APP) is a mediator of such plasticity-related signaling," suggesting an etiology analogous to chronic illness such as osteoporosis and arthrosclerosis. Osteoporosis occurs when there is a chronic imbalance between osteoblastic and osteoclastic signaling and new bone formation is exceeded by old bone resorption. "By analogy, in Alzheimer's disease, there is a fundamental age-associated imbalance between the dynamically opposed physiological processes that mediate plasticity, i.e. between synaptoblastic and synaptoclastic activity" (Bredesen, 2014).

Aβ is a peptide produced by the proteolytic cleavage of its precursor protein, APP, mediated by γ-secretase and β-secretase 1 (Heneka et al., 2014). This integral membrane protein can be cleaved via two alternative pathways and in this way act as a molecular switch to mediate neuroplasticity. Amyloidogenic processing of APP leads to cleavage at the beta, gamma, and caspase sites to produce pro-AD peptides sAPPβ, Aβ, Jcasp, and

C31 - all of which have been shown to mediate neurite retraction and caspase activation. In contrast, the non-amyloidogenic processing of APP through cleavage at the alpha site results in the formation of sAPPα and αCTF, peptides which mediate neurite extension and inhibit Aβ production and caspase activation (Bredesen, 2014; Chen, 2017).

In studies using regular and transgenic mice, both genetic and pharmacologic methods were used to manipulate the APP derivative peptide balance and were found to cause predictable effects on learning and memory. This suggests that the APP cleavage pathway may be a potential target to inhibit AD pathophysiology. A number of agents that affect this pathway, including nitrin-1 and Aβ, have been identified, however, targeting any of these agents individually has had limited success. Additionally, other potential intervention targets, aside from Aβ oligomers, including inflammatory mediators, apolipoproteins, trophic factors and their receptors, and axoplasmic machinery have been identified. Although targeting any one of these pathways shows great promise in preclinical studies, it has not proven effective in human studies. This inconsistency seems to suggest that a "network-based therapeutics approach, rather than a single target-based approach, may be more effective for the treatment of cognitive decline due to Alzheimer's disease" (Bredesen, 2014). The complex, multifactorial nature of the disease may require interventions that target several risk factors and disease mechanisms simultaneously for optimum effect. Comprehensive combination therapies have been shown to greatly improve treatment of other chronic illnesses, such as HIV and cancer. In the case of HIV, the development of highly active antiretroviral therapy, a form of combination therapy, caused a significant decline in death rates for a disease that had been minimally treatable for decades (Brady et al., 2010).

The Finger Study

The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) assessed a multi-domain approach towards improvement of cognitive functioning and prevention of cognitive decline in the first extensive, long-term, methodologically rigorous trial to date. They conducted a two-year trial to confirm the associations of several modifiable factors, such as diet and physical inactivity, with Alzheimer's disease.

Participants were required to be between 60-77 years of age and have a minimum CAIDE (Cardiovascular Risk Factors, Aging, and Dementia) Score of 6 points. The Consortium to Establish a Registry for Alzheimer's disease Neuropsychological Battery Test was used for cognitive screening. Only participants who tested at or slightly lower than the mean level of cognitive performance, as compared to the normal population, were included in the study. Individuals with diagnosed or suspected dementia, and several other disorders or impairments were excluded.

Participants were randomly assigned to the intensive multi-domain intervention or regular health advice group (control group) using computer-generated allocation at each of six testing centers. The trial was double-blinded to the maximum possible extent, wherein allocation was not disclosed to either participants or outcome assessors. Participants were restricted from interacting with each other and advised not to discuss their experiences.

Procedures

All participants met the study nurse five times during the two year trial where they received advice on a healthy diet, and physical, cognitive, and social activities to manage risk factors and prevent cognitive impairment. The intervention group received four additional components, including a nutritional intervention, a physical exercise training program, cognitive training, and social activities.

Results

There was significant improvement for the primary outcome in the intervention group, which showed 25% greater improvement in the neuropsychological test battery (NTB) total score than the control group. The intervention group also showed higher improvements in executive function and processing speed than the control group, 83% and 150% respectively. There was no evidence, however, of significant change in memory for the intervention group (Ngandu et al., 2015).

A More Comprehensive Multi-Domain Approach to Treatment of Alzheimer's Disease

The ReCODE Protocol

The ReCODE Protocol outlines a more comprehensive therapeutic system that targets as many as 36 risk factors or disease mechanisms to prevent and reverse cognitive decline. While the individual effects of any one of these factors may not be significant, the protocol targets many factors simultaneously to create a synergistic effect capable of reversing the imbalance of synoblastic/synoclastic activity mediated by APP. Typically, the evaluation of patients with cognitive decline does not include testing for genetics, inflammation, infection, homocysteine, fasting insulin level, toxic exposure, blood brain barrier or body mass index, all of which are known risk factors for AD. Patients following the ReCODE Protocol undergo extensive lab and genome testing, referred to as a "cognoscopy," to identify genetic risk factors and suboptimal physiological, metabolic, and cognitive parameters. The results are used to generate a personalized ReCODE Report™ which designs an individualized protocol targeting the identified risk factors in order to synergistically achieve an optimum outcome (Bredesen, 2017).

Three Subtypes of Alzheimer's

To optimize treatment development, Bredesen et al. uses metabolic profiling to distinguish between three subtypes of

Alzheimer's disease, each with its own characteristic biomarkers. Although there may be overlap, each subtype requires remediation of specific suboptimal indicators.

Subtype 1: Inflammatory

Carriers of the apolipoprotein E4 (ApoE4) gene are at increased risk for developing AD, particularly the inflammatory subtype. Carriers homozygous for the ApoE4 allele often become symptomatic as early as late forties or fifties, single ApoE4 allele carriers in late fifties or sixties, while symptom onset for those without the gene is typically in the sixties to seventies. This subtype is associated with biomarkers of systemic inflammation such as increased hs-CRP, IL-6 and TNFα as well as a decreased albumin:globulin ratio. Symptomatically, this subtype is characterized by the loss of the ability to form new memories while retaining long-term memories and the ability to calculate, spell, and write. Brain atrophy of the hippocampus is evident early in the disease progression (Bredesen, 2017).

The Inflammation Hypothesis

Inflammation has been implicated in AD mechanisms and is believed to play a critical role in the pathogenesis of the disease. When Aloysius Alzheimer studied the post-mortem brain of Aguste Deter in 1907, he found microglia, the primary cells of the innate immune system involved in the brain inflammation response, clustered around the amyloid plaques. Initially, the inflammation hypothesis was regarded as unlikely or even impossible due to the view of the brain as an "immune-privileged organ," allowing for inflammation only "through direct infection or after the breakdown of the blood brain barrier and subsequent infiltration of peripheral immune cells" (Heneka et al., 2014). However, extensive research over the last decade has confirmed the presence of neuroinflammation in AD and suggests that its presence may even begin in preclinical stages of the disease (McCaully & Grush 2017). PET imaging has shown evidence of significantly increased levels of neuroinflammation markers in brain areas affected by AD neuropathology while almost no neuroinflammation was found in individuals without dementia, despite high plaque burden (Krstic et al., 2012). Furthermore, several known risk factors for AD, such as a history of systemic infection, obesity, and reduced physical activity, are associated with some component of inflammation (Heneka et al., 2014). Increasing evidence suggests that neuroinflammation seems to correlate more directly with cognitive decline than plaque deposition alone.

Innate Immunity: Microglial Contribution to AD Pathogenesis

Innate immune cells, particularly microglia, have been implicated as mediators of neuroinflammation in AD and as contributors to its pathogenesis. Microglial cells are an essential part of the central nervous system and serve to protect the brain from injury and pathogenic invasion. In brain development, microglia assist in synapse formation and eliminate dysfunctional synaptic connections (Van Eldik et al., 2016). Rice, et. al. (cited in Spangenberg & Green, 2017) found that healthy adult mice, without a proliferation of microglia, showed 35% more synapse-bearing dendritic spines than mice with significant microglial proliferation, indicating that microglial participation in synaptic maintenance may continue even into adulthood through the dismantling of dendritic spines in the AD brain.

In the homeostatic adult brain microglia are in a "surveillance state," constantly surveying the brain parenchyma to detect abnormalities and supporting neuronal health and function by regulating synaptic plasticity and pruning unnecessary synapses. Upon detection of an insult, such as Aβ plaques, these immune cells are activated and assume an entirely different phenotype with both chemical and morphological changes, including the retraction of their processes. This phenotypic change may inhibit the ability of microglia to alter and adapt synapses and contribute to the impaired synaptic plasticity characterized by AD (Van Eldik et al., 2016). Once activated, microglia secrete pro-inflammatory cytokines, including TNFα, IL-6, and IL-1β to induce other cells to migrate to the injury site and mediate clearance of the invasive material through phagocytosis. In acute inflammatory events, the inflammatory response is resolved through regulatory proteins which induce microglia to secrete anti-inflammatory factors to promote tissue repair. The microglia then revert to their "surveillance state". However, in AD, microglia are unable to phagocytose Aβ, as evidenced by the presence of plaques surrounded by microglial cells in post-mortem AD brains (Spangenberg & Green, 2017). This leads to the sustained release of pro-inflammatory mediators which "has been shown to be involved in the suppression of axonal transport and adult neurogenesis" (Heneka et al., 2014).

Inflammatory dyshomeostasis drives microglia into a state of chronic activation in which they no longer function properly, resulting in aberrant synaptic pruning, neuronal loss, and accumulation of Aβ, eventually leading to cognitive dysfunction (Spangenberg & Green, 2017). Cytokines, for instance, have been linked to cytoskeletal and synaptic alteration through increased tau phosphorylation and decreased synaptophysin levels.

Experimental Correlation Between Innate Immune Activation and Neurodegenerative Disease

Numerous animal studies have linked immune responses to neurodegeneration. In one study, the NLRP3 gene, an important component of the neuroinflammatory pathway, was removed from APP/PS1 mice. This gene knockout resulted in reduced inflammation, enhanced Aβ clearance, as well as improved synaptic plasticity and cognitive function (Heneka et al., 2013). In another experimental study, inflammation caused by systemic

immune challenges alone was revealed to trigger Alzheimer-like neuropathology in mice. Mice exposed to systemic immune stimulation prenatally and then again later in adulthood were predisposed to develop sporadic-like AD during aging. They displayed chronic microglial activation, increased Aβ deposition, and working memory impairment (Krstic et al., 2012). In another study, neutralizing increased levels of the inflammatory cytokine, IL-1β, was found to reduce Aβ and p-tau in triple-transgenic AD mice (Ferreira et al., 2014).

A recent study found a correlation between increased proliferation of microglial cells in human AD and disease severity. To establish the influence of inflammation over the onset and progression of AD they targeted the colony-stimulating factor 1 receptor (CSF1R), a receptor protein that regulates the activation and proliferation of microglial cells. A tyrosine kinase inhibitor was administered to APP/PS1 to induce prolonged inhibition of CSF1R. This inhibition resulted in decreased microglial proliferation and improved performance in memory related tasks. These results support the "link of the inflammatory response generated by microglia...with the observed synaptic and behavioural deficits" (Olmos-Alonso, et. al. 2016).

In their review of innate immune activation in neurodegenerative disease, Heneka et al. conclude that neuroinflammation is likely not only a consequence but also an early cause of the pathology (Heneka et al., 2014).

Key Measures of Inflammation

C-reactive protein (CRP) is produced by the liver in response to inflammation. ReCODE protocol advises testing for hs-CRP (high sensitivity) as the standard CRP test cannot always distinguish optimal levels, below 0.9 mg/dl, from mildly abnormal levels. The albumin to globulin ratio is a complementary measure of inflammation and should be at least 1.8. Fatty acids omega-6 and omega-3 are pro and anti-inflammatory respectively. Their ratio should be between 0.5 and 2.9. Levels of cytokines such as IL-6 and TNFα are also important indicators of inflammation. Concentration of IL-6 should be less than 3.0 pg/ml and that of TNFα should be less than 6.0 pg/ml. (Bredesen, 2017)

Reducing Inflammation

The ReCODE protocol recommends a three-pronged approach to reducing inflammation. Treating inflammation is of no use if the trigger is still present, so first, it is critical to remove the sources of inflammation by preventing exposure to "inflammagens". There could be several sources, including chronic infection, viruses, a diet high in simple carbohydrates, leaky gut, and even poor oral hygiene. The second step is to resolve the inflammation through specialized pro-resolving mediator (SPM) supplements. SPMs are small cell signaling molecules such as resolvins, and maresins that have been identified during active inflammation resolution in the body and mediate the return to tissue homeostasis (Serhan et al., 2011). Chronic inflammation indicates the inability to return to homeostatic condition and SPM supplements can provide the missing resolution agonists. The third step is to inhibit new inflammation through ingestion of anti-inflammatories such as omega-3 and curcumin (Bredesen, 2017).

Subtype 2: Non Inflammatory or Atrophic

Homozygous and heterozygous carriers of the ApoE4 allele are at increased risk for this subtype as well, though symptoms typically begin about a decade later than the inflammatory subtype. Symptoms are similar to those in inflammatory Alzheimer's, with an impaired ability to form new memories but retained ability to write and calculate. Inflammation is not present and some of its biomarkers may actually be suboptimal. Rather, this subtype is associated with reduced overall support of synaptic plasticity marked by an atrophic profile, including insulin resistance, hypovitaminosis D, hyperhomocysteinemia, and reductions in hormonal support from molecules such as estradiol, progesterone, and testosterone (Bredesen, 2017). While the extent to which each of these risk factors contributes to AD pathology is still being studied, scientists have proposed theoretical mechanisms to support the contribution of many of these mediators, including homocysteine and insulin, to its pathogenesis.

Homocysteine

Epidemiological studies have associated increased levels of homocysteine (HC) with Alzheimer's disease progression (Morris, 2003). According to one study, homocysteine contributes to AD pathology through an Aβ-fibrinogen interaction. This interaction induces the oligomerization of fibrin and the formation of abnormal fibrin clots which are resistant to fibrinolysis. Accumulation of fibrin clots leads to inflammation and disruption of the blood brain barrier. In their study, AD patients with high levels of homocysteine showed increased Aβ plaques and fibrinogen levels in the brains. Similarly, researchers induced hyperhomocysteinemia in an AD mouse model by administering a high methionine diet for several months. These mice displayed severe Aβ plaque deposition along with learning and memory impairments (Chung et al., 2016). Homocysteine is a marker of both inflammation and suboptimal nutritional support. Sufficient amounts of vitamins B6, B12, and B9 (folate) are required to maintain an optimal level, below 6 mM/L (Bredesen, 2017).

Insulin Resistance

Insulin resistance is another significant risk factor for AD. Insulin is degraded by a protein known as insulin degrading enzyme. This enzyme has also been linked to the decomposition of Aβ plaques. High levels of insulin require insulin degrading enzyme to be constantly breaking down excess insulin, thus limiting its opportunity to decompose Aβ plaques. High levels of glucose

also lead to the production of advanced glycation end products. These molecules trigger inflammation when they bind to their receptors, cause the formation of free radicals, and damage blood vessels, thus reducing nutritional support to the brain. Fasting insulin level should be 4.5 mIU/ml or below and fasting glucose level should be between 70-90 mg/dL (Bredesen, 2017).

Subtype 3: Cortical or Toxic

Subtype 3 is characterized by biomarkers of toxicity and typically occurs in carriers of the ApoE3 rather than the ApoE4 allele. Symptom onset is often earlier, in the late forties to early sixties, and following a period of great stress, sleep loss, anesthesia, or menopause. Cortical Alzheimer's is completely different than the other two types; it is not predominantly amnestic in the early stages and presents itself instead with cortical deficits such as dyscalculia and aphasia. In addition to short-term memory loss, long-term memory loss, including procedural memory, is also affected (Bredesen, 2017). In these cases, PET imaging often indicates more general cerebral atrophy compared to the more restricted temporoparietal reduction typically seen in patients with subtypes 1 and 2 (Bredesen et al., 2016).

Current research seems to suggest a possible link between mercury toxicity and AD. In vitro studies have found that mercury increases secretion of Aβ, causes structural changes in mitochondria which induce a response from the innate immune system. Mercury also interferes with membrane structures leading to the aggregation of neurofibrillary fibers and degeneration of neuronal axons (Walach et al., 2015).

Exposure to other biotoxins such as arsenic has also been shown to affect neurological function. Arsenic exposure most commonly comes from groundwater, a phenomenon particularly prevalent in western United States where certain areas are estimated to have over 300 times the permitted EPA level of arsenic in the drinking water. Gong et al. proposed the Arsenic Exposure Hypothesis for AD based on premises of existing hypotheses for the disease. They find correlations between arsenic toxicity and several typical pathological markers of the disease. These include the following: over transcription of APP, brain inflammatory response, and the generation of free radicals which cause oxidative stress and neuronal death (Gong et al., 2010).

Although much of the literature on arsenic toxicity details its cognitive effects on children, recent studies have correlated it with altered adult cognition as well. The FRONTIER Project is an ongoing study being conducted on residents living in West Texas, an area that contains significant levels of arsenic in the groundwater. Participants with long-term chronic exposure to arsenic scored lower on tests for global cognition, processing speeds and immediate memory (Tyler & Allan, 2014).

It has been suggested that Toxic Alzheimer's is a phenotypic manifestation of chronic inflammatory response syndrome (CIRS), commonly caused by exposure to biotoxins such as molds or tick-borne pathogens. Cognitive decline in both patients with CIRS and Type 3 Alzheimer's is not limited to amnestic symptoms; it includes executive dysfunction often concomitant with hypozincemia, dysfunction of the hypothalamic-pituitary-adrenal axis, and psychiatric effects such as depression. In the case studies of patients displaying type 3 symptoms, all of them had a history of significant toxic exposure (Bredesen, 2016). Patients with the toxic subtype of Alzheimer's also often present significantly high levels of copper and low levels of zinc which can cause increased sensitivity to toxins such as mercury and mold. A copper:zinc ratio of 1.4 or higher has been associated with dementia.

ReCODE recommends treating metal toxicity in one of several ways including chelation, or a gentler method called Detox Qube which helps optimize the natural detoxification process of the body to eliminate potentially toxic metals such as mercury and arsenic. Treatment for toxicity from other toxins such as mold are more complicated and exposure specific and thus should be treated by a physician experienced in biotoxin-associated illness (Bredesen, 2017).

Additional Factors Addressed by ReCODE

The primary characteristic of the ReCODE protocol is its attempt to simultaneously address as many potential disease mechanisms as possible, including diet, exercise, and cognitive training, among others.

Ketogenic Diet: A Neuroprotective Mechanism

The protocol recommends that patients adhere to a ketogenic diet which has been associated with neuroprotective benefits. Ketogenic diets have been linked to several neuronal and synapse supporting mechanisms. Studies have associated a ketogenic diet with antioxidant effects, increased cerebral ATP indicating metabolic effects, and decreased expression of pro-apoptotic factors clusterin and caspase-3, implicating anti-apoptotic mechanisms as well. Additionally, it has been found that the presence of β-HB increases the synthesis of BDNF, a trophic factor which mediates neuroprotection and is associated with cognitive improvement (Bredesen, 2017). Growing evidence of brain glucose hypometabolism in AD brains may provide a mechanism to explain this correlation; namely that the abundance of ketone bodies (KB) made available by a ketogenic diet may compensate for the decreased glucose uptake in the brain by acting as a fuel replacement, allowing the brain to work more efficiently.

Ketone Bodies and Brain Glucose Hypometabolism

The adult brain uses close to 23% of the body's total energy requirement, despite representing only about 2% of total body weight, with glucose used as the predominant form of fuel. It is well documented that glucose metabolism is significantly deteriorated in patients with AD. PET imaging has even shown significant

hypometabolism in subjects with risk factors for AD prior to the manifestation of any symptoms of cognitive decline. A recent review of several independent studies conducted on various groups at risk for AD shows evidence of increased presymptomatic brain glucose hypometabolism, ranging from 12-20% as compared to the control, in all at-risk groups (Cunnane et al., 2016).

For instance, subjects who were homozygous for the E4 allele with a family history of AD, both risk factors for developing AD, were screened for glucose hypometabolism against a control group. Despite no difference in cognitive test results, the experimental group displayed decreased glucose metabolism in the same brain regions identified in AD subjects, specifically in the posterior cingulate and prefrontal, parietal, and temporal cortices (Reiman et al., 2004). This indicates the possibility that PET scans measuring the cerebral metabolic rate of glucose (CMRg) can even be used to predict the onset of the disease.

Given the necessity of the brain for a constant energy supply, Cunnane et al. has proposed that glucose hypometabolism in the brain causes chronic, gradual brain energy starvation and may be part of the disease etiology of AD. Thus, it seems reasonable for potential therapeutic strategies to be aimed at correcting the underlying problem of deteriorating brain fuel supply, specifically by studying the body's normal method of coping with decreased glucose availability which occurs during fasting, malnutrition, or strenuous exercise. When glucose supply is severely compromised, the brain uses ketone bodies, specifically β-hydroxybutyrate and acetoacetate, as the main reserve fuel (Owen et al., 1967).

Ketone vs Glucose Metabolism and Uptake

A study comparing brain glucose and ketone uptake was conducted to determine whether hypometabolism in the brain is generalized to include both glucose and ketone metabolism or a condition specific to glucose. Results showed that global brain FDG (glucose analogue used in PET imaging) uptake was 14% lower in early AD patients than the control group while no significant difference in C-Ac (acetoacetate analogue) uptake was found. Thus, it seems possible that providing more KB to offset the deficit in energy caused by glucose hypometabolism in aging brains may decrease neuronal shrinkage and lower the risk of developing AD. Indeed, clinical studies suggest that nutritional treatments to increase plasma KB concentration may be effective against cognitive decline in early stages of AD (Cunnane et al., 2016).

This theory has been tested in a cell culture model of AD with positive results. Hippocampal cells of embryonic rats exposed to Aβ42 demonstrated a 50% decrease in cell number. When these cells were simultaneously exposed to β-HB, cell survival doubled, indicating the potentially protective nature of β-HB from amyloid toxicity (Henderson, 2008). The effectiveness of ketone body treatment in human AD subjects has also been tested in clinical trial. In this study, subjects with mild to moderate probable AD consumed a beverage containing emulsified medium-chain triglycerides (MCTs) to induce elevated serum KB levels. Due to its shorter chain length, MCTs generate the rapid production of ketone bodies, unlike longer fatty acid chains. After 90 minutes, neurophyscological testing showed a significant correlation between increased serum β-HB concentration and memory improvement, specifically for subjects without the ApoE4 allele (Reger et. al, 2004).

The Ketoflex 12/3 Diet

Due to significant evidence indicating the positive effects of a ketogenic state on cognitive function, the ReCODE Protocol posits the need for a ketogenic nutritional plan, referred to as the "Ketoflex 12/3" diet. The typical western diet is high in carbohydrates which inhibit ketogenesis and the utilization of KB. A ketogenic diet promotes the release of free fatty acids to be synthesized into KB, a process which is normally inhibited by insulin signaling in the presence of carbohydrates.

The Keto in Ketoflex 12/3 refers to the induced state of ketosis through a high-fat, low carbohydrate, plant-based diet. Consumption of MCTs has also been shown to help induce mild-ketosis and is recommended as well. These include coconut oil and palm kernel oil, of which approximately 10% consist of these medium chain fatty acids, or MCT oil which is a concentrated form of the fraction of medium chain fatty acids in coconut oil (Cunnane et al., 2016).

The flex in the Ketoflex 12/3 diet reflects the flexibility and variety of food choices. The diet is mainly plant-based with consumption of protein limited to just a few ounces a day. The consumption of too much protein causes some of it to be converted to carbohydrates. In his book, The End of Alzheimer's, Dr. Bredesen outlines some specifics regarding food choices. These include the following: choosing mainly foods with a glycemic index lower than 35, as these foods do not require significant insulin release; eating detoxifying plants such as cilantro, cruciferous vegetables, kale, maca, and avocados to help eliminate toxins such as heavy metals and Bisphenol A; and getting probiotics and prebiotics either in pill form or from fermented foods such as kimchi and sauerkraut, to help optimize bacteria in the gut. He also recommends reducing cooking times and temperatures to reduce the production of advanced glycation end products which create inflammation, and optimizing nutrition with supplements such as reservatol, nicotinamide riboside, citricoline, ubiquinol and polyquinoline quinine (Bredesen, 2017).

The 12/3 refers to the fasting times; namely a 12 hour fasting period between the last meal at night and the first meal of the next morning, and a 3 hour minimum between dinner and bedtime. As previously discussed, fasting is a highly effective way to induce ketosis.

Stress Management

There are several mechanisms that may account for the association of chronic unresolved stress with cognitive decline. Stress activates the HPA axis to stimulate the release of stress related hormones, such as cortisol, from the adrenal glands. Increased levels of cortisol can lead to neuronal damage, particularly in the hippocampus, thus contributing to cognitive and memory decline. Stress also increases several risk factors for AD including blood glucose levels and hyperstimulation of neurons. Stress is most closely linked with Type 3 Alzheimer's, where onset of cognitive decline often coincides with a stressor. Therefore, a program for stress reduction is included in the ReCODE protocol, including meditation and yoga to lower cortisol levels and prevent hippocampal atrophy (Bredesen, 2017)

Sleep Optimization

There are several neural mechanisms through which sleep affects cognition. Sleep is associated with reduced amyloid plaque formation and induces autophagy in the brain, recycling dysfunctional components and improving cellular health. Production of growth hormone increases during sleep allowing for cell repair and production of supportive brain cells. More hours of sleep allow for a longer period of fasting which helps improve insulin sensitivity. ReCODE recommends at least 8 hours of sleep and the use of melatonin if falling asleep is difficult (Bredesen, 2017).

Physical Activity

Exercise has been linked to many benefits related to cognition. Among them are: reduced insulin resistance, increased ketosis, stress reduction, improved sleep, and increased size of the hippocampus which is a key region in memory and shows atrophy in AD. For optimal cognition benefits, ReCODE recommends combining aerobic exercise with weight training 5 days a week for 45 - 60 minutes per day (Bredesen, 2017).

Table 1. Summary of patients treated with the therapeutic system described

Patient	History, evaluation	Diagnosis	Status
67F 3/3	2yr memory ⇓; FH+	aMCI	Normal x 2.5 yrs; working
69M 4/3	12yr memory ⇓; FDG-PET+, NPsych+	Early AD	"Clearly improved;" working
70M 4/3	4yr memory ⇓; NPsych+, failed MemTrax	AD	Improved; MemTrax passed
75M 3/3	1yr memory ⇓	SCI	Improved; working
75F C677T	1yr memory ⇓	aMCI/early AD	Improved
55F 3/3	4yr memory ⇓	aMCI/early AD	Normal; working
72M 3/3	7yr memory ⇓	aMCI	Improved; working
55M 4/3	2yr memory ⇓	SCI	Normal; working
63F 4/3	FH dementia, mild memory ⇓	SCI	Normal, negative amyloid PET; working
60F 4/3	4yr rapid decline; MoCA 6, amyloid PET+	Late AD	Decline

F, female; M, male; 3/3, ApoE 3/3; 4/3, ApoE 4/3; C677T, the C677T mutation in methylene tetrahydrofolate reductase (MTHFR); FH, family history; aMCI, amnestic mild cognitive impairment; SCI, subjective cognitive impairment; FDG-PET+, fluorodeoxyglucose positron emission tomography interpreted as typical of Alzheimer's disease; amyloid PET+, amyloid PET scan read as abnormal, indicative of amyloid accumulation; NPsych+, quantitative neuropsychology tests showing abnormalities typical of AD; MoCA, Montreal Cognitive Assessment; MemTrax, an iPhone application that quantitates memory.

Cognitive Training

Although the effects of brain exercises are controversial, cognitive training was one of the additional intervention elements given to the intervention group of the FINGER study which suggest a positive correlation between brain exercises and cognition (Ngandu et al., 2015).

Patient Case Studies

The results of the multi-domain therapeutic system utilized by Bredesen et al. on 10 patients is summarized in the table below. As indicated, patients either reverted to their normal mental status prior to exhibiting symptoms of cognitive impairment, or showed improvement in cognition. One patient with advanced AD, however, showed decline. These results suggest that memory loss in cases of Subjective Cognitive Impairment (SCI), Mild Cognitive Impairment (MCI), and early AD may be reversed and show sustained improvement using the therapeutic program described by the ReCODE protocol (Bredesen, 2017).

Conclusion

Although preliminary results of this study seem promising, unlike the FINGER study, the results are only anecdotal and fail to include predefined criteria for success, any indication as to how patients were selected for inclusion, or clear descriptions of the exact protocol followed for each patient. Additionally, the protocol addresses many biomarkers that have been implicated in AD without proving whether they are etiologically or epiphenomenally linked. There is also no control group for comparison. Thus a more extensive, controlled clinical trial is required to determine the broad range effectiveness of the ReCODE protocol on cognitive impairment.

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