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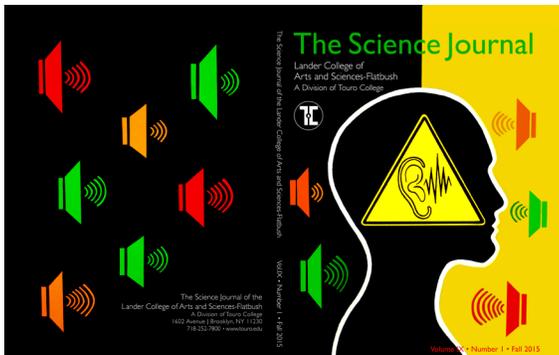
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# Mechanical Plasticity: Skeletal Muscle Adaptations

By Naomi Broker

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## Abstract

The purpose of this paper is to investigate the adaptations that occur in human skeletal muscle in response to endurance and resistance exercises. The advances made in science over the past several decades increased the number of methods available for the classification of muscle fibers, resulting in many fiber type classes and their corresponding characteristics. This allows for the tracking of changes that occur within the muscle fibers. The heterogeneous collection of fiber types found within a muscle allows for its dynamic nature. Myosin form expression varies according to the muscle's changing functional demands. In response to endurance training, muscle fibers adapt by changing in composition, converting between type IIB and type IIA (i.e. the fast to slow direction). In response to resistance training, muscle fibers undergo both fiber-type shifting and hypertrophy. This muscle plasticity allows for the physiologic changes that take place in athletes and in physical therapy patients. The research available allows for the designing of interventions specific to increasing one's endurance or power.

## Introduction: Muscle fiber and motor unit typing

Muscle fiber types can be described using morphological, histochemical, immunohistochemical and biochemical characteristics. Clear morphological differences were seen in birds, with fast muscles appearing white and slow muscles appearing red. The redness of the slow muscle is due to the greater myoglobin and capillary content that permits a greater oxidative capacity of the muscle (Staron, 1997). Research has long shown that there is a clear correlation between myosin ATPase activity and muscle contraction speed. Studies in rats, measuring isolated muscle units, lead to initial classification of muscle fibers based on their isometric twitch contraction speed as fast, intermediate and slow (Pette et. al., 1999). Presently, muscle fibers are classified by means of 3 different techniques: histochemical myosin ATPase staining, immunohistochemical myosin heavy chain isoform identification, and biochemical identification of metabolic enzymes.

Acronyms:	
Myosin ATPase	MATPase
Myosin Heavy Chain	MHC
Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoretic	SDS-PAGE
Fast-Twitch Glycolytic	FG
Fast-Twitch Oxidative	FOG
Slow-Twitch Oxidative	SO
Slow-Twitch	S
Fast-Twitch Fatigue-Resistant	FR
Fast-Twitch Fatigue-Intermediate	Fint
Fast-Twitch Fatigable	FF

## Histochemical Classification

Myosin ATPase histochemical staining confirmed the diversity of muscle fibers. The stain's intensity differs based on the myofibrillar differences in pH sensitivity. Type I fibers were found to be alkali labile (lighter stain, low ATPase activity) and acid stable (intense stain, high activity), while type II fibers were alkali

stable and acid labile (Staron, 1997, Sieck, Prakash, 1997). At first, fibers were identified as slow type I, and fast types IIA and IIB (Pette et. al., 1999). However, advances in the histochemical staining technique has led to the identification of a total of 7 human muscle fiber types (Staron, 1997). The newly identified fibers, types IC, IIC, IIAC, and IIAB, have intermediate myosin ATPase staining characteristics. Type IC, the slowest fiber, stains most similarly to type I fibers, while type IIAB fibers have intermediate staining characteristics between type IIA and IIB fibers. Thus, the 7 human muscle fiber types (from slowest to fastest), as identified by myosin ATPase histochemical staining are: types I, IC, IIC, IIAC, IIA, IIAB, and IIB (Pette et. al., 1999). As seen in the literature, some researchers do not use all 7 fiber types, but rather place all fibers into the original 3 categories (Staron, 1997).

## Immunohistochemical Classification

A second fiber type classification is based on the identification of different myosin heavy chain (MHC) isoforms (Pette et. al., 1999). Immunohistochemical analysis of human muscle began after scientists discovered antigenic differences between the myosin types of different human muscle (Staron, 1997). Isoforms were identified using antimyosin antibodies or by sodium dodecyl sulfate-polyacrylamide gel electrophoretic (SDS-PAGE) separation (Pette et. al., 1999). A general correlation was discovered between the histochemically classified fiber types and the MHC isoforms they express (Sieck, Prakash, 1997, Fry et. al., 1994). The original 3 myosin isoforms identified were MHC I, MHCIIa, and MHCIIb, corresponding to those identified by myosin ATPase staining as pure types I, IIA, and IIB, respectively. However, each muscle fiber can contain more than one myosin heavy chain isoform, forming hybrid fibers. This phenomenon explains the existence of more muscle fiber types than the amount of pure isoforms. These mixed fibers almost always contain "neighboring" myosin heavy chain isoforms (i.e., MHC I and MHCIIa or MHCIIa and MHCIIb) (Pette et. al., 1999). Type IIAB fibers that have a predominance of the MHCIIa isoform stains more like type IIA fibers, but fibers that have a predominance of the MHCIIb isoform stains more like type IIB fibers

(Staron, 1997). Consequently, the histochemical myosin ATPase types express their isoform genes to varying degrees, according to the variable ratio of isoforms present in the fiber. Due to its quantitative nature, the SDS-PAGE technique is perhaps the best method for muscle fiber typing, as electrophoretic separation permits the recognition of the relative concentrations of different myosin heavy chain isoforms in mixed muscle fibers (Pette et. al., 1999, Fry et. al., 1994).

A fourth myosin heavy chain isoform, MHCIIx or MHCIIc, and its corresponding fiber type IIX, is present in small mammals. Evidence shows that MHCIIb in humans is homologous to MHCIIx/d of small mammals (Pette et. al., 1999, Hilber et. al., 1999). In actuality, MHCIIb in humans is really MHCIIx/d, as humans do not express MHCIIb, the fastest myosin heavy chain isoform (Pette et. al., 1999). In fact, recent data shows that fibers that were histochemically identified as type IIB in humans contain low amounts of MHCIIa, and therefore, in reality, are hybrid type IIAB fibers (Staron, 1997). Therefore, the 3 myosin heavy chain isoforms present in human limb muscles are (from slowest to fastest): MHCI, MHCIIa, and MHCIIx/d (previously incorrectly known as MHCIIb).

**Biochemical Classification**

A third classification technique reflects the energy metabolism of the muscle fibers. Histochemical myosin ATPase fiber typing of type I or type II corresponds to slow and fast muscle fibers, respectively, and the enzymes involved reflect the metabolic pathways that are either aerobic/oxidative or anaerobic/glycolytic (Pette et. al., 1999). This classification scheme leads to 3 fiber types: fast-twitch glycolytic (FG), fast-twitch oxidative

(FOG), and slow-twitch oxidative (SO) (Pette, Staron, 1997). Type I and IIA fibers have a greater mitochondrial density and oxidative capacity than type IIB and IIX fibers (Sieck, Prakash, 1997). Accordingly, there is a good correlation between type I and SO fibers, but correlations between type IIA and FOG and type IIB and IIX and FG fibers are more diverse (Hamalainen, Pette, 1995). This can be explained by the fact that changes in oxidative capacity can take place without changes in MHC isoforms (Staron, 1997). Generally, type I fibers rely primarily on aerobic/oxidative energy metabolism and type II fibers rely primarily on anaerobic/glycolytic metabolism, but as it cannot be assumed, the terms type IIB and FG or type IIA and FOG cannot be used interchangeably (Pette et. al., 1999).

**Myosin Light Chains**

The myosin light chains also exist in different isoforms, slow and fast, and affect the contractile speed of the fiber (Talmadge, Pette, 1995). A homogeneous/pure myosin heavy chain isoform can be heterogeneous in regard to its myosin light chain isoform. Usually, however, fast heavy chain isoforms fuse with fast light chain isoforms, and slow heavy chain isoforms fuse with slow light chain isoforms (Pette et. al., 1999, Jostarndt-Fogen et. al., 1998).

**Motor Unit Classification**

The proper functional unit of the neuromuscular system, the motor unit, reflects the characteristics of its individual fibers. Motor units are categorized based on their contractile speeds and fatigue characteristics. Contractile speed classifies motor units into slow-twitch (S), usually comprised of type I fibers, or fast-twitch (F), usually comprised of type II (Burke, 1999). The

<b>Table 1: Summary of fiber types and their corresponding characteristics</b>				
	<b>Type I fibers (I, IC)</b>	<b>Type IIA fibers (IIC, IIAC, IIA, IIAB)</b>	<b>Type IIX fibers</b>	<b>Type IIB fibers (not present in humans)</b>
<b>MHC isoforms</b>	MHCI	MHCIIa	MHCIIx/d	MHCIIb
<b>Contraction time</b>	Slow	Moderately Fast	Fast	Very Fast
<b>Oxidative Capacity</b>	High	High	Intermediate	Low
<b>Glycolytic Capacity</b>	Low	High	High	High
<b>Biochemical Fiber Type (generally)</b>	SO (slow oxidative)	FOG (fast-twitch oxidative)	FG (fast-twitch glycolytic)	FG (fast-twitch glycolytic)
<b>Resistance to Fatigue</b>	High	Fairly High	Intermediate	Low
<b>Motor Unit Class (generally)</b>	S (slow twitch)	FR (fast-twitch fatigue-resistant)	Fint (fast-twitch fatigue-intermediate)	FF (fast-twitch fatigable)
<b>Activity used for</b>	Aerobic	Long-term anaerobic	Short-term anaerobic	Short-term anaerobic
<b>Mitochondrial Density</b>	High	High	Intermediate	Low
<b>Capillary Density</b>	High	Intermediate	Low	Low

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F motor units are additionally classified by their type II fiber subdivisions. Fast-twitch fatigue-resistant (FR) are comprised of type IIA fibers, fast-twitch fatigue-intermediate (Fint) are comprised of type IIX, and fast-twitch fatigable (FF) are comprised of type IIB (Burke, 1993). The fatigue resistance of the muscle unit is explained by the mitochondrial density and oxidative capacity of the muscle fibers. The motor unit typically reflects the function of the muscle. The diaphragm muscle employs its fatigue-resistant S and FR units to produce normal breathing, but must recruit its Fint and FF units for actions that require greater force, such as gagging or sneezing. Contrariwise, the extensor digitorum longus muscle of the limb, which is used for motor activities demanding short periods of greater force production, is composed mostly of FF type IIB fibers. Consequently, type I and IIA fibers are intended to sustain longer periods of activation, while type IIX and IIB fibers are intended to produce shorter bursts of activation (Sieck, Prakash, 1997).

Therefore, the overall properties of a muscle reflects the properties of its heterogeneous collection of fiber types and their overall proportions. It is this range of fiber types that allows for the wide range of muscle function a single muscle can display. Muscle fibers adapt to their motor unit's changing stimuli not only by changing their size, but by converting their fiber type composition to suit the functional demands. This muscle plasticity serves as the physiologic basis for the adaptations of skeletal muscle to resistance and endurance exercises.

## Methods

EBSCO multi-search, ProQuest Medical Library, Wiley Library, Journal of Applied Physiology, MEDLINE, and PubMed databases were used to find material for this paper. Access was gained to these databases through the Touro College Online Library and Einstein Online Library websites. Key words, such as "muscle fiber types" and "effects of exercise on muscle" were used to search for scientific articles. After reading through these articles, other key words were identified, such as "fiber-type shifting", and "resistance vs endurance". Additionally, sources listed as references for review articles on the APTA website (American Physical Therapy Association) were searched for to find the original papers.

## Discussion

The plasticity of the heterogeneous composition of muscle fibers allows for adaptations in the contractile and metabolic properties of skeletal muscle. Various phenotypes are therefore possible in response to different workloads. This plasticity has practical implications, both for athletes and rehabilitation patients alike.

This range of myosin form expression in response to exercise

regimes is evident in the vastus lateralis. In untrained, physically active subjects, the vastus lateralis is made up of roughly 50% of slow type I fibers and 50% type II fibers (40% type IIA and 10% IIX). Power athletes, such as sprinters, power weight lifters and throwers, are constituted chiefly of IIA and IIX fibers, with a percentage of type I as little as 20%. Ultra-endurance athletes, such as long-distance runners, have a 95% constitution of type I fibers. The variability of the composition of the vastus lateralis muscle is due to fiber type shifting. Long-term endurance training induces a substantial transformation from fast muscle fibers to slow muscle fibers. Slow muscle fibers rely mainly on aerobic metabolism and, for that reason, are vital for endurance activities such as swimming, cycling and marathon running. The shift in MHC isoform type depends on changes at the molecular level. New slow MHC polypeptides, which are still categorized as type IIA fibers, are produced, marking the start of their transition towards the slow type I fibers (Zawadowska et. al., 2004).

In response to training, the most common fiber conversions are between type IIB and type IIA. Slow to fast/ type I to type II conversions are possible in response to the loss of function related to deconditioning, as shown in studies on humans with spinal cord injury and microgravity exposure during their time in space. Detraining in humans (i.e., decreased use of skeletal muscle following a formerly high activity level) leads to shifts MHCIIa to MHCIIx/d and possibly MHCI to MHCIIa. Additionally, there is a related decrease in aerobic-oxidative metabolic enzymes (Pette, Staron, 1997). In short, immobilization of skeletal muscle may cause conversions in the slow to fast direction. Scientists assumed that power training may also cause the transformation in slow to fast direction. This was a logical assumption, since fast muscle fibers depend mainly on anaerobic metabolism and are consequently essential for power training, such as sprinting and weight lifting. Additionally, IIX fibers produce a greater maximal power output than IIA fibers and are therefore essential for such activities. However, studies have proven that all methods of training (both endurance and power/resistance) cause a conversion toward the slow direction (Zawadowska et. al., 2004).

## Endurance

Many studies have proven that endurance exercises (low-resistance, repeated contractions that require the muscle to produce a high aerobic metabolic rate) lead to several adaptations within the muscle to augment aerobic metabolism and resistance to fatigue (Staron et. al., 1990). Firstly, the oxidative capacity of all fiber types will increase by increasing the amount of mitochondria, oxidative/aerobic enzymes and capillaries in the muscles being trained (Holloszy, Booth, 1976, Fitts, Widrick 1996). Based on metabolic classification scheme, there is a transition from the FG fiber type to the FOG fiber type without, necessarily causing a change in the myosin heavy chain composition (Pette, Staron, 1997).

Secondly, there are alterations in myosin heavy chain isoform. It has been suggested that there is an increase in the percentage of type I fibers following aerobic training, and a subsequent decrease following detraining in elite endurance athletes. Additionally, within type II fibers, MHCIIx/d (IIB) are converted to MHCIIa (IIA). In other words, there is a decrease in the population of pure type IIB fibers and an associated increase in the population of pure type IIA fibers. It also seems that there is an increase in the hybrid population of type I and type II fibers, known as type IIC (Staron et. al., 1990). Evidence as to the conversion of type II to type I fibers, as stated previously, is lacking (Ricoy et. al., 1998). The transformation of type IIB fibers to type IIA in response to endurance training is fairly logical. Although there are variations on the oxidative capacities within a muscle type, as a class, type IIA fibers generally have a greater oxidative capacity than type IIB fibers. Therefore, an increase in the population of type IIA fibers makes the muscle more oxidative. In fact, there is a negative correlation between the percentage of type IIB fibers and maximum oxygen intake. Therefore, as stated previously, detraining and immobilization cause a conversion in the slow to fast direction, from type IIA to type IIB (Staron et. al., 1990).

Thirdly, there can be a transformation in myosin light chain isoforms. Type I fibers show an increase in contractile speed in response to endurance exercises, but show a decrease following deconditioning in humans. Logically, this change cannot be explained by a conversion of fiber type, but rather, by a conversion in the myosin light chain isoforms from slow to fast and from fast to slow, respectively (Larsson et. al., 1996, Widrick et. al., 1996). The conversion from slow to fast allows the fibers to maintain their properties of efficient energy usage while increasing their contractile speed to keep up with the demands of the exercise (Fitts, Widrick, 1996). Such a conversion would not be detected by the histochemical technique, as there is no change in the myosin ATPase (Pette, Staron, 1997). To summarize, muscle fiber adaptations to endurance exercise varies based on fiber type. In all types, the oxidative capacity of the fibers increases. Type II fibers shift in the type I direction, leading to slower, oxidative types. Type I fibers convert their myosin light chains to increase their contractile speeds.

### Resistance/Power

High-intensity resistance strength training involves short, maximal contractions that require the muscle to produce a large amount of anaerobic energy (Staron et. al., 1990). Resistance training, such as high-load-low-repetition exercises, leads both to the fiber type shifting seen with endurance training and to muscle hypertrophy, which plays a significant role in increasing force production (Kraemer et. al., 1996). Initial strength gains made with high-intensity resistance training are caused

by neural factors, rather than hypertrophy of the actual muscle fibers. However, adaptations may also be occurring in the contractile proteins of skeletal muscle within a short duration of training, even two weeks, with sufficient intensity (McArdle et. al., 1994). Visible hypertrophy is not evident until later in the training period (>8 weeks) (Kraemer et. al., 1996), around the same time researchers found a shift in muscle fiber type composition from MHCIIx/d to MHCIIa (Staron et. al., 1994, Kraemer et. al., 1995, Staron et. al., 1990).

To explore this plasticity, twenty four male subjects were categorized into three groups according to their sports/physical activity. Group A was made up of untrained students, group B of national and sub-national level endurance athletes (7.8+/-2.9 years of specialized training) and group C of sprint-power athletes (12.8+/-8.7 years of specialized training). Biopsies of the vastus lateralis muscle were obtained and immunohistochemically analyzed for fast/slow MHC composition. This muscle is easily accessible and easily trained. Most importantly, it expresses all three myosin isoform types at specific amounts, and so its phenotype visibly mirrors any adaptive modifications that occur after different forms of exercises. Unpredictably, group C sprint-power athletes (such as ice hockey, volleyball, karate, soccer players and modern dancers), who were expected to display the highest percentage of MHCIIx, were no different in this aspect from group B endurance athletes (such as marathon runners, cyclists and cross country skiers). The muscle phenotypes of both groups were similar, containing a small proportion of the MHCIIx isoform and a predominance of slow MHCII isoform. Clearly, the muscle phenotype was adapted for long lasting, sustainable activities (similar to what happens in endurance athletes), rather than activities that require a maximum power output in minimal time. Moreover, the fastest isoform, MHCIIx, was relatively lower in group C athletes than in group A students. This myosin profile in group C athletes is unfavorable to their sport. Muscles that contain a higher percentage of type IIX fibers have a greater maximum shortening velocity, which is the most important factor in maximum power output and therefore vital for their sports regime. This is a possible explanation for why, despite years of training, these athletes could not reach international level (Zawadowska et. al., 2004).

A study performed on women proved similar to the results shown by men. To examine the adaptations that take place following a high-intensity resistance strength training program (i.e. hypertrophy and fiber type shift), twenty-four women participated in a 20-week program for the lower extremity. Biopsies were obtained both before and after the training program from the superficial part of the vastus lateralis. Once again, this muscle was chosen due to its easy accessibility, broad

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fiber type composition, and potential for training (Staron et. al., 1990).

Based on staining intensities using MATPase histochemistry, six fiber types (I, IC, IIC, IIA, IIAB, and IIB) were distinguished, and three groups were determined based on fiber type (I, IIA and IIA+IIB). Dramatic hypertrophy of all three groups (I= 15%, IIA= 45%, and IIAB +IIB= 57%) following a high-intensity strength training regime showed that fast twitch fibers are not the only fibers affected. There appears to be an increase in contractile elements within the muscle fibers, leading to an increase in both muscle strength and size, similar to the adaptations found in men following strength exercises (Staron et. al., 1990).

Additionally, the data shows that strength training causes muscle fiber-type conversions. In the pre-training biopsy sample, group 1 (type I) had the largest cross-sectional area ( $4253 \pm 949 \mu\text{m}^2$ ), group 2 (type IIA) had intermediate ( $3370 \pm 1048 \mu\text{m}^2$ ), and group 3 (type IIAB+IIB) had the smallest ( $2697 \pm 931 \mu\text{m}^2$ ). Despite a hypertrophy of all three groups, the “hierarchy of fiber sizes” was changed following training. The areas of group 1 and 2 were not significantly different from each other (type I =  $4893 \pm 770 \mu\text{m}^2$ , type IIA =  $4888 \pm 967 \mu\text{m}^2$ ), and the area of

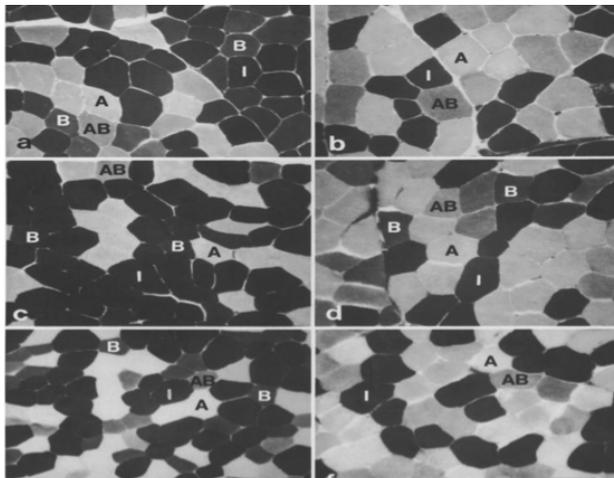


Figure 1: Cross sections of pre (left) and post (right) biopsies examined for MATPase activity from three different women. The dark staining type IIB fibers have visibly disappeared in the post-biopsy biopsy (Staron et. al., 1990).

group 3 was still significantly smaller ( $4233 \pm 1433 \mu\text{m}^2$ ) than the areas of both group 1 and 2. Therefore, despite the fact that the total area of type IIB fibers increased, the percentage of IIB fibers (MHCIIx/d) significantly decreased, with a concomitant increase in the percentage of IIA (MHCIIa) fibers (Staron et. al., 1990, Figure 1).

It is assumed that the recruitment of the infrequently activated IIB fibers caused their transformation to type IIA fibers. This increases the oxidative capacity of strength-trained muscle, as supported by the findings of significantly greater volumes of mitochondria in weightlifters' muscles compared to untrained subjects. This also explains the observation of an increase in short-term endurance following an intense-resistance training routine (Staron et. al., 1990). As a result of this shifting, reductions in myosin heavy chain “coexpression” has been reported both after endurance and resistance training, with a corresponding increase in pure fibers (Williamson et. al., 2000).

### Conclusion

The study of muscle fiber types has constantly been evolving over the past several decades. New systems of classification that categorize the fibers into more specific groups enables researchers to accurately track the changes that occur within a muscle's fibers. Endurance training is known to increase the muscle's endurance by increasing the oxidative capacity of its fibers, as supported by the increase in type I fibers. Resistance/power training is known to increase the volume of the contractile proteins in the muscle fibers, promoting hypertrophy of the muscles. However, research shows that rather than increasing the percentage of fast muscle fibers, as expected, resistance exercises leads to fiber type shifting in the slow direction, increasing the oxidative capacity of the muscle.

While research has proven that resistance exercises leads to hypertrophy and muscle type shifting in both men and women, such studies are fairly new. Research conducted on the effects of endurance exercises far exceeds the research conducted on the effects of resistance exercises. As such, studies pertaining to resistance exercises do not always agree, and the pool of available research is not large enough to evaluate which studies are most accurate. For example, some papers claim that fiber type shifting causes type IIB to convert to type I fibers, while other papers say that type IIA is the farthest it can go. Therefore, additional research must be conducted to shed light on this topic and determine which theory is correct. However, it is clear that just like endurance exercises, resistance exercises lead to conversions in the fast to slow direction, and not in the slow to fast direction (as was originally assumed).

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# Noise Induced Hearing Loss: The Impact of Acoustic Trauma on the Ear

By Aviva Levihaiem

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## Abstract

Noise-induced hearing loss (NIHL) is a pervasive disability that affects millions of people across the world. It is characterized by a decrease in hearing sensitivity to sounds that fall between 3000–6000 Hz (known as the “notch”). NIHL is caused by either a sudden acoustic trauma or long-term exposure to noise levels above 85 dB. The noise exposure induces overproduction of free radicals within the cells of the cochlea, overpowering of the ear’s antioxidant defense systems. The free radicals then destroy cell membranes and hair cell nuclei, causing necrosis and apoptosis, leading to hair cell death. This upsets the mechanical structure and the metabolic homeostasis within the ear, impeding hearing function. Studies have shown that some people are more susceptible to NIHL, either due to environmental factors or genetic factors. NIHL is preventable; OSHA and the EPA have sought to regulate sound levels on job sites and encourage the use of hearing protective devices at work, and researchers are developing new imaging tests to accurately diagnose NIHL before severe hearing loss sets in. There are also ongoing studies regarding the prophylactic and therapeutic use of pharmacological agents (antioxidants and glucocorticoids) and vitamins in the prevention and treatment of NIHL. This paper is a review of the literature available on NIHL and the ongoing studies related to diagnosis, treatment, and genetic factors in the disorder. The literature was found on a number of research databases, with most of the source material coming from the Academic Search Complete (EBSCO) database.

## Introduction

Millions of people across the globe are exposed each day to acoustic overstimulation – noise – in their daily activities, whether at their jobs or during their leisure activities. Noise is one of the most common sources of environmental stress, and it can cause both mechanical and metabolic damage to an individual’s ear. As a result, noise-induced hearing loss (NIHL) is a disability that is widespread among individuals exposed to either prolonged loud noise or excessively loud noise. More than 600 million people around the world endure excessive noise at their jobs and are thus at risk for NIHL. Millions more expose themselves to excessive noise during leisure activities, particularly when listening to or playing music, and they too place themselves at risk to developing NIHL.

The susceptibility to NIHL has been shown to have both environmental and genetic factors. Environmental variables that increase a person’s likelihood of developing NIHL include noise exposure that is combined with extreme temperatures, chemical solvents, and prolonged vibration. Individual environmental factors including smoking, high blood pressure, and high cholesterol levels, have also been shown to increase an individual’s risk of developing NIHL. Currently, the genetic factors that increase a person’s susceptibility to NIHL are largely unknown. Many animal studies are currently ongoing to identify genetic polymorphisms linked with NIHL.

This paper will explore how noise impacts both the mechanical structure of the ear and the metabolic processes within the ear. Additionally, it will examine recent and ongoing studies regarding the genetic factors that increase a person’s susceptibility to developing NIHL, as well as the preventative and therapeutic interventions that are currently in use or being tested in experimental stages.

## Methods

The information in this paper was synthesized from literature reviews and studies published in peer-reviewed journals. The articles were selected using databases, primarily the Academic Search Complete (EBSCO) database made available through the Touro College virtual library. Data was extracted according to relevance, with special attention given to NIHL statistical data; mechanical and metabolic disruption in the ear as a result of noise trauma; and the diagnosis, treatment, and genetic factors related to NIHL.

## Discussion

The word noise is derived from the Latin word *nausea*, which means “impulsive, unwanted, and unpleasant” (Taneja, 2014). In medical terms, noise has been defined as a “physical agent capable of causing damage to the human body with the short and medium term effects” (Metidieri et al., 2013). Sound is measured in units known as decibels (dB), and normal sounds are generally less than 75 dB and are unlikely to cause any damage to the ear or cause hearing loss. However, sounds that are higher than 85 dB can cause physical damage to the structures of the ear. This includes excessively loud noises that are short and sudden, such as gunshots or explosions. Moreover, an individual who is frequently exposed to noises that are continuously and moderately loud can experience damage to hearing structures that results in NIHL. NIHL can involve functional limitations for a number of hearing processes, including “changes in frequency selectivity, temporal and spatial resolution, recruitment, and tinnitus as well as changes in hearing sensitivity” (Metidieri et al., 2013). NIHL is the second most common hearing loss after age-related hearing loss (Levey et al., 2012).

Excessive noise in public areas has been called noise pollution, and it is considered an environmental problem along with other forms of pollution, such as smog or water pollution. In fact, noise pollution affects the largest number of people worldwide, according to the World Health Organization (WHO) (Metidieri et al., 2013). In research on NIHL, noise is classified in three categories: (i) continuous noise, which is noise that persists for minutes or hours at about the same level of intensity; (ii) impact noise, which is a high level but short duration noise that occurs when two objects collide; and (iii) impulse noise, which is a high level but short duration noise that results from an explosion or gunfire (Bielefeld, 2015). NIHL can be diagnosed as either acoustic trauma or chronic NIHL. Acoustic trauma is defined as a sudden but permanent hearing loss caused by a one-time exposure to a forceful sound, with sound pressure levels of approximately 130-140 dB. The extent of damage after an acoustic trauma will vary from mild to profound. In contrast, chronic NIHL results from gradual exposure to less forceful noise over an extended period of time, and this can result in either mild or profound hearing loss (Rajguru 2012).

Approximately 25% of workers across the globe are exposed to noise levels that can induce NIHL. It is the most common work injury (Metidieri et al., 2013). Noise exposure is often experienced with other causal agents of NIHL, including chemical exposure. For example, when an individual is exposed to carbon monoxide and noise simultaneously, he has a higher risk of developing NIHL, even though exposure to carbon monoxide alone rarely induces hearing loss (Kashani et al., 2011). Smoking cigarettes while being exposed to noise both raises the risk of NIHL and increases the extent of the hearing damage. This may be due in part to the carbon monoxide exposure related to cigarette smoke (Liuyan et al., 2013). Other causal agents include vibrations, medications, and one's individual susceptibility to hearing loss (Metidieri et al., 2013).

The National Institute for Occupational Safety and Health (NIOSH) estimates that in the United States alone, there are 5–30 million workers who are exposed to dangerous noise levels each year (Bielefeld, 2015). NIHL is an insidious condition, as those exposed to loud noise are generally unaware that damage is occurring until it is too late (Levey et al., 2012). It is characterized by a decrease in hearing sensitivity to sounds that fall between 3000–6000 Hz (known as the “notch”). The decrease in hearing sensitivity is often asymmetric. Typically, there is greater hearing loss in the left ear than in the right ear (Phillips et al., 2010).

Individuals from all segments of the population are at risk for NIHL, though there is a higher prevalence among men (Pelosi, 2014). Among Americans aged 20-69, there are 23 million people (12.8% of this demographic) who have NIHL (Bielefeld,

2015). However, the risk of developing NIHL is unrelated to age (Phillips et al., 2010). In fact, the pervasiveness of NIHL rises steadily over childhood and adolescence (Pelosi, 2014). The American Academy of Audiology estimated in 2008 that one in eight children has sustained NIHL. In 2010, a similar estimate was made in which 16% of teenagers were thought to have sustained NIHL. Among younger demographics, NIHL is typically caused by exposure to excessively loud music over an extended period of time, particularly on personal listening devices such as iPods, MP3 players, or CDs. Children's toys that emit loud sounds have also been found to cause NIHL. A study conducted with 329 student musicians between the ages of 18 to 25 years found that 45% of the students had sustained NIHL, with 78% of the hearing loss happening at 6000 Hz (Levey et al., 2012).

Noise exposure at work is often associated with factory workers who spend their working hours in cramped spaces with loud, industrial machinery. However, there are less conventional occupations that have high risk of NIHL as well. As many as 60 percent of military personnel involved in active duty return home with NIHL or tinnitus, and have both (Pelosi, 2014). This is likely due to their excessive gun use (Berg et al., 2014). Firefighters, too, are at high risk for NIHL, as the noise they are exposed to regularly is usually high in intensity and short in duration (Hong et al., 2013).

Individuals who do not work in noisy environments or listen to loud music can also be at risk for NIHL in their daily lives. Neitzel et al. found that New York City subway riders are exposed to noise louder than the limits recommended by WHO and the United States Environmental Protection Agency (EPA). This presents a heightened risk for NIHL to those who use mass transit often, especially those who remain on the subways for a longer period of time (Neitzel et al., 2009).

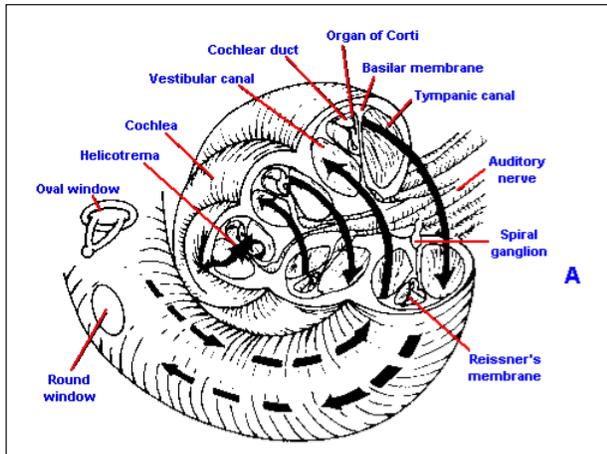
### **Mechanical Structure and Metabolic Processes of the Ear**

The prevalence of NIHL demonstrates how the ear is a complex and vulnerable organ. The cochlea is the inner ear organ that converts sound for the process of hearing. The cochlea holds the organ of Corti, which is comprised of hair cells that serve as the sensory receptors of sound. There are two types of hair cells: inner hair cells and outer hair cells. The inner hair cells use the auditory nerve to communicate with the brain to create sound perception. The outer hair cells amplify sound, increasing hearing sensitivity by ~40 dB. The organ of Corti also contains a number of supporting cells that form the physical structure of the ear that is necessary for the mechanical movement that occurs when sound waves reach the ear.

The organ of Corti requires metabolic homeostasis to properly convert sound waves and auditory signals. Noise exposure can

# The Impact of Acoustic Trauma on the Ear

**Figure 1**



**Figure 2**

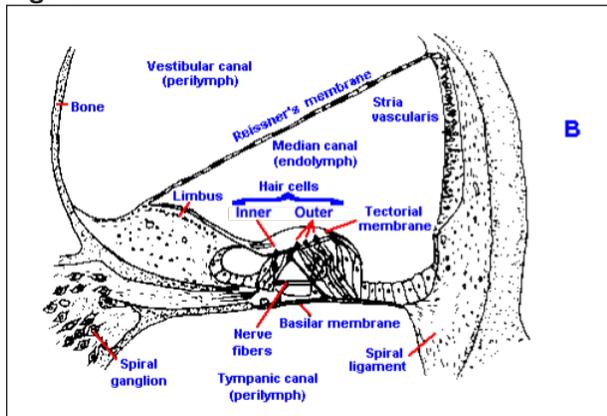


Figure 1: Diagram of the cochlea cut through along the modiolus. Arrows demonstrate the pathway of pressure waves that originate in the oval window. (Curtis et al., 1970)

Figure 2: Cochlear partition and the organ of Corti. (Curtis et al., 1970)

damage the cochlea both metabolically and mechanically, and NIHL can occur from either metabolic or mechanical deterioration, as well as from a combination of both (Bielefeld, 2015). (Figures 1 and 2)

## Normal Ear Function

The hearing process is a complex series of steps that occurs when a sound is emitted. Sound waves travel to an individual's ear, where the hair cells act as receptors for the energy from sound waves and then convert the sound waves into nerve signals that reach the brain (Pelosi, 2014). Sound waves first enter the outer ear and then traverse the ear canal to reach the eardrum. When the sound waves strike the eardrum, it begins to vibrate, and the vibrations reach the middle ear. In the middle

ear, bones (also known as "ossicles") called the malleus, incus, and stapes, amplify the sounds. The vibrations are then sent to the inner ear, known as the cochlea, which is a fluid-filled organ with an elastic membrane known as the basilar membrane. Attached to the basilar membrane, there are sensory cells, known as outer and inner hair cells. The vibrations that reach the inner ear generate a traveling wave that runs along the basilar membrane as a result of the electromotility of the outer

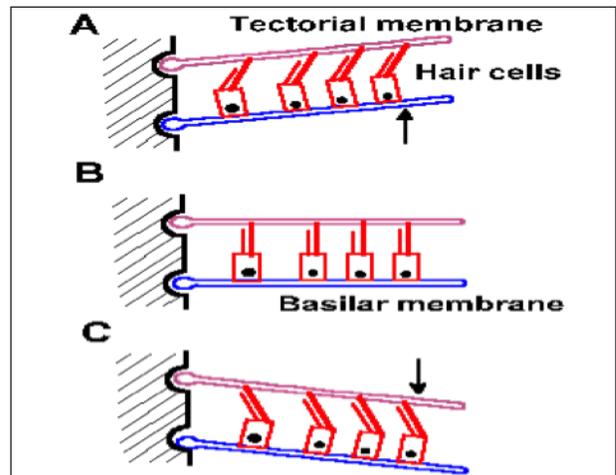


Figure 3: Movements of the basilar membrane and the deflection of the stereocilia:  
 A. Upward movement of the basilar membrane (arrow) deflects hairs away from the modiolus.  
 B. The basilar membrane is at rest is  
 C. Downward movement (arrow) deflects hairs toward the modiolus. (Klinke, 1978)

hair cells. The movement induces stereocilia, spiky structures on the apical surface of the inner hair cells, to rub up against the tectorial membrane and thus release neurotransmitters that travel across the synapse between the inner hair cell and the auditory nerve. Figure three depicts these movements. This process allows for sound waves to change into electrical signals that are carried to the brain by the auditory nerve, and the brain allows the individual to perceive the sound he is hearing (Levey et al., 2012).

## Development of NIHL

Most cell populations in the cochlea are vulnerable to noise damage. Noise can impair or kill: the pillar cells, which provide the structural support for the organ of Corti; the fibrocytes of the lateral wall, which allow potassium recycling necessary for the depolarization of the hair cells; and the outer and inner hair cells, which are vital for reducing hearing thresholds, discriminating between sound frequencies, and delivering auditory information to the brain. The synapses between the inner hair

cells and afferent auditory nerve neurons are also susceptible to noise damage, and damage to these synapses can result in long-term auditory nerve neuropathy (Bielefeld, 2015). An individual with NIHL will experience deterioration of the ciliated cells of the organ of Corti (Metidieri et al., 2013). Figure 4 shows the destruction of a region of the organ of Corti. The noise exposure induces overproduction of free radicals. While free radicals are often present in cells, they can be destructive when they are overproduced, resulting in an overpowering of the ear's antioxidant defense systems. These free radicals are made up of reactive oxygen species (ROS) (Levey et al., 2012). ROS are molecules with an odd number of electrons and thus highly reactive and destructive to the body. The accumulation of ROS causes damage to the mitochondria, which is the driving force of the cell and produces more than 98% of the energy used in our bodies. When ROS impair the mitochondria, they lose their capacity to produce energy. Ultimately, this reduces the amount of energy available for normal inner ear function, causing hearing loss (Seidman, 1999).

When the ear is exposed to excessive noise, the outer hair cells in the cochlea require high levels of energy and consume more oxygen. This generates a number of byproducts that mix with other molecules in the cochlea to generate high levels of ROS. This upsets the metabolic homeostasis of the cochlea. Additionally, the ROS then destroy cell membranes and hair cell nuclei, which causes tissue death (necrosis) and apoptosis (cell self-destruction), ultimately leading to hair cell death. Once it is dead, the hair cell will break into pieces, and this upsets the mechanical structure of the ear. Eventually, the hair cells are replaced with scar tissue. While the scar tissue ensures that the basilar membrane of the ear remains intact, it cannot play a role in the hearing process, and thus the individual with NIHL will experience hearing loss (Levey et al., 2012).

Cochlear microcirculation is vital for hearing transduction. Sensory hair cells of the cochlea are at risk of ischemia during noise exposure, and studies have shown that inadequate blood flow is associated with the development of NIHL. An important component of cochlear microcirculation is the diameter of the spiral modiolar artery, a branch of the anterior inferior cerebellar artery, which meets the lateral cochlear wall to form the striavascularis. The spiral modiolar artery has been shown to constrict during and after noise exposure (Arpornchayanon et al., 2013), and this is an additional cause of NIHL.

Another contributing factor in the development of NIHL is calcium homeostasis in the cochlea. Calcium plays the role of second messenger in a cell's physiological activities, and an upset calcium homeostasis has been linked to trauma-induced neuronal injury (Bao et al., 2013). In the cochlea, calcium participates

in a number of functions, including mechano-electric transduction, hair cells' voluntary contractions, the inner ear's frequency selection, basal membrane vibration, and the release of neurotransmitters (Liu et al., 2012). Calcium homeostasis is sustained through a number of different calcium channels, including voltage-gated calcium channels (VGCCs). VGCCs are comprised of two groups: high-voltage-activated (L-type) and low-voltage-activated calcium channels (T-type) (Bao et al., 2013). L-type VGCCs provide access for calcium to enter hair cells and are essential for the regulation of calcium homeostasis. After noise exposure, the levels of calcium in hair cells have been shown to increase, and an excessive amount of calcium in a cell has been linked to cell injury and cell death. Thus, too much calcium in the cochlea, brought on by excessive noise, has been linked to NIHL (Liu et al., 2012).

### Genetic link

Although there is much research about the link between environmental agents and NIHL, less is known about the genetic basis for the impairment. Various animal studies have provided significant evidence of a genetic predisposition for NIHL. Mitochondrial dysfunction and ROS production have been shown to play significant roles in neurodegenerative conditions. As ROS overproduction is a result of noise exposure, mitochondrial genes and endogenous antioxidant defense-related genes have been thought to be the genes associated with NIHL. There are a number of antioxidant enzymes at work in the cochlea. Glutathione-S-transferase (GST) enzymes catalyze the conjugation of glutathione with xenobiotics and are vital for antioxidant protection. GST is comprised of a number of gene classes (GSTA, GSTM, GSTP, GSTT, GSTZ, GSTS, GSTK, GSTO). Humans have genetic variability of GSTM1 and GSTT1. For example, approximately half of the European population has null genotypes for GSTM1, and 25–40% of Europeans do not have GSTT1. A person with a null genotype is unable to conjugate certain metabolites for these enzymes, and this makes him or her more susceptible to damage from oxidation. Therefore, it has been suggested that an individual with a null genotype is more likely than his peers to develop NIHL when exposed to excessive noise (Liu et al., 2012).

There are also genetic mutations that reduce susceptibility to NIHL. A1555G in MTRNR1 is the mitochondrial mutation most frequently linked to hearing loss, typically with aminoglycoside-induced deafness and non-syndromic deafness. MTTSI is another mitochondrial gene with a number of mutations. A1555G or other mitochondrial variants in the MTRNR1 and MTTSI have been suggested as factors that lessens susceptibility to NIHL.

Abreu-Silva et al. conducted a study to evaluate genetic factors

in susceptibility to NIHL. The researchers compared persons with NIHL and persons without NIHL, as well as their family history of hearing loss and whether they had genetic mutations that have been linked with hearing loss (including deletions in GSTM1 and GSTT1, A1555G in the MTRNR1 gene). Their sample was comprised of 255 bus drivers and workers at a printing facility in Saõ Paulo, Brazil. Both groups were regularly exposed to excessive noise. The subjects of the study included 107 people whose audiograms showed NIHL, 44 people with hearing impairment and audiograms that did not show NIHL, and 104 people with normal hearing. Data was collected regarding each subject's age, regularity of noise exposure, family history of hearing loss, alcohol consumption, smoking habits, and geographic origin of their ancestors. Subjects also provided blood samples for DNA screening and genotyping. The researchers found that family history of hearing loss is significantly associated with NIHL, which supports a genetic susceptibility to the impairment. Moreover, the researchers found that the existence of at least one allele in both genes (GSTM1 = 1/? and GSTT1 = 1/?) increases the risk of NIHL, and that individuals of mitochondrial haplogroup L1 were disproportionately affected by NIHL. However, no significant findings were made regarding variants in the MTRNR1 and MTT51 genes (Abreu-Silva et al., 2011).

Additional research on the genetic susceptibility to NIHL has focused on Human 8-oxoG DNA glycosylase I (hOGG1), a key enzyme in the human base excision repair pathway. NIHL occurs as a result of DNA damage to cochlear hair cells, causing necrosis and apoptosis. At the same time, a number of DNA repair pathways, including base excision repair, help to maintain the integrity of the cell and stave off cell death. This DNA repair has been shown to be essential in maintaining hearing after noise exposure.

The 8-oxoguanine (8-oxoG) DNA lesion, which is caused by the overproduction of ROS, is a common cause of oxidative damage associated with NIHL. HOGG1 in the base excision repair pathway eliminates 8-oxoG. Research has suggested that the Ser326Cys polymorphism (rs1052133) in exon 7 of hOGG1 gene can impact the activity of hOGG1 enzyme and thus may be useful as a genetic marker for vulnerability to different diseases. Shen et al. conducted a study to evaluate whether there is a link between the hOGG1 Ser326Cys polymorphism in the human base excision repair pathway and susceptibility to NIHL. The researchers genotyped 615 workers with NIHL and 615 normal hearing workers in China. The workers had not been regularly exposed to any other causal agents of NIHL, like chemical solvents, heat, or vibrations, and they had no other medical conditions that could have impacted their hearing.

The researchers found that the hOGG1 Cys/Cys genotype was statistically associated with NIHL. There was a higher number

of persons with the hOGG1 Cys/Cys genotype among NIHL workers than among normal hearing workers, suggesting the hOGG1 Cys/Cys genotype may have a lesser capacity for restoring 8-oxoG damage, and thus a higher susceptibility for NIHL. The researchers also found that the hOGG1 Ser326Cys polymorphism had a synergistic effect when combined with noise exposure time, noise exposure level, whether the individual is a smoker, and whether the individual drinks alcohol regularly (Shen et al., 2014).

### Diagnosis

Early detection of NIHL would help decrease the social and economic burdens related to the condition (Meinke et al., 2013). Moreover, early detection can help stop the progression of hearing loss and prevent damage to speech frequencies (i.e., 500, 1000, 2000 and 3000 Hz) (Mehrparvar, 2011). NIHL is diagnosed when an individual is found to have a hearing level change, compared to a baseline audiogram, of an average of 10 dB or more at 2000, 3000, and 4000 Hz in either ear (Levey et al., 2012). Generally, NIHL results in hearing loss in the higher frequencies (3000-6000 Hz), so during hearing tests, hearing loss is observed at 4000 Hz with improvement in hearing at 8000 Hz. However, among elderly patients, the improvement at 8000 Hz is often lost, and this presents difficulty in determining whether the hearing loss is from NIHL, age, or another cause. Tympanometry is used to test the condition of the middle ear. Asymmetric hearing losses are evaluated using a brainstem auditory evoked response or a high resolution CT scan of the inner ear and internal auditory canal (Seidman, 1999).

Researchers are currently evaluating a potential new means of diagnosing and monitoring NIHL known as distortion product otoacoustic emission (DPOAE) level mapping. The goal of DPOAE is to detect early signs of cochlear damage and then track changes in cochlear function, but the maps have mostly been used in trials with animals. A study was conducted in which they performed DPOAE level mapping on 17 individuals without a history of noise exposure and then compared the results to maps created with 19 individuals with mild moderate NIHL. The DPOAE maps presented accurate data in a color pattern, a "visual snapshot" of cochlear function between the frequencies of 0.5 kHz and 6.0 kHz. This graphic form of data presentation enables for more detailed data collection and supports rapid comparison of maps for an individual. With further research, DPOAE maps may become the standard tool for detecting and monitoring NIHL (Meinke et al., 2013).

### Prevention

NIHL is not a new phenomenon. Reports from as early as 600 B.C. tell of the Sybarites, who prohibited metal-working or the possession of roosters within their city limits due to the dangers

of excessive noise. Other historical documents show that individuals living near the waterfalls of the Nile River experienced more hearing loss than those at a greater distance from the waterfalls' noise (Seidman, 1999). NIHL is an impairment that is both avoidable and foreseeable (Metidieri et al., 2013). The implications of NIHL can extend beyond hearing loss; it can result in the development of a range of secondary diseases, including hyperacusis (over-sensitivity to certain frequency and volume ranges of sound), tinnitus, or depression related to social isolation (Tziridis et al., 2014).

The federal government has long sought to regulate noise exposure and prevent NIHL. In 1936, the Walsh-Healey Public Contracts Act was instituted to oversee workplace noise exposure, though it was not properly enforced. In 1970, the Occupational Safety and Health Administration (OSHA) was founded to both monitor and regulate workplace noise exposure. Currently, OSHA allows for exposure to 90 dB for 8 hours a day, while the EPA allows for a level of 85 dB. However, on-the-job inspections are typically infrequent, and fines for noise-level violations are often low.

The key to preventing NIHL is avoiding damaging noise exposure (Seidman, 1999), beginning with an assessment of risk of an environment. It is advisable to have workers rotate tasks to ensure that no individual is exposed to continuous hazardous noise over an extended period of time (Singh et al., 2012). Additionally, acoustic attenuation devices or engineering controls can be used to reduce the noise dose to which an individual is exposed (Bielefeld, 2015). If sounds often go above 85 dB, hearing protection is essential to preventing damage. OSHA mandates the distribution of free hearing protective devices (HPDs) in workplaces such as these. Protective devices may include ear plugs or ear muffs (Pelosi, 2014). Earplugs can reduce between 10 dB and 22 dB of sound diminution, and protective earmuffs can reduce sound by 20-55 dB. When earplugs are used together with earmuffs, the benefit is additive (Seidman, 1999).

However, there are a number of barriers to the use of HPDs. First, many work sites require ongoing communication with colleagues, and this is hindered by the use of ear protectors. Additionally, Reddy et al. (2012) conducted surveys with workers in high-noise environments, and their findings suggest that there is a "fatalist belief among workers that noise is an acceptable and unavoidable part of the job." This perception often leads workers to avoid using HPDs, not recognizing that these devices can prevent permanent hearing loss (Reddy et al., 2012). Hong et al. (2013) explain that the perceptual factors involved in HPD use include perceived self-efficacy, perceived benefits, perceived barriers, perceived susceptibility to hearing loss, and perceived severity of hearing loss. In a study conducted by Nodoushan et al. (2014), HPDs were found to be used more frequently and more

effectively when workers received training about the reasons for HPD use, as well how they can be properly fitted in the ear.

In addition to HPD use, scientists continue to investigate a number of less conventional approaches to reducing the damage from noise exposure. Experimental methods for preventing NIHL include reducing body temperature, stimulating a number of neural structures, treatment of the ear with a number of compounds, increasing oxygenation, interfering with ROM activity, and sound conditioning. In some cases, these alternative approaches have achieved partial protection. In particular, the concept of sound conditioning has received a lot of attention from scientists. Conditioning allows for the toughening of the cochlea through exposure to non-damaging levels of sound before being exposed to noise trauma. Resistance can develop after the exposure to stimuli that varies in duration, frequency, and continuity. This is due to brain and nerve adaptability; during sound conditioning, the cochlea's outer hair cells perform similarly to a muscle (Seidman, 1999).

### Treatment

Research has also focused on the use of pharmacological agents in the prevention and treatment of NIHL. While there is no widely-accepted protocol for pharmacological treatment of NIHL, there are a number of ongoing research studies that are testing substances on both animals and humans, including antioxidants that decrease oxidative stress by eliminating ROS; glucocorticoids; and agents that improve cochlear blood flow, stimulate inhibitory transmitter systems, or obstruct apoptosis pathways in hair cells (Tziridis et al., 2014).

There are a number of antioxidants currently being tested for their efficacy in both preventing and treating NIHL. The antioxidant  $\alpha$ -tocopherol has been shown to reduce the noise-induced damage in guinea pigs (Kashani et al., 2011). N-Acetyl-L-Cysteine (NAC), an antioxidant which has been shown to inhibit lipid peroxidation and destroy ROS by increasing levels of glutathione within the cell, has been found in a number of animal studies to be effective in treating NIHL if given immediately after noise exposure (Rajguru, 2012).

Antiepileptic drugs and glucocorticoids are also being tested in the treatment of NIHL. Bao et al. conducted a study regarding prophylactic treatment of NIHL. Their study sought to reduce the risk of NIHL by administering anticonvulsant drugs to block T-type calcium channels in the organ of Corti, and thus prevent an injurious disturbance to calcium homeostasis that may occur after exposure to noise. This approach had been found to be effective in protecting neurons after a stroke. The researchers administered to mice two anticonvulsant drugs - ethosuximide and zonisamide - to block T-type

calcium channels, and then exposed them to high levels of noise. The mice were also administered synthetic glucocorticoids, dexamethasone and methylprednisolone (Bao et al., 2013). Glucocorticoids were used because they activate the enzyme Na, K-ATPase, which has been linked to restoring cellular osmolarity and neuronal conduction. Additionally, glucocorticoids have been found to be effective in both cells and dendrites (Rajguru, 2012).

In Bao et al.'s (2013) study, the anticonvulsant drugs and the glucocorticoids were administered after the noise exposure as well to assess whether the drugs had any therapeutic effect after noise-induced trauma. The researchers found that anticonvulsant and glucocorticoid drugs could have both a prophylactic and therapeutic effect, and that there was a synergistic prophylactic effect when the drug families were used together (Bao et al., 2013). Further studies are necessary to ascertain whether the drugs would have the same effect in preventing and treating NIHL in humans.

Vitamins and minerals are also being tested in the prevention and treatment of NIHL. Studies show that magnesium has both a prophylactic and therapeutic effect on noise-induced injuries. In the cochlea, levels of magnesium are essential for preventing disruption in cochlear microcirculation and oxygenation and for protecting against free radicals. Clinical trials are ongoing to assess whether application of magnesium to the inner ear, together with the administration of an antioxidant, can prevent and reduce hearing damage after a noise-related trauma (Rajguru, 2012).

Tziridis et al (2014) conducted a study on the use of ginkgo biloba extract EGb 761 to prophylactically treat NIHL and tinnitus in the Mongolian gerbil. Their study results demonstrate the neuroplastic effects of EGb 761 on central and peripheral levels of auditory processing when given prophylactically to gerbils exposed to noise. The researchers posit that the antioxidant effect of EGb 761 provides protection against NIHL. Additionally, EGb 761 was shown to increase blood flow, so it provides a protective effect against noise exposure, which can decrease blood flow in the cochlea. While EGb 761 has not yet been tested on humans, the results of the gerbil study are promising (Tziridis et al., 2014).

Another agent being tested in the prevention and treatment of NIHL is vitamin B12. Vitamin B12 is an essential cofactor in methylation of myelin basic protein and cell membrane phospholipids and also important in methionine synthesis from homocysteine. There have been studies that have shown that individuals who have low levels of vitamin B12 and folic acid are more susceptible to hearing loss caused by noise exposure. When there is a B12 deficiency, there can be axon degeneration and neuronal loss. Kibar et al. (2013) theorize that supplementary B12 can decrease

homocysteine synthesis in the cochlea, increase concentration of nitric oxide, and thus cause the blood vessels to dilate. As such, the B12 may be able to induce increased vascular perfusion and cellular metabolism within the cochlea. In a study Kibar et al. (2013) conducted with guinea pigs, they were not able to show a significant decrease in hearing loss when the guinea pigs were treated with B12. However, studies are ongoing to determine the efficacy of B12 treatments in preventing and minimizing the damage of NIHL (Kibar et al., 2013).

Hyperbaric oxygen therapy (HBOT) is also being considered for its use in treating NIHL. HBOT can be used to improve oxygenation in the inner ear, and thus improve microcirculation and attenuate injured cochlear hair cells. HBOT can infuse hypoxic areas of the cochlea with oxygen, which can work to speed up cellular mechanisms necessary for recovery. There are ongoing studies that are evaluating the impact of HBOT on NIHL, as well as the combined use of HBOT and glucocorticoids (Rajguru, 2013).

### Conclusion

Though the effects of noise exposure have been known within the medical community since the 1960s, the general public remains largely unaware of the long-term effects of both sudden and gradual noise trauma. In a study conducted with American respondents, only 16% of those surveyed reported that they had read about or heard about NIHL in newspapers or other media forums. In another American-based study, 72% of respondents admitted that they never wear hearing protection when they are exposed to loud noises, even though they are aware that noise exposure can be harmful (Ramma, 2011).

It has been said that an ounce of prevention is worth a pound of cure, and this maxim is especially relevant in regards to NIHL. It is essential that the general public is made aware of the damaging effects of noise exposure, both on the job and during recreational activities. While studies related to the prophylactic and therapeutic intervention for NIHL are promising, there is currently no widely-accepted treatment protocol and no known cure. As such, it is imperative that OSHA and EPA guidelines are adhered to in all areas of work, and that HPDs are employed during occupational hazards and leisure activities. Moreover, it is essential that people recognize that their ears are vulnerable to injury, and they should be protected from unnecessary – and often irreversible – damage.

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# The Mechanisms of Weight Gain in Sleep-Deprived Individuals

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## Abstract

The obese population in America has grown during the last century. During these years as well, Americans have been sleeping less. Cross sectional studies show that there is a correlation of the two factors, and indeed find a greater number of overweight individuals amongst the sleep-deprived population. Though they are unclear, studies attempt to establish possible mechanisms through which weight gain occurs. Results of studies show that sleep deprivation may influence leptin and ghrelin levels, which can cause hunger, and excessive caloric intake. Sleep-deprived individuals also have an increased opportunity to eat during the wakeful nighttime hours. In the sleep-restricted state, activity levels may decrease, and though the extra hours spent awake also cause a small increase in energy expenditure, there is over-compensation in energy intake, leading to weight gain. Obstructive Sleep Apnea also plays a role in obese individuals' sleep patterns, and may lead to further weight gain. Without excessive caloric intake it may be possible to maintain weight in the sleep-deprived state. This paper will review a number of studies and give an overview of some of the possible methods through which sleep-deprivation causes obesity.

## Introduction

Sleep was once thought of as a process during which the both the body and brain are at rest and inactive. But in modern times, starting in approximately 1950, sleep has been studied and discovered to be an active process, necessary to maintain many of the body's functions (National Institute of Neurological Disorders and Stroke, NINDS, 2014).

In 1937, Alfred Loomis, along with E. Newton Harvey, and Garret Hobart, used the EEG to map out what they considered five stages of sleep, A-E. In 1953, Eugene Aserinsky and Nathaniel Kleitman established the REM sleep stage. In 1957 Dement and Kleitman mapped out the sleep stages as they are known today, dividing sleep into NREM (non-rapid eye movement) and REM (rapid eye movement) sleep. NREM sleep consists of four stages, each with varying brain wave activity, and increasing in depth as the stage number increases. These stages are cyclical, repeating throughout the night. Following the four stages, there is a period of REM sleep (Shepard et al., 2005). REM sleep is associated with rapid eye movements, increased heart rate, and dreaming (NINDS, 2014).

With vast research and many studies conducted since, the understanding of the roles that sleep plays in the body has been greatly advanced and its necessity has been studied extensively (Shepard et al., 2005).

Sleep is both a circadian rhythm and a homeostatic cycle. A circadian rhythm is natural rhythm, approximately twenty-four hours long, and controlled primarily by the suprachiasmatic nucleus. The natural timing of the cycle is based on the light-dark cycle (NIGMS, 2012). Though they are naturally occurring, circadian rhythms can be altered and affected by environmental factors. One of the circadian rhythms is the body's drive for alertness and wakefulness, which gets higher throughout the daytime hours and decreases during the night. The circadian sleep-wake cycle, along with the body's homeostatic drive for sleep, which increases based on the

amount of prior wakeful hours, keep the body in a proper rhythm, maintaining the appropriate amount of necessary sleep (Dijk and Lockley, 2002).

The necessary amount of sleep in humans varies with age, ranging from 16–18 hours in infancy to 7-8 hours in the average adult (NINDS, 2014). In the last century, America has seen an approximate 20% decrease in the amount of sleep it gets. This has had economic, social, and health consequences. Sleep deficiency has been associated with numerous health risks such as hypertension, stroke, obesity, and diabetes. Sleep is necessary to support many vital systems in the body, and adequate sleep is necessary for optimal cognitive performance, without which many accidents may occur (Institute of Medicine (US) Committee on Sleep Medicine and Research, 2006).

Parallel to the decrease in sleep, the incidence of obesity in America has drastically increased. Studies conducted by the National Health and Nutrition Examination Survey (NHANES) show that more than one third or 34.9% of adults over age 20 are considered obese. Additionally, of American children and adolescents ages 2–19, approximately 16.9% are obese (Ogden et al., 2014). Obesity is a result of excessive weight gain, which occurs primarily when the energy expenditure of the body is less than energy intake, resulting in a net gain. Over time, if the gain continues, the weight accumulates and results in obesity. Other factors that may cause obesity are environmental factors, genetics, illnesses, and medications (CDC 2011).

The correlation between sleep deficiency and weight gain has been examined and many cross sectional and epidemiological studies found a relationship of sleep deprivation with obesity. Studies show an association of a higher body mass index (BMI) in individuals who generally sleep fewer hours than the recommended sleep time. Over all, the studies point at a relationship

of sleep deprivation to obesity primarily in the pediatric and young adult population (Beccuti and Pannain, 2011). Though these results were most evident in the young and middle-aged population, one study found that there is a greater likelihood (3.7 times in men and 2.3 times in women) for obesity in older individuals who sleep less than 5 hours per night (Patel et al., 2008). Though the data is evident of a correlation between the two, there are many factors that may contribute to the obesity and the findings are not entirely conclusive (Patel and Hu, 2008). This paper will investigate the mechanisms involved in sleep deprivation that result in weight gain and obesity?

### Methods

Information was obtained online with access to many online publications through the Touro College library. Key words included sleep-deprivation, obesity, weight gain, leptin, ghrelin, energy expenditure, OSA. Additionally, references made by literature reviews were helpful in conducting research.

### Discussion

Many studies have been performed in an effort to establish the relationship of decreasing sleep with increasing likelihood of obesity. The studies examine whether the shortened sleep times have an effect on weight, and via which mechanisms this may occur. Three commonly discussed factors that may contribute to the weight gain in sleep-deprived individuals are energy intake (consumption of food), energy expenditure, and hormonal leptin and ghrelin levels.

### Leptin and ghrelin

Leptin and ghrelin are two essential hormones in the maintenance of the body's energy balance. These two hormones have opposite effects on the body. Leptin induces a satiety and fullness, and ghrelin, hunger and appetite. Leptin is primarily released by adipose tissue into the bloodstream and send the brain signals regarding the energy state of the body. Leptin signals receptors at the hypothalamus, which affect hypothalamic neurons and various neuropeptides. Leptin is known to have anorexic (appetite suppressing) effects. Ghrelin, is released primarily by the stomach and signals the hypothalamus, which thereafter has orexic (appetite inducing) effects (Klok et al., 2007). Because of the correlation found between sleep and weight gain, it is presumed that perhaps leptin and ghrelin, which are closely related to food intake and regulation, are affected by sleep restriction. Studies examine this detail, and show inconsistent results.

One study that examined the sleep-weight gain relationship was performed on 12 average men, 22 +/- 2 years of age, and with average BMIs 23.6 +/- 2 kg/m<sup>2</sup>. The participants did not smoke or take any medication. They all regularly slept 7 to 9 hours, and they had not crossed time zones in the last four weeks before the study.

The study consisted of two randomized periods, six weeks apart. Weight did not change during the time between the two studies. For the first night of study, participants were given a restricted diet, while during the day, they ate their regular diets. During the second day of study, they received a glucose infusion of 5 g/kg/ 24 hours. After both periods of the study- two nights of 10 hours in bed, and two nights of 4 hours in bed, blood samples were taken at 20-minute intervals, from 8 am to 9 pm. Results showed that in the sleep deprived state, leptin levels were 18% lower and ghrelin levels 28% higher than in the normal sleep state (Spiegel et al., 2004). These results accounted for the elevated hunger levels in the sleep-deprived state. Consequently, increased hunger usually causes increased food intake. However, these studies did not measure exact food intake of the participants, so there is no clear evidence of the increased energy intake.

Another study performed on 1,024 volunteers in the Wisconsin Sleep Cohort Study found a decrease in leptin levels with sleep deprivation. They underwent polysomnography and answered questionnaires to measure sleep. In the sleep-deprived state, (5 hours versus 8) a morning blood test showed a 15.5% decrease in leptin and 14.9% higher ghrelin levels (Taheri et al., 2004). Consistent with the findings by Spiegel et al. these results can likely cause increase in hunger and food intake, leading to weight gain.

Though these results show a clear effect of sleep on leptin and ghrelin, not all studies yielded such results. Numerous reasons may be responsible for the discrepancy in the results. One reason might be that in the study by Spiegel et al., the blood samples were taken after participants underwent constant glucose infusion, which could perhaps yield results that differ from those of a regular food diet.

Another study was in search of a relationship of partial sleep deprivation to weight gain and insulin sensitivity in women. It was performed on 14 healthy women, age 23-38, who were weight stable, with no recent illness, non-smoking, non-lactating, and not on any medication besides oral contraceptives. They were all in the follicular phase of the menstrual cycle, slept 7.5 to 9 hours on an average night, did not cross time zones in last month, and had no sleep complaints. Participants were studied after 2 nights of 8 hours of sleep, after 4 nights of consistent decrease in sleep (minus one additional hour nightly) and then 2 nights of recovery sleep. Food was not restricted, though alcohol was. Their diet was carefully recorded using computer software. After performing a glucose tolerance test, blood was collected in 15-minute intervals for 90 minutes. Leptin levels showed a 24% increase after 2 nights of approximately 5.5 hours in bed versus 9 hours in bed. Ghrelin levels were stable. This may be due to the extra caloric intake during those days of study, as will be discussed below. Insulin

sensitivity did not appear to be affected by sleep restriction (Bosy-Westphal et al., 2008).

Numerous other studies also yielded results that were inconsistent with each other. Certain studies show increase in leptin, others show a decrease, while others do not show any significant change.

One study was performed on eleven people, men and women, with an average BMI of 26.5, for two two-week periods in random order. In the restricted sleep they slept 5.5 hours, compared to 8.5 hours in the habitual sleep. They had an unrestricted diet throughout the study. The results showed that in the sleep deprived state, leptin and ghrelin levels were not affected significantly. These results show that perhaps in response to sleep loss, the body may have an altered response to restricted food, and the hormonal levels remain more stable than in the body's usual state. This would account for the stable leptin and ghrelin levels across the two periods of study (Nedeltcheva et al., 2009).

One other study observed that in nine average weight men after one night of normal sleep, one of partial sleep restriction and one total sleep deprivation, leptin levels did not show a significant change, while ghrelin levels were 22% higher in total sleep deprivation than in normal sleep (Schmid et al., 2008).

Clearly, many studies yield conflicting results on the effect of sleep on leptin and ghrelin. Two studies we discussed show entirely opposite results (Spiegel et al. 2004 and Bosy-Westphal et al., 2008). Why are the observations to the various studies inconsistent?

Perhaps it is the fact that certain factors were sometimes unaccounted for in these particular studies, such as genetics, individual's environment, race, and height (Spiegel et al., 2004). Additionally not all of the studies measured and accounted for precise weight of the participants, which is perhaps necessary to determine the effect of one factor on another. Additionally, as mentioned above, the diets of the participants in the studies were not consistent with each other, which may have had an effect on the body's endocrine response.

The inconsistent results of the many studies suggest that the leptin and ghrelin levels may or may not change and contribute to weight gain in response to sleep restriction. However, even studies that do not show a direct change in leptin and ghrelin, do show greater caloric intake, likely due to hunger and increased opportunity to eat.

The effect that sleep has on energy expenditure must also be taken into account. Intuitively, in the sleep-deprived state,

because of fatigue, energy expenditure should decrease. However, studies show that a sleep deprived individual experiences extra wakeful hours during which he/she is burning more calories than if he/she had been sleeping. However, because during those wakeful hours the individual is likely fatigued, activity levels are lower than regular wakeful hours. Therefore, the overall energy expenditure in the sleep-deprived state may increase only slightly.

### Energy intake versus expenditure

To maintain weight one must be in energy balance, when energy consumed is equal to energy expended. Weight gain occurs when the body is in positive energy balance, when the person takes in more calories than they burn. Perhaps even without an observed hormonal change, the body reacts to sleep deprivation with increased hunger, and thus increased caloric intake. Sleep may also have an effect on metabolism and energy expenditure? Many of the studies mentioned above, searched for the effect of sleep deficiency on hunger levels, energy intake, and energy expenditure, in addition to its role in leptin and ghrelin levels.

According to Spiegel et al. participants recorded their hunger levels after both periods of the study- restricted and normal sleep times- by answering how hungry they felt at every hour from 9 am to 9 pm, after the second night of sleep. They were also asked to describe their appetite, answering how much they would like to eat 7 different foods (sweet, salty, starchy, fruits, vegetables, meat, dairy etc.) Results showed that consistent with the decreased leptin and elevated ghrelin levels found in this study, 24% higher hunger levels were found, as well as 23% higher appetite levels, in the sleep deprived state. The appetite increase was highest for calorie and carbohydrate dense foods. Increased appetite for protein rich foods was not significantly increased in sleep-deprived state (Spiegel et al., 2004). The study by Schmid et al. also showed an increase in average hunger ratings of 3.9 after total sleep deprivation as opposed to 1.7 after a 7-hour time in bed, consistent with increased ghrelin (Schmid et al., 2008).

Presumably, the increase in hunger accounts for greater caloric intake in sleep-deprived individuals, causing weight gain. However, in the studies by Spiegel et al. (2004) and Schmid et al. (2008), exact caloric intake during the study periods was not measured, and thus we cannot necessarily assume that there was extra consumption of calories or how many. Additionally, during the study periods, activity levels were kept low (reclining, sitting etc.), and they did not examine whether or not there is a change in energy expenditure or energy balance. Without this information we cannot determine if there would be a theoretical net weight gain. To examine these

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details, further study on energy expenditure is needed.

Energy expenditure can be measured using indirect calorimetry, metabolic chambers, heart rate and other methods. Indirect calorimetry and metabolic chambers measure the level of carbon dioxide production to assess energy expenditure.

In the study by Bosy-Westphal et al. careful measurement of caloric intake was measured during the entire study via computer. Resting energy expenditure was measured using indirect calorimetry, and total energy expenditure was measured using 24-hour heart rate monitoring. Physical activity was also measured with pedometers. Energy intake significantly increased from baseline to sleep deprived state, while energy expenditure did not show a significant change. The average increase in calories was 415 +/- 471 kcal/day, and caused a net weight gain (Bosy-Westphal et al., 2008). Because there was no change in energy expenditure seen, we can assume that in prolonged sleep deprivation the individuals would experience significant weight gain. This provides further evidence that sleep deprivation causes an increase in caloric intake and body weight, despite the fact that there was no significant endocrine response to sleep deprivation.

However, the study did observe a change in thyroid hormone levels, so further research is required to examine more specific results of the effect sleep restriction may have on energy expenditure, such as measuring total energy expenditure using a caloric chamber (Bosy-Westphal et al., 2008).

This leads us to another study that did indeed measure specific total energy expenditure, using a caloric chamber. The study shows a significant increase in energy expenditure in the sleep-deprived state due to the extra hours of time spent awake. Ten women, with average BMIs were studied under shortened sleep and habitual sleep, 4 and 8 hours, 3 days of each, while kept on a strictly controlled diet. The results showed significantly greater energy expenditure of approximately 92 kcal in the shortened sleep period. This study provides evidence that there may be an increase in energy expenditure in the sleep deprived state, however, there is an overcompensation of energy intake, which leads to weight gain (Shechter et al., 2013).

Another study that measured energy intake versus expenditure found that with unlimited food allowance, the participants did not consume a significant amount of extra calories during meals, however, they did increase their intake of snack calories between the hours of 7pm-7am, with more carbohydrates and less protein/fat snacks (Nedeltcheva et al., 2009). The fact that the increase was only significant between

7pm-7am, not 7am-7pm, highlights the fact that during the nighttime hours when sleep is restricted participants found time to consume significantly more snacks, leading to an increase in daily calories.

When one is sleep deprived and awake during nighttime hours, not only is there elevated hunger during the day, but there is also increased opportunity to eat during the extra hours spent awake. More so, the nature of nighttime eating is snacking, which can cause unintentional excessive caloric intake.

The total energy intake was 297 kcal more in sleep-deprived state. Additionally, during both sleep deprived and habitual sleep state, there was a surplus of calories, due to the participants' eating habits. Some individuals had a greater 'propensity' to eat, while others not. The study also found a slight change in energy expenditure between the two sleep states. The difference, which they considered insignificant, was 136 +/- 437 kcal more per day in the restricted sleep period (Nedeltcheva et al., 2009). Even if the change in total energy expenditure was considered significant, there is a surplus of an average 160 kcal per day in the sleep-deprived state. If we were to apply this information to a general population one can assume that the overcompensation in calories would cause weight gain in individuals who are consistently sleep deprived.

Perhaps if participants were more active, the studies would find a significant increase in energy expenditure, and there would no longer be the overcompensation of calories. However, because of fatigue and sleepiness, activity levels should presumably be lower when one is sleep-deprived. Studies show that the activity levels of sleep-deprived individual are indeed lower.

Another study involved 30 men and women, ages 30-49, and with BMIs between 22-26. No shift workers, or individuals that had travelled across time zones within 4 weeks of the study were allowed. Smokers, diabetics, those with excessive caffeine intake, or neurologic issues were also disqualified. The study was divided into two stages of 6 days each. In the shortened and normal sleep stages, the participants were given 4 hours in bed and 9 hours in bed, respectively, with no naps during the day. They measured sleep by polysomnography. For the first 4 days of each period, their food intake was allotted and strictly measured. The last two days were also carefully measured, but participants were allowed to make their own food choices. On day 4 they were asked how they would rate their hunger, how full they felt, how energetic, how much they could eat, and how much they would like to eat

foods that were sweet, salty, savory etc. After measuring the energy intake and expenditure, results showed that women consumed 15.3% more in the restricted sleep period, with a 39% increase in fats. Men consumed 9.2% more. Additionally they ate more often in the sleep-restricted stage. While they consumed more, no significant change in energy expenditure was found, though the point of peak activity observed was higher in the normal sleep period. In the normal sleep period, participants spent a greater percentage in sedentary activity, a lesser percentage in light activity, and a significantly greater percentage of time doing heavy activity, than in the restricted sleep period. Overall the study showed greater feelings of sluggishness and less energy during the shortened sleep period and there was no significant change in total energy expenditure over the course of the study (St.-Onge et al., 2011).

This is intuitive, as a sleep-deprived individual is tired and consequently has less energy for intense activity. Low intensity activity leads to less energy expenditure, and opportunity for more weight gain.

Surprisingly, there was no net change in energy balance observed, after controlling for phase order and gender. However, like Nedeltcheva et al., this study showed an increase of approximately 300 kcal per day in the shortened sleep period. Due to the fact that they are primarily due to the increase in fat and saturated fat calories, the people would also be at risk for cardiovascular disease (St-Onge et al., 2011).

Even though, unlike previous studies, this study did not show a significant effect on participants hunger ratings, there was an observed increase in energy intake. This suggests that the relationship of sleep deprivation and obesity is one primarily based on excessive food intake. The continuous surplus of calories in the sleep-deprived state may eventually lead to obesity, if not coupled with an increase in energy expenditure (St-Onge et al., 2011).

### **Obstructive Sleep Apnea**

There is reason to believe that perhaps the relationship of poor sleep and obesity is a reverse process. The cross sectional data showing a relationship between obesity and sleep deprivation may be due to the fact that as a result of being overweight, obese individuals experience shortened sleep, with poorer quality. This is indeed true in the case of Obstructive Sleep Apnea (OSA). Obese people are twice as likely to experience Obstructive Sleep Apnea, a significantly higher risk, than average weight individuals. OSA occurs in approximately 45% of obese individuals. Additionally, their risk for OSA is increased six times, if they gain even 10% of their normal (baseline) weight. On the other hand, the OSA can show an improvement with weight loss of

the same amount. One reason that obesity increases the risk for OSA is that fat situated in the upper airways can cause an increase in the collapsibility of the airway, and increase the risk for apnea. A patient with sleep apnea has interrupted sleep and decreased sleep quality. This leads to sleep deprivation, and the sleep deprivation leads to additional weight gain. The process is cyclical. An obese individual is at risk for OSA. OSA causes sleep deprivation and an increased risk for weight gain. The weight gain causes further worsening of OSA symptoms. Studies propose that weight loss can effectively help the obesity, as well as help improve symptoms of OSA (Romero-Corral et al., 2010).

In light of the above, we can say that the relationship between sleep deprivation and obesity is indeed cyclical. However, as our discussion shows, even amongst the average weight population, individuals who are sleep deprived tend to increase energy intake and gain weight.

### **Further Research**

Though studies have established correlation of the above factors, further research would be helpful to provide more conclusive evidence of the mechanisms involved in sleep deprivation that cause obesity. Discrepancies amongst the studies may be due to environmental factors, genetic predisposition, and metabolic differences that vary with each individual participant. Additionally as mentioned above, participants each have varying propensity to eat more, and gain or lose weight, independent of sleeping habits. Furthermore, the studies discussed were performed on relatively small samples of people. Additional studies with a greater number of participants, and in a broader population of ages could perhaps provide more conclusive results. Moreover, perhaps there is a way to study populations in a free-living environment, rather than in a lab setting, and assess under those circumstances, which mechanisms resulting from sleep deprivation lead to the development of obesity.

### **Conclusion**

Obesity is an epidemic that has many economic, social, and health costs. The effects it has on health and healthcare costs are tremendous. Obesity not only requires treatment itself, but it also leads to many health problems such as cardiovascular disease, hypertension, type two diabetes, stroke, osteoarthritis, gallstones, certain types of cancer, fertility problems in women, as well as other health issues (Kopelman, 2007).

Studies show that if the growing obesity epidemic continues, 86.3% of American adults will be overweight or obese, with 51.1% obesity by year 2030. This could lead to health care costs projected at 860.7-956.9 billion dollars (Wang et al., 2008). Obesity also has many social costs, amongst adults and children alike. Obese people may experience limitations on

physical activity, and there are stigmas associated with being heavily overweight that may cause obese people to be treated differently in the workplace and social forums (Seidell, 1998). In light of the above, if sleep deprivation has a role in the growing obese population, not only is intervention necessary to treat obesity and its side effects, but there must also be intervention to counsel people regarding positive sleeping habits. Beginning with school aged children, they must be educated regarding the benefits of getting enough sleep. Parents should try to assist children in creating healthy sleeping habits. Adults should be careful to try to generally maintain a proper sleep schedule, when social and work responsibilities do not demand late bed times. Overall, a greater awareness must be made to the public that sleep deprivation has serious negative side effects.

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# Biological Reasons for the Neurotoxic Effects of MDMA ('Ecstasy') on the Developing Fetus

By Leah Schneeweiss

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## Abstract

MDMA (3, 4-methylenedioxymethamphetamine) is an illicit, recreational drug known by many individuals as 'Ecstasy.' MDMA has gained popularity over the past decade and has become a drug of choice at dance parties and clubs because of the stimulating and hallucinogenic effects that it has on the central nervous system. Scientists have determined that MDMA causes neurotoxic damage to adults by harming the serotonergic system in the adult brain. Researchers discovered that embryos exposed to MDMA while in utero also suffer neurotoxic deficits, although not due to impairments in the embryos' serotonergic systems. These deficits arise because of the cortisol increase that is found in adults after ingestion of MDMA, which can be transmitted to a developing fetus and thereby lead to a reprogramming of the hypothalamic-pituitary-adrenal axis in the fetus. In addition, MDMA can cause changes in the norepinephrine and/or dopamine systems in a developing fetus and thus create lasting neurological damage. MDMA-induced elevation of Atg5, a protein involved in autophagy, leads to teratogenesis in a developing fetus by inhibiting neuronal growth and differentiation. In another vein, 3, 4-Dihydroxymethamphetamine (HHMA) and malondialdehyde (MDA), two main metabolites of MDMA, have toxic effects on an embryo and are another mechanism via which Ecstasy can cause impairments in the fetal brain. While these and other hypotheses are currently under much investigation, scientists are approaching this topic with the understanding that it is most probably an interplay of many biological changes that result from fetal exposure to MDMA that together create the neurological defects observed in these fetuses. The various aspects of MDMA and the damage it can have on a fetus have been researched by the author using the Touro database and various links to journals and articles that this database provided.

## Introduction

MDMA, known scientifically as 3, 4-methylenedioxymethamphetamine, is an amphetamine derivative drug increasing in popularity amongst teenagers and young adults (Broening, et al., 2001). Amphetamines are a class of molecules that have stimulating effects on the central nervous system (CNS). MDMA is known by recreational users as 'Ecstasy' because it has stimulating and hallucinogenic effects on the CNS while engendering immediate feelings of ecstasy and openness upon ingestion (Singer, et al., 2012).

Studies show that adults taking MDMA have a decrease in serotonin (5-HT) in the brain, a reduction in 5-hydroxyindoleacetic acid (5-HIAA), a main metabolite of serotonin, and a reduction in tryptophan hydroxylase (TPH), an enzyme involved in the synthesis of serotonin (Broening, et al., 2001). This serotonergic reduction is harmful to the exposed adult. Interestingly, though the effects of MDMA on adults have been well researched, few studies have been conducted on the effects of MDMA on prenatally exposed children and on the reasons for these effects. Due to its popularity amongst teenagers and young adults, it has become increasingly prevalent to find developing fetuses exposed to MDMA while in utero. Scientists are determining the long term neurological effects of Ecstasy-exposed fetuses by conducting post parturition studies on both rats and children that have been prenatally exposed to MDMA. Some long term neurotoxic effects include sequential and spatial learning delays (Broening, et al., 2001) milestone delays at 4 months of age, and poorer mental development index scorings at 12 months of age (Singer, et al., 2012). The question stands as to why these and

other neurotoxic effects develop in both rats and children that have been exposed to MDMA in utero. Scientists are hypothesizing numerous reasons for these effects, including serotonin (5-HT) reduction (Broening, et al., 2001), elevated levels of cortisol (Parrott, et al., 2014), changes in the norepinephrine (NE) system (Thompson, et al., 2011) and in the dopamine (DA) system (Thompson, et al., 2009), an increase in autophagy protein 5 (Atg5) (Chae, et al., 2009), and embryotoxicity due to MDMA metabolites (Barenys, et al., 2012). This paper explores various venues theorized to be the cause of the cognitive and neuronal impairment observed in Ecstasy-exposed fetuses.

## Methods

Research on the topic of the biological reasons for the neurotoxic effects of MDMA on the developing fetus was compiled from various sources found in the Touro database. Information was obtained regarding general usage of MDMA as well as the observations noted in children of women who ingested MDMA while pregnant. Finally, data about various experiments performed on rats was obtained in order to gain insight into possible biological causes for the neurotoxic deficits noted in MDMA exposed fetuses. The information was arranged into a comprehensive order and edited by the author.

## Discussion

One strong hypothesis as to how Ecstasy induces long term brain damage in a fetus is that MDMA-exposed fetuses are harmed due to a decrease in 5-HT in the brain. This hypothesis is based on the established fact that MDMA causes serotonergic reductions in the adult brain. In a study done on Sprague-Dawley

rats, researchers in the University Of Cincinnati College Of Medicine sought to determine whether the effects of MDMA on exposed fetuses are indeed caused by a 5-HT reduction. Sprague Dawley rats were mated and their litters were reduced to 8 pups, 4 males and 4 females, with parturition day termed P0. The rats were divided into 2 groups. One group received two subcutaneous injections of MDMA daily on P1-P10 while the second group received similar MDMA injections on P11-P20. These intervals of P1-P10 and P11-P20 correspond to early and late third trimester development in humans, respectively.

Between P60 and P80, the rats were tested in various assigns. The results of the tests illustrated that the rats in the P11-P20 group had significant decreases in sequential learning, spatial learning, and memory abilities, while those in the P1-P10 group did not show significant decreases in any of these areas. Neither group showed a significant decrease in cued learning. These tests demonstrate that the neurotoxic effects that MDMA has on a developing fetus are more likely to occur when MDMA is taken during late third trimester development and that these effects are long term, as seen from the presence of the impairments in rats that had already reached adulthood.

The researchers sought to determine whether these cognitive impairments in the rats were due to serotonergic decreases in the rat brains. In order to investigate this hypothesis, the rats were decapitated on P105 and their brains were dissected. Reductions in 5-HT in the hippocampus were found for both the P1-P10 and P11-P20 groups, findings that do not correlate with the cognitive impairments seen only in the P11-P20 groups. In addition, the frontal cortex of the rats displayed 5-HT reductions only in the P11-P20 group, but these reductions were insignificant as seen with a correlation coefficient between these reductions and the spatial learning deficits exhibited by the rats that did not approach significance. Therefore, while this study shows that MDMA has long term negative effects on spatial and sequential learning and memory, it also illustrates that these cognitive deficits are not due to serotonergic reductions. The cause of fetal brain damage by MDMA must be via a mechanism other than the 5-HT reduction that causes MDMA induced damage in the adult brain (Broening, et al., 2001).

This idea that prenatal MDMA exposure and the long term damage that it causes is not due to 5-HT reduction can be supported by the mechanism via which MDMA causes 5-HT reduction in the adult brain. Studies indicate that MDMA metabolites form free radicals upon further metabolism and thereby damage serotonergic neurons. This is supported by the presence of malondialdehyde in rat brains after exposure to MDMA. Malondialdehyde is an end product of lipid peroxidation, the process during which lipids in cell membranes are broken down

via free radicals. Because free radicals are hard to measure due to their short half-lives, the by-products of these radicals known as TBARS (thiobarbituric acid reacting substances) are used to measure the damage induced by the free radicals. The TBARS Assay is a test that reacts thiobarbituric acid with malondialdehyde in order to quantify the amount of free radicals found in a specimen. This reaction yields a florescent product that indicates the presence of free radicals. Thus, a high yield of malondialdehyde from a TBARS Assay found in adult rats exposed to MDMA indicates the presence of free radicals in these rats. Another indication of the presence of free radicals in rats exposed to MDMA is that 5-HT reduction is prevented from occurring in MDMA-exposed rats that are injected with alpha-phenyl-N-tert-butyl nitron (PBN), a free radical scavenger. This indicates that the 5-HT reduction occurring in adult rats may be happening in part because of free radicals. A study exposing both neonate and adult rats to MDMA showed no significant increase in the presence of malondialdehyde in the hippocampus and cortex of the neonate rats while the adults' brain cortices did demonstrate a significant increase in malondialdehyde (Figure 1). Free radicals cause the serotonergic reduction in adult rats, and it can be inferred that cognitive defects found in adult humans exposed to MDMA are caused by this 5-HT

Figure 1

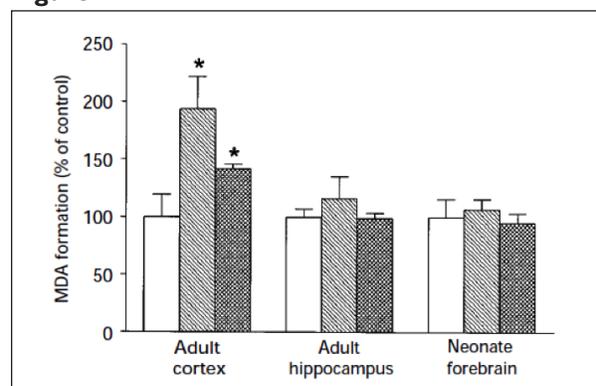


Figure 1 - Formation of thiobarbituric acid reacting substances (TBARS) in the hippocampus and cortex of adult rats and cortex of neonate rats 3 h (hatched columns) and 6 h (cross-hatched columns) after MDMA (40 mg kg<sup>-1</sup>, i.p.) administration. Results show % change in brain malondialdehyde (MDA) formation compared to saline-injected control animals (open columns), as mean±s.e.mean, n=8± 10. \*Significantly different from saline (Colado, et al., 1997).

reduction. In contrast, neonatal rats and, by inference, humans prenatally exposed to MDMA, have cognitive impairments that cannot be attributed to 5-HT reductions (Colado, et al., 1997). Other factors must be explored to lend reason for these long term neurotoxic effects.

Another possible cause for the cognitive deficits recognized in children prenatally exposed to MDMA is elevated levels of

## Biological Reasons for the Neurotoxic Effects of MDMA

cortisol caused by ingestion of Ecstasy. As noted, MDMA has been shown to cause impairments in long term memory, learning, and locomotor activity in children exposed to this drug in utero. Researchers hypothesize that these impairments may be due to the increase in cortisol that is present. Individuals ingesting Ecstasy display higher levels of cortisol in comparison to drug-free individuals with baseline cortisol levels. In addition, increased cortisol levels may be damaging to fetal development. Therefore, if the Ecstasy user is a pregnant woman, her MDMA usage may put her fetus at risk (Parrott, et al., 2014).

Cortisol is a glucocorticoid steroid hormone produced by the adrenal cortex in response to bodily stress and/or low blood glucose levels. The body releases this hormone in order to provide additional metabolic resources that enable an individual to function properly while under stress. The hypothalamic-pituitary-adrenal (HPA) axis is a systemic interaction of endocrine glands that utilize a negative feedback mechanism in order to control the hormonal activities of the adrenal cortices (Mosby's Medical Dictionary, 2009).

In a recent study performed on MDMA users, light usage of MDMA (ingestion of MDMA 1-4 times within the past 3 months) led to a mean increase of 50% in cortisol levels, in contrast with a control group of MDMA-free individuals. Heavier Ecstasy users (ingestion of MDMA 5+ times within the past 3 months) were noted to have a mean increase of 400% in their cortisol levels in comparison with a control group. While these statistics definitely illustrate radical increases in cortisol levels in MDMA users, a 10% increase of maternal cortisol leads to a mere 1% increase in fetal cortisol. This is due to the placental glucocorticoid barrier that prevents most of the cortisol from reaching the fetus. However, while a small increase in maternal cortisol levels may not cause damage to a fetus, a 400% increase in maternal cortisol can possibly have long term deleterious effects on the child.

According to the scientific review by Parrott, et al. (2014), "... HPA basal hyperactivation... may represent a complex neuro-endocrine dysfunction associated with MDMA use." They imply that elevated cortisol levels caused by MDMA ingestion may lead to HPA axis impairments, specifically in a developing fetus. This idea is supported by a study on the effects of maternal stress on a fetus, because researchers have found that similar to ingestion of MDMA, an increase in a mother's stress level causes more cortisol to be produced, and this hormone is then passed to the fetus via the placenta. As noted, although most of the cortisol is prevented from reaching the fetus due to the placental glucocorticoid barrier, about 1/10th of the cortisol will still reach the fetus. This hormone may then reprogram the HPA axis activity of the developing fetus which can cause long

term damaging effects on the child. Studies illustrate that the negative effects of stress on the developing fetus include poorer attention spans, sleeping, feeding, and activity problems, greater impulsivity, and a higher pervasiveness of Attention Deficit Hyperactivity Disorder (ADHD). Because ingestion of MDMA causes an increase in cortisol in the same way that maternal stress does and because cortisol has been shown to cross the placental barrier, researchers infer that it is the elevated cortisol levels found in MDMA users that are responsible for the neurotoxic deficits found in Ecstasy-exposed fetuses and that the long term effects of MDMA may mirror the effects of maternal stress on a fetus.

It is interesting to note that increased cortisol levels can be seen in an individual for up to 3 months after he/she has stopped using MDMA. Thus, it follows that a woman who stops MDMA usage prior to pregnancy may still be liable to inflict lasting damage to her fetus unless she has had several months of drug abstinence prior to conception (Parrott, et al., 2014).

A study researching the connection between elevated cortisol levels and memory problems supports the idea that elevated cortisol levels may be the cause of long term damage in MDMA-exposed fetuses, specifically in the area of memory impairment. Researchers tested healthy individuals with a mean age of 61.8 years to determine if memory problems are linked to elevated cortisol levels. This study used a special testing method in order to control for any stress that would develop in the subjects because of the inherent knowledge of their memory loss. The study demonstrated that women with memory complaints had significantly higher levels of cortisol than women without memory complaints, although no such difference was seen in men. In addition, individuals who complained of memory issues also had increased activity of their HPA axes (Wolf, et al., 2004). Similar to the adults in this study, fetuses exposed to MDMA also had elevated cortisol levels and hyperactivity of their HPA axes (Parrott, et al., 2014). In a similar vein, a study performed on cocaine dependent individuals served to analyze the cause for the learning and memory dysfunctions observed in these drug addicts. Researchers determined that cocaine dependent individuals have higher levels of cortisol than individuals in a 'cocaine-free' control group, and this increase was associated with the poorer learning and memory skills observed in these individuals (Fox, et al., 2009). Thus, it can be inferred that the memory impairments noted in children prenatally exposed to MDMA may be caused by the elevated cortisol levels that these fetuses are exposed to.

Another hormone that may be linked to the damage induced by MDMA in the developing fetus is norepinephrine (NE). NE is a noradrenergic hormone and neurotransmitter made from

dopamine (DA) by the enzyme dopamine beta hydroxylase (DBH). It is released from the adrenal medulla into the bloodstream and acts as a systemic hormone, and it is also released from noradrenergic neurons in the locus ceruleus of the brain where it acts as a neurotransmitter. Scientists have found that a correlation exists between prenatal exposure to MDMA in rats and changes in the structure and function of the NE system in these prenatally exposed rats. NE system dysfunction has been associated with exaggerated behavioral responses to new stimuli as well as a decrease in habituation to these stimuli, impairments which are attributed to attentional processing deficits. Because researchers have observed these behaviors in prenatally exposed rats, they infer that the damage induced by MDMA exposure to a developing fetus may be linked to abnormal wiring of the fetus's NE system (Thompson, et al., 2011).

In order to test this hypothesis, pregnant Sprague-Dawley rats were randomly assigned to two groups. One group was injected with 15mg/kg of MDMA twice a day at 8 hour intervals, a dose consistent with the typical MDMA dosage taken for human consumption, while the other group received subcutaneous injections of saline two times a day at corresponding 8 hour intervals. These injections were administered from embryonic day 14 (E14) to embryonic day 21 (E21). The pups were born on E21 and the first day after parturition was termed postnatal day 1 (P1). The litters were reduced to 4 males and 4 females for the MDMA group and 5 males and 5 females for the saline group. The pups were anesthetized on P21 and their brains were removed and cut into specific sections for observational analysis.

Among the different areas of the brain that were analyzed, three sections of the rostral hippocampus, CA1, CA2, and CA3, were scrutinized for NE abnormalities. This section of the brain was studied because it receives noradrenergic innervation from the locus ceruleus and because it is involved in spatial memory and responses to novelty, abilities that have been found lacking in MDMA exposed fetuses. The rats prenatally exposed to MDMA exhibited a 32.1% increase in DBH fiber density in the CA1 region of the hippocampus in comparison with the control group. In addition, norepinephrine transporter (NET), a molecule that regulates NE signaling by clearing NE from the synaptic cleft, increased in MDMA rats by 39.3% in the CA1 region and 32.1% in the CA3 region. In spite of these increases, the rostral hippocampus displayed no elevation in NE levels and levels of 3-methoxy-4-hydroxyphenolglycol (MHPG), a main metabolite of NE. The locus ceruleus displayed no increase in DBH fibers, and NET binding did not increase in this area. However, in the prelimbic (Cg3) region of the prefrontal cortex, there was a 69.2% increase in DBH neurites. This was accompanied by a 15% increase in NE in the prefrontal cortex as a whole, although there was no increase in NET binding or MHPG levels in the

prefrontal cortex.

These findings suggest that MDMA can cause NE system dysfunction in the developing fetus. As an example, the increase in NET binding observed in the CA1 and CA3 regions of the hippocampus illustrate that MDMA caused more NE to continue to be present in these areas even after exposure, or that the high levels of NE found in the hippocampus during MDMA exposure led to a sustained increase in NET binding in these areas as a form of systemic regulation. Because NE in the CA1 region of the hippocampus plays a big role in communication signaling between the hippocampus and entorhinal cortex, NE abnormalities in this region may lead to communication deficits which in turn may lead to impairments in spatial learning and working memory. In fact, as illustrated in other studies previously mentioned, these deficits have been found in rats exposed to MDMA while in utero. Also, as mentioned previously, NE increases have been related to exaggerated responses to novelty and attentional processing impairments. These deficits also correlate with a study that observed that rats exposed to MDMA while in utero had increased levels of responsivity to a new cage at P21, an increase that persisted into adulthood as far as P61-P62. Therefore, it can be theorized that NE system changes caused by rat embryonic exposure to MDMA, and, by inference, NE system changes caused by human fetal exposure to MDMA, lead to many of the impairments observed in children prenatally exposed to MDMA. Changes in the noradrenergic system that develop in the fetus as a result of MDMA exposure have direct links to the deficits noted in these children post-parturition.

It is worth mentioning that the results of the MDMA exposure are noted on P21, and that this corresponds to the early teenage years in humans. Thus, NE system changes and the resulting impairments can be effective in this time period. These systemic deficits may be the cause of the impulsivity and 'search for novelty' that is present amongst many young adults that often leads to substance abuse or reckless behavior (Thompson, et al., 2011). Perhaps the mothers ingesting MDMA while pregnant are unknowingly setting the stage for substance abuse in their children many years down the line.

In a similar study, scientists determined that fetal rat exposure to MDMA affects the fetus's dopamine (DA) system. These effects can be seen by a five-fold increase in dopamine neuron fibers in the prefrontal cortex of the rat brain as well as by smaller increases in DA fiber density in the striatum and nucleus accumbens of rats exposed to MDMA while in utero. DA has many pathways in the brain, one of which is the mesocortical tract, a pathway that transports DA from the ventral tegmentum to the frontal cortex. The pathway is known to

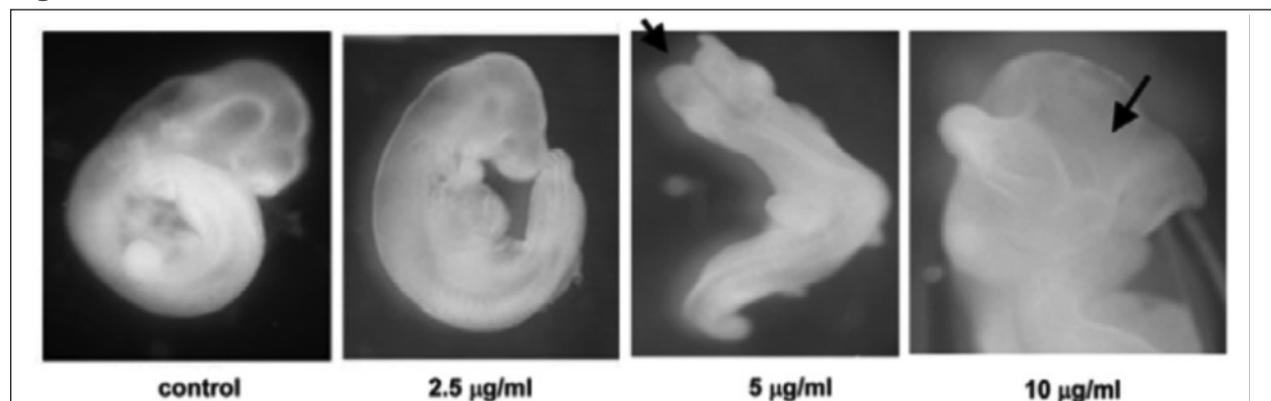
## Biological Reasons for the Neurotoxic Effects of MDMA

influence exploratory behaviors, impulsivity, and a search for novelty. When this system is impaired, the results can cause hyperactivity and are sometimes linked to behavioral issues such as ADHD. Because changes in the DA system of both humans and rats is associated with decreased habituation to novelty and increased locomotor activity, this study sought to discover whether the changes in the DA system noted in the rats exposed to MDMA could be correlated with significant changes in these particular behavioral areas (Thompson, et al., 2010).

The study tested Sprague Dawley rats that were injected with MDMA on embryonic day 14-20, as well as a group of 'control' rats, and measured their behavior on postnatal day 21. Many

packaging unneeded cytoplasm and organelles into vesicles known as autophagosomes in preparation for degradation so that the cell can recycle these particles. After an isolation membrane elongates and surrounds unneeded cellular particles, an autophagosome is formed which fuses with a lysosome, eventually leading to the catalytic breakdown of the extraneous cellular particles. Seven proteins known as autophagy-related proteins regulate this process. These proteins are grouped into two distinct categories – the Atg12 system (comprised of Atg 5, 7, 10, and 12) which involves regulating the elongation of the autophagosome isolation membrane, and the Atg8 system (comprised of Atg 3,4,7, and 8), a system involved in regulating the attachment of the phospholipids in the autophagosome

**Figure 2**



*This diagram illustrates images of fetal brain development of various rat embryos that received specific doses of MDMA on gestational day 8.5. Note how the teratogenic effects pointed out by the arrows are in concordance with the higher dosages of MDMA (Chae, et al., 2009)*

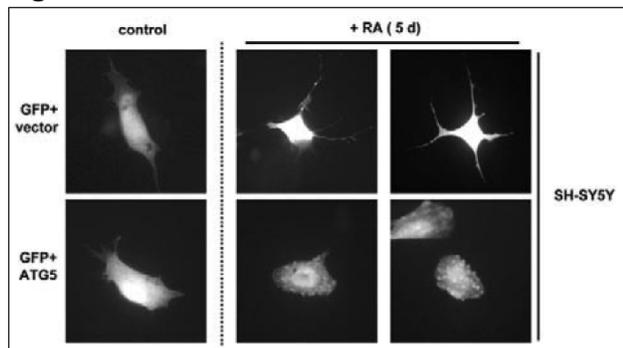
behavioral areas such as home cage locomotor activity, running wheel activity, gravitation towards a high fat diet, and cocaine self-administration levels where tested. However, the only areas of behavior that had significant results in comparison with the control group were the rats' decreased acclimation to new environments, increased perseverance to find a cued platform, and increased locomotion in the center of an open field which served as an indication of the rats' decreased levels of anxiety. Thus, the researchers hypothesized that the rats exposed to MDMA in utero had alterations in their dopamine systems, specifically in the mesocortical pathway. They conjectured that a possible cause for the decreased habituation to novelty and increased perseverance seen in the rats was caused by impairments that the MDMA wreaked on the fetal rats' developing DA systems (Thompson, et al., 2010).

Another study researching deficits found in fetuses exposed to MDMA explores impairments in neuronal differentiation that arise due to an increase in an autophagy-related protein 5 (Atg5). Autophagy is an intracellular process that involves

membrane (Chae, et al., 2009).

Defects in the autophagy system have been linked to various physiological impairments. In this study, researchers discovered that rats embryos injected with MDMA had elevated levels of Atg5. The study explanted rat embryos after 8.5 days of gestation and administered doses of 2.5, 5, and 10 micrograms of MDMA to the embryos. When the embryonic mRNA was sampled, it was noted that Atg5 expression had doubled after 48 hours of MDMA exposure and that extreme teratogenesis and fetal brain damage was observed in the rats (Figure 2). In order to determine if this brain damage occurred due to impairment of neuronal differentiation, human neuroblastoma SH-SY5Y cells were divided into two groups, a control group and a group injected with 10 micrograms of MDMA. The group of cells administered with MDMA illustrated an increase of 1.8 times the amount of Atg5 mRNA expression after 48 hours. Furthermore, when a sample of SH-SY5Y cells was induced with a green fluorescent protein vector (GFP), a protein used as a marker of genetic expression, in the presence of a plasmid encoding for Atg5, the cells did not express

Figure 3



This figure depicts the SH-SY5Y cells that were induced with GFP vector and retinoic acid for 5 days. The control group (top row) was imaged after 5 days while the test group (bottom row) was induced with Atg5 and also imaged after 5 days. Neurite extension and arborization is present and can be seen in the control group while no extension and arborization is present in the test group (Chae, et al., 2009).

any neurite extensions or arborizations (fine branching at the end of the neurite fiber). This was in the presence of retinoic acid, a metabolite of vitamin A that usually aids in growth and development. In contrast, a control group of SH-SY5Y cells induced with a GFP vector and retinoic acid in the absence of Atg5 exhibited extensive neurite extension and arborization (Figure 3). This demonstrates that an increase in Atg5 impairs neuronal growth and differentiation. Therefore, because an increase in Atg5 is found to impair the differentiation of SH-SY5Y neuroblastoma cells, it can be inferred that the teratogenesis observed in rats exposed to MDMA that is accompanied by an increase in Atg5 can be attributed to a similar mechanism of impairment of neuronal differentiation (Chae, et al., 2009).

An additional area that can be explored in order to explain the neuronal impairments discovered in fetuses exposed to MDMA is the metabolites that MDMA forms upon ingestion. As stated previously, when MDMA enters the body, it is broken down into substances that are further metabolized into free radicals. This is supported by the presence of malondialdehyde (MDA) in rat brains after exposure to MDMA, because MDA is one of the end products of lipid peroxidation, a process during which lipids in cell membranes are broken down via free radicals. However, MDMA forms other metabolites aside from MDA, depending on which enzymes are present to break it down. Therefore, while the formation of the MDA metabolite accounts for the breakdown of 20-90% of MDMA ingested by rats, 3, 4-Dihydroxymethamphetamine (HHMA) is a metabolite of MDMA that is formed upon human consumption and accounts for 53-81% of MDMA metabolism in humans. The difference in MDMA metabolism that results in the formation of MDA or HHMA is very slight. Ortho-demethylation of MDMA, which occurs in humans, leads to the formation of HHMA, while

Figure 4

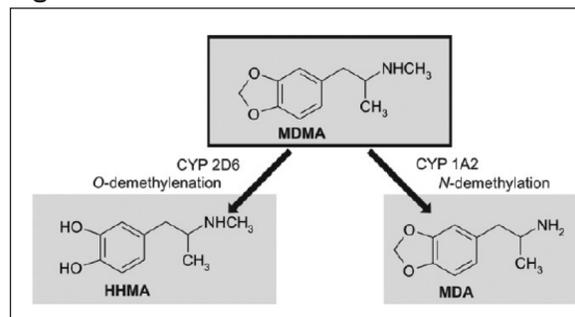


Figure 4 - This diagram illustrates the two main metabolic pathways of MDMA. O-demethylation produces HHMA and is the main pathway that occurs in humans while N-demethylation produces MDA and is the main metabolic pathway that occurs in rats (Barenys, et al., 2012).

N-demethylation, occurring in rats, leads to the formation of MDA (Figure 4). MDMA does metabolize into MDA in humans, as does MDMA metabolize into HHMA in rats, but only trace amounts of these metabolites are found in humans and rats, respectively (Barenys, et al., 2012).

Researchers have found that the presence of HHMA or MDA while a fetus is developing can lead to embryonic defects in the fetus. Scientists explanted embryos from Sprague Dawley rats after 9.5 days of gestation and injected various embryos with MDMA, MDA, and HHMA. The concentrations of the injections for each substance were 5, 15, 25, and 50 micrograms. They discovered that the embryos injected with 25 and 50 micrograms of MDMA had a decrease in crown-rump length (CRL), a measurement of the fetus from the top of the head (crown) to the bottom of the buttocks (rump). In addition, abnormal tissue formations, known as dysmorphogeneses, were observed in the rats injected with 50 micrograms of MDMA. These dysmorphogeneses displayed themselves as an opening in the superior frontal tissue of the head, termed an open cranial neural pore, an abnormal prominence in the inferior frontal part of the head, termed a protuberant nasal placode, and a disproportionately small forebrain in comparison with the midbrain and hindbrain (Barenys, et al., 2012).

Similarly, the embryos injected with doses of MDA displayed a decrease in their CRL after receiving a dose of 50 micrograms of MDA. These rats also exhibited dysmorphogeneses such as the posterior part of the embryonic trunk located behind the embryo instead of in front of it, termed an abnormal flexion, an abnormal caudal part of the embryo that was marked by a bend in the distal part of the embryo's trunk, abnormally shaped somites, and unusual narrowing of the otic vesicles that represented the formation of irregular ears (Barenys, et al., 2012).

The embryos injected with doses of HHMA also displayed

## Biological Reasons for the Neurotoxic Effects of MDMA

similar anomalies. Interestingly, the group of embryos injected with 50 micrograms of HHMA died upon exposure to this concentration of metabolite. Therefore, the doses were modified to 10, 20, 30, and 40 micrograms of HHMA per group of embryos. At both 30 and 40 micrograms of HHMA, the CRL of the embryos significantly decreased. Additionally, dysmorphogeneses such as abnormal flexion, a protuberant nasal placode, and an abnormal yolk sac (an anomaly marked by a yolk sac that was not properly rounded or was missing vasculature) were noted in the embryos injected with 40 micrograms of HHMA (Barenys, et al., 2012).

This research indicates that MDMA severely damages a developing fetus due to the toxic metabolites that are formed upon MDMA ingestion. However, it must be addressed why the dysmorphogeneses noted in the fetuses exposed to MDMA differed from those noted in the fetuses exposed to MDA and HHMA. While some of the abnormalities such as a protuberant nasal placode and decreased CRL were seen in both the MDMA-exposed embryos and the MDA and/or HHMA-exposed embryos, there were anomalies noted in the MDA and HHMA-exposed embryos that were not found in the MDMA-exposed embryos. This presents a problem, because MDA and HHMA are metabolites of MDMA and therefore, all of the anomalies found in an embryo exposed to these metabolites should be found in an embryo exposed to MDMA. Perhaps this can be explained by the fact that MDMA forms many metabolites upon ingestion, aside from MDA and HHMA. It is possible that the 'other' metabolites of MDMA interact with one another and 'change' the irregularities noted in embryos exposed to MDA or HHMA to represent those seen in embryos exposed to MDMA.

Additionally, it is worth focusing on the fact that the HHMA killed the embryos that received injections of 50 micrograms while this did not occur to the embryos receiving injections of 50 micrograms of MDA. HHMA is the main metabolite of MDMA in humans, and this finding suggests that it is more toxic than MDA, the main metabolite of MDMA that is found in rats. Because many of the studies available today about MDMA and its harmful effects have used rats as their method of research, perhaps humans need to be extra cautious when ingesting MDMA. This is because the negative effects that MDMA has been shown to display in rats is probably greater in humans since HHMA is the main metabolite of MDMA in humans and appears to be a more toxic metabolite than MDA.

### Conclusion

MDMA is an illicit, amphetamine derivative drug known as 'Ecstasy' that causes harm to a developing fetus exposed to this drug while in utero. Scientists have confirmed that fetuses exposed to MDMA exhibit shorter attention spans, poorer

memory, decreased habituation to novelty, poorer mental development index scorings, and other neurological impairments. While researchers have determined the negative effects that MDMA has on a fetus, the biological reasons for these effects are still being researched extensively. Scientists have determined that these deficits are not due to 5-HT reductions in the brain. However, these defects may be caused by changes in the fetuses' cortisol levels, systemic NE or DA changes that are caused by exposure to MDMA, an increase in Atg5 that leads to inhibition of neuronal growth and differentiation, or the embryotoxic effects of HHMA and MDA, two main metabolites of MDMA. These explanations require further research to determine how they interrelate with one another. Additional studies can investigate the interactions of all of the systemic changes caused by fetal exposure to MDMA to explore how these interactions bring about the cognitive deficits noted in MDMA-exposed fetuses. While this area of research has yet to be explored, individuals should take note that the harmful effects of MDMA have already been determined with certainty and that consumption of this drug should be avoided.

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# Human Animal Chimeras For Therapeutic Protocols

By Bracha Sklar

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## Abstract

Research on humans is limited, therefore human animal chimeras have been used to study human systems. A Human animal chimera is an animal containing animal and human cell lines. The primary goal of human animal chimera research is to establish an animal with human cellular characters, which can and should more realistically be able to imitate as closely as possible the in vivo situations in humans. This research is very important, because it allows scientists to study human systems in vivo using a humanized animal model. However ethical issues arise when experimenting with humans and animals being mixed together. Using information found on Touro College's database, this article reviews studies done using human animal chimeras, and their potential benefits for therapeutic protocols.

## Introduction

A primary goal in biological research is to understand human development, however research on humans is restricted. Although isolation of human embryonic stem cells (hESCs) and the generation of human induced pluripotent stem cells, have led to more options in the field of human biology, there is still need to analyze these cells in an in vivo setting. As a result, many researchers are now turning to human animal chimera research (Hermerén, 2015). The definition of a chimera, suggested by Lensch et al. (2007), is an organism containing cells from two or more individuals of the same or different species. Behringer (2007) proposed, that a chimera is an organism with two cell lines, where the individual is "composed of somatic and, in certain cases, germline tissues derived from more than one zygote."

There are different types of chimeras, Tetragametic chimeras, arise in many cases from in vitro fertilization (IVF), a route of fertilization, in which an egg and sperm are combined in a laboratory dish, and the resulting embryo is then transferred into the uterus. Because the ensuing embryos were grown in close contact, Tetragametic chimeras can result (Granzon, R.R., 2014). Tetragametic chimeras also occur when twin zygotes fuse together and develop into one body. As a result, the individual's tissues are derived from more than one zygote, and contain two sets of DNA. There have been cases where this happened, and affected the individual later on in life, when she has her own child and if a DNA test is done, it might not match up. It can be her 'sisters' genes that she fused with that matches her children's. Further testing will prove it's her child by matching the same genotype in some parts of her body. A person who has two different eye colors, can be from the fusion of two zygotes, among other causes. Consequently, someone born with male parts and female parts can be male and female early embryos that merged together (Norton and Zehner, 2008). But here, chimeras spoken about are human animal chimeras, which are being studied more by scientists in the hope to discover cures for diseases, such as Alzheimer's and Parkinson's and to develop vaccines, for example to help fight HIV.

Human animal chimeras have been created by "grafting human cells and tissues into the embryos, fetuses, or adults of vertebrate model organisms (Behringer, 2007)." Currently researchers and

scientists are experimenting how the use of chimeras can advance the field of medicine. Scientists are utilizing the unique ability of stem cells to proliferate and differentiate into various cells. They are using the idea of stem cell research, but with a twist. Scientists are injecting human stem cells (hCSs) into animal embryos for the purpose of studying human diseases. When a fetus is forming, the immune system is not mature enough to attack anything foreign, therefore modifications can be made in an embryo. For example, early discovery of an abnormality can be treated with stem cell administration. The injected cells can freely proliferate and/or differentiate into new cells, replace the diseased ones, and regenerate damaged tissue. Although this concept might sound relatively new, the idea of chimeras are already being practiced today, by using pig heart valves for humans and using animals for skin grafts (Cooper, 2012).

The study of biological systems in humans is largely regulated to in vitro models that lack the mechanisms and intricacy of living organisms. The complexity of a biologic structure can only be precisely and realistically replicated by an in vivo system. In vivo studies in laboratory animals are routinely used to model human biology and disease but they are not human and therefore cannot fully replicate human functioning. Transplanting human cells into animals provides a way to study human systems in vivo (Sun et al., 2007). Humanizing an animal, by implanting human blood, neurons, germ cells and other tissues into it, results in a chimera. Human animal chimeras, containing animal and human cell lines have been generated for decades to facilitate human biological studies and therapeutic strategies for disease (Behringer, 2007).

This paper reviews studies done using human animal chimeras. Although research with chimeras shows great potential for insight into diseases and medicine, it also raises unique ethical issues that must be considered. Are the benefits of using human animal chimeras for therapeutic protocols worth the possible risks?

## Materials and Methods

In order to answer the question proposed above, many research papers and journal articles with relation to this area have been read. Touro College's library data base (tourolib.org) was utilized to search for relevant studies and reviews, with

databases such as ProQuest, and EBSCO. Further research was done by looking up references cited from review articles to find original papers. All research accumulated was used in an attempt to determine if the benefits of humanized animals outweigh the possible risks.

### Discussion

There have been a number of studies using human animal chimeric mice as an *in vivo* system to study how stem cells proliferate, to test diseases, and to study in more detail the biologic systems. Often mice are used, however fetal sheep and fetal goat, during the pre-immune stage of development, have also been used as a surrogate animal model for human stem cells. The pre-immune stage allows for the transplant of human stem cells, and the resulting human animal chimera serves as a unique and clinically relevant xenograft animal model for assessing the differentiation potential of hSCs *in vivo*. However from a scientific perspective, large animals are not a suitable model for mechanistic research and the experiments reviewed here utilize mice, as a host for human cells. Small animal models, such as mice and rats according to scientists are ideal models to use. The advantages of using small animals is that they contain naturally occurring migration patterns of stem cells, and provide the availability of extending homing and engraftment sites. Additionally, the presence of tissue and organ specific signals from niche, greatly facilitate the widespread distribution of human donor cells. It is these reasons that compelled scientists to develop an influential small animal model, also known as a humanized animal for pursuing hSC basic research (Sun et al., 2007).

### Stem Cells

In one study, a human/rat xenograft animal model was generated by transplanting human low-density mononuclear cells (hMNC's) from human umbilical cord blood (hUCB) into fetal rats to study how the human donor stem cells behave *in vivo*. Numerous methods including flow cytometry, Polymerase Chain Reaction (PCR), and immunohistochemistry (IHC) assay were used to test the human donor contribution. Flow cytometry detected that out of 29 recipients, 19 had human leukocyte common antigen, CD45+ cells in peripheral blood (PB). PCR analysis on 11 different adult tissues showed that 14 out of 19 CD45+ animals possessed donor derived human engraftment in multiple tissues for example, the liver, spleen and thymus. The differentiation of human donor cells in the liver was assessed by IHC with the use of human specific antibody against the hepatocyte marker; CK18. The recipient liver, and the human liver had cells that expressed human CK18, while normal control rats did not. IHC examination revealed that in the chimeric adult spleen of recipients, many donor derived human cell populations expressed CD45 marker indicating the human spleen-specific differentiation of donor derived human

cells. Differentiation of hMNC's was evaluated in the rat thymus using IHC with a human specific antibody against CD45. At 3 weeks, 2 months and 6 months after *in utero* transplantation, multiple human cells recognized in the recipient thymus strongly expressed CD45. Human umbilical cord derived cells expressing CK18 underwent site specific differentiation into CK18+ human cells in the recipient liver and CD45+ human cells in the recipient spleen, which demonstrates that after *in utero* transplantation of hMNC's, they successfully engraft into the liver and spleen of recipients. "Subsequently, the long term that survive in these organs are then actively influenced by niche signals to contribute in organogenesis (for example liver and spleen) recipients in the xenogeneic competitive settings." This experiment concluded that a human rat chimera was successfully developed in which xenogeneic human cells exist for up to 6 months. This provides a great *in vivo* model to study how stem cells work (Sun et al, 2007).

In another study, hESCs were implanted into the brain ventricles of embryonic mice, and found to differentiate into functional neural lineages, generating mature active human neurons that successfully integrate into the adult mouse forebrain. This study reveals insights to recognition of common signals for neural differentiation throughout mammalian evolution. The results showed that hESCs, when transplanted into the ventricle of the developing mammalian brain, can give rise to neuronal and glial lineages, suggesting that they are "responsive to environmental cues that regulate cell fate determination and differential migration." This human animal chimera model provides an *in vivo* approach to study human neural development. An approach which can be used to study human neurodegenerative and psychiatric diseases, and a prospective technique to speed up the screening process for therapeutic drugs in the future (Muotri et al., 2005).

### Disease Model

In another experiment, an animal with a humanized immune system was used to serve as a human disease model for Human Immunodeficiency Virus type 1 infection (HIV-1). Rag2<sup>-/-</sup>vc<sup>-/-</sup> mice, when neonatally injected with human CD34+, develop a functional human immune system, with human hematopoietic cells found in the thymuses, PB, spleen, and bone marrow (BM) of the animals. Rag2<sup>-/-</sup>vc<sup>-/-</sup> mice become infected with HIV-1 when injected with CCR5 tropic HIV-1. HIV-1 infection is characterized by constant virus replication and a gradual loss of CD4+ T cells and T-cell function. After being injected with CCR5 tropic HIV-1, there was a productive infection of human cells in PB, thymus, spleen tissue, and BM, and ratios of CD4 (+) T cells to CD8 (+) T cells declined. Infection of the mice with 5000 TCID<sub>50</sub> of R5 HIV-1 resulted in a productive infection in 3/3 animals. HIV replication was detected in PB, thymuses and spleen and BM was also positive for HIV-1. When a 10 fold

smaller amount 500 TCID50 was used for infection, only 2/5 were found to be productively infected with HIV-1 in PB and all lymphoid organs. This study demonstrated that HIV-1 can be detected in multiple lymphoid tissues of Rag2<sup>-/-</sup>vc<sup>-/-</sup> mice, using low doses of CCR5-tropic HIV-1. This mouse, consisting of a human hematopoietic system, can serve as a small animal model for investigating HIV-1 pathogenesis and testing potential HIV-1 therapies (An et al., 2007).

In the next study, a chimeric mouse, that sufficiently mimics the pathophysiological micro-environment in human liver, was established as a unique experimental model to study human liver cancer metastasis (Fujiwara et al., 2012). This was discovered by expression of an albumin-urokinase plasminogen activator (Alb-uPA) transgene in the mouse that is hepatotoxic, resulting in the progressive destruction of the mouse liver. It was found that when primary healthy normal human hepatocytes were transplanted into this mouse, the human hepatocytes were capable of reconstituting the host liver, highlighting the generous capacity of hepatocytes to regenerate. This liver reconstitution mouse model has led to a mouse with a humanized liver, by the development of mice with livers that are reconstituted by engrafted human hepatocytes. This model can be useful for in vivo testing of anti-cancer drugs and for studying the mechanisms of human cancers (Behringer, 2007). Based on this, scientists theorized that the chimeric mouse can be used as an animal model to investigate the underlying mechanisms of tumor metastasis into the liver, where the "parenchyma is composed largely of normal and healthy human hepatocytes (Fujiwara et al., 2012)."

### Human Organs

Human animal chimeras can be used to create complete human organs by growing organs made exclusively from human cells in a chimeric animal, such as a pig, that could potentially be used for organ transplants (Hermerén, 2015). It was shown, that when a mouse that is deficient in T and B cells was injected with mouse embryonic stem cells, the T and B cells generated were the donor derived mouse embryonic stem cells. The donor stem cells compensated for the lack of T cells by producing T cells. It was hypothesized that the same may be true for organs. That if an animal that is deficient in an organ is injected with stem cells, the stem cells might compensate for what its lacking and grow into that organ.

A study was done where mouse wild-type pluripotent stem cells (PSCs) were injected into Pdx1<sup>-/-</sup> mouse blastocysts, (pancreatogenesis-disabled) and found that they developmentally compensated vacancy of the pancreatic "developmental niche," generating almost entirely PSC-derived pancreas. The organ generation system described may be useful to treat organ failure in humans if pigs or other large animals are used.

However, several concerns have to be addressed first to bring this idea into practice. For example, if interspecific chimeras between mouse and rat were to be generated, their "embryonic lethality is high and maturation into adulthood is uncommon (Kobayashi et al., 2010)."

In another study the immune-deficient athymic nude mouse was used as a recipient of human tissue grafts, to generate human animal chimeras. Because this mouse lacks T cells, it allows for many types of xenograft tissues to survive and grow (Behringer, 2007). Four to seven grafting experiments using human fetal material in the nude mouse have been successful. In 1969 the first successful transplantation of human malignant tumor tissue in the nude mouse was reported. Since then, the number of tumors transplanted has increased steadily. The nude mouse with transplanted malignant tumors is a new alternative model in oncologic research. It can be applicable to: "basic studies of the genesis of cancer tissue, immunology, and cell kinetic research (Spang-Thomsen and Visfeldt, 1976)." The nude mouse was used as a model for cancer, by grafting pieces of human tumors under the skin of the nude mice, in that way, providing a "bio incubator" for tumor growth. This bio incubator gives scientists a glimpse into how to treat diseases in humans. The introduction of the nude mouse significantly enabled these types of studies and this in vivo assay is one of the fundamental experimental models for cancer research (Behringer, 2007).

Other experiments include studying mice with human germ lines. By subcutaneously transplanting human pre-pubertal and adult testicular tissue fragments into immune deficient mice, it is seen that human spermatogonia can survive in mice. Hopefully with more research, it may be possible to come up with a strategy to preserve the germline of young boys undergoing chemotherapies and radiotherapies that cause sterilization (Behringer, 2007). This study utilized Nude mice and severe combined immunodeficiency- non-obese diabetic mice, (SCID-NOD), two immunodeficiency recipients to compare the grafting of pre-pubertal and adult murine and adult human testicular tissue. The survival of pre-pubertal and adult murine testicular tissues, and of adult human testicular tissue was evaluated after subcutaneous grafting to immune deficient mice. This xenograft model in an immune deficient mouse with pre-pubertal human testicular tissue is a theoretical strategy for restoring fertility in childhood cancer patients, while circumventing the risk of malignant recurrence (Geens et al., 2006).

### Ethical issues

These different types of human animal chimeras have provided important insights into fundamental biological mechanisms and the development of therapeutic protocols for human disease. Chimeras have compensated as human animal models for

the study of human processes in vivo. However there has been a lot of ethical debate concerning this research. These experiments reviewed have been approved, because the carefully transplanted human cells aren't large enough to make the animal human. They only humanize certain parts, and the resulting chimeras are euthanized after a number of days. Many boundaries had to be set with this type of research. In these studies, the human donor contribution is very low compared to the animal host. Although cells express an evidently higher degree of activity than genes, they also are intricately dependent on their surroundings. When hSCs are transplanted into animals, although they are free to proliferate and differentiate they are still in an animal body, and are reliant on their environments. They help to humanize an immune system if the animal is lacking one, however the host still remains an animal, with a distinct cell line of human cells, resulting in a chimera. Only in a receptive host, local niches regulate key developmental changes for several adult stem cell types, termed as an "inseparable relationship", besides for bone marrow transplantation, in which the hematopoietic stem cell is an "agent" in blood reconstitution (Hyun, et al 2007).

Research with human animal chimeras become a problem when cognitive capacities of the animal is changed, and when the germ line is affected. Therefore, boundaries have been put into place. Precautions in this field of research include, using progenitors rather than PSCs to avoid germ line contribution, which pose the risk of producing human embryos in animals. And treating the humanized mice, as a modified crop, by keeping it isolated to prevent it from mating with other mice, as well as euthanizing it immediately when research is concluded (Hermerén, 2015).

### Conclusion

The above studies reviewed involved reconstituting animal models by transplanting human stem cells, HIV, cancer, and growing human organs in mice. The experiments resulted in a human animal chimera, an animal with two cell lines. Human animal chimeras, animals carrying human tissues were used as an alternative model to investigate human specific biological processes without experimentation on human individuals. Although human animal chimeras provide numerous possibilities, there is still a lot of controversy and risk associated with it. Since research is developing rapidly, and people's values and perception of risk and benefit change, not all issues can be settled. This approach to studying human pathways in animals concerns many people, however with the proper care and thought-out methods, research should be able to proceed without extreme regulation, and more experiments using human animal chimeras for therapeutic protocols should be approved.

### Abbreviations

HSCs- Human Stem Cells  
HESCs- Human Embryonic Stem Cells  
hMNC's- Human Low-density Mononuclear cells  
hUCB- Human Umbilical Cord Blood  
PCR- Polymerase Chain Reaction  
IHC-Immunohistochemistry  
PB- Peripheral Blood  
HIV-1-human Immunodeficiency Virus type 1 infection  
BM- Bone Marrow  
Alb-uPA- Albumin-Urokinase Plasminogen Activator  
PSCs- Pluripotent Stem Cells  
SCID-NOD- Severe Combined Immunodeficiency- Non-Obese Diabetic

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# Human Stem Cells: Is it Possible to Limit Pluripotent Human Stem Cells to Differentiate into a Specific Tissue or Organ?

By Chaya Korf

Chaya Korf graduated in June 2015 with a B.S. degree in Biology.

## Abstract

It would seem that differentiation of stem cells is the remedy of modern times. Yet stem cell research is more complex than the public may recognize and is still in the developmental stage. Recent breakthroughs show promise; various guidelines provide structure to this growing field but it is not ready for gross public application. The objective of this paper is to research various different methods of stem cell production and application. Methods included reviewing articles and studies to evaluate the process in production of affective stem cells. There are many therapies that illustrate this research. Upon completing and explaining this phase, further analysis of research presented risk factors such as negative immune responses, tumor, and teratoma formation are discussed. This paper further examines the different complications that may be triggered by stem cell therapy. The danger analysis is briefed to describe the risks involved in stem cell therapy while explaining the ongoing research and discoveries to assist in limiting these risks. This paper culminated with the success of the most affective form of therapy to date. There are ongoing studies directed to limiting stem cell therapy for safe and risk-free application. Nevertheless, no definite conclusion can be reached as to how stem cells can be limited to the point of remaining simultaneously pluripotent and effective.

## Introduction

A young woman walks into her doctor's office complaining of what seems to be a pimple that does not go away. She initially treated it with over counter creams, then a facial and many dermatologist visits. Upon a more extensive examination the specialist realized that this was not the average case of adolescent acne and ordered blood work. The test revealed that this girl has type-one diabetes. Her plans to go to college were placed on hold and her life completely changed. What if diabetes didn't have to be a life altering disease? What if glucose monitoring and insulin injections were replaced with a one-time stem cell therapy that could replace the damaged cells? This example did occur and the girl is being treated with subcutaneous insulin infusion therapy. Stem Cell Research has not reached this breakthrough but the hope is that in the future stem cells will be used to test medical treatments and replace cells lost by devastating diseases that currently have no sustainable cure.

The first several divisions of the mammalian fertilized oocyte give rise to totipotent cells. These cells can form cells in the placenta, embryonic sack, and any cell in the body. Once cells are designated for the body they are termed pluripotent cells. These embryonic stem cells have virtually limitless potential and are an invaluable resource for the biomedical community. Along with the importance of these cells arises a responsibility of using them correctly. The process of understanding how these stem cells work and the services they can provide has not been an easy feat. This battle has been fought in both the courtrooms and laboratories. The legal system bears the responsibility of creating a system in which guidelines are being followed and ethics upheld. This is an ongoing battle, and there is no undermining this work. Regardless of several ongoing debates and some legal verdicts, the biomedical community is addressing stem cell research, cell regeneration, and organ construction

from two different perspectives. There is ongoing promising research being done on organ and cell regeneration with the use of somatic stem cells. Specific mature, undifferentiated, adult cells are reprogramed to function as stem cells and referred to as induced pluripotent stem cells (iPSC). These cells, generally found in the epithelial cells of an adult organ, have already been assigned a task and are therefore limited to the regeneration of that particular cell or organ. However, being that they are extracted from the patient, it is assumed, although not tested, that these cells will face less rejection after implantation because of the body's familiarity with the cells. An alternative form of adult stem cells is activation of somatic stem cells (Stem Cell Information, n.d.) within the body. These cells reside within a healthy body and function as a natural maintenance system by regenerating aged or damaged tissue and replacing lost cells. Examples of the somatic stem cells are cord blood and bone marrow. The third of these general stem cell approaches allows for more potential but at a greater risk; pluripotent embryonic stem cells (ESC) can be used to create any cell or organ but at a higher probability for tumor growth. There are several distinct differences between embryonic and adult stem cells. Adult stem cells are rare cells that require extraction, isolation, and then in vivo cell culturing, to multiply. This cell culture process has not fully developed and is a great deal more complicated than the growth of ESCs (Herberts et. al. 2011., Shanthly et. al. 2006).

This paper will explore the potential of regenerating tissue by means of pluripotent stem cells, as well as discuss literature on growth of these cells to build tissues and replace cells. Upon exploring the processes of in vitro and in vivo differentiation, in both induced pluripotent stem cells (iPSC) and human embryonic stem cells (hESC), one can question the technology needed to operate these cells. Is it possible to limit stem cells to differentiate, as they would in utero, to create a select tissue or organ?

# Stem Cells Differentiation into a Specific Organ

## Methods

This document was amassed by referencing original research and peer reviewed scholarly articles. US government websites and portions of scholarly articles were accessed to gain knowledge about the background, history, and general methods involved in stem cells therapies. Specific topic information, regarding the research question, was collected, reviewed, and analyzed. Google Scholar, and databases that are made accessible through the Touro College online library, such as Proquest and Ebsco, were used to search and access documents related to the research topic. The following are examples of key words were used: stem cell and apoptosis, c-myc, tumorigenic factors, in vivo growth, nodal signaling. There was no discrimination made regarding the date of publication on articles.

## History

Initial stem cell research dates back to 1870, with in vitro fertilization of an oocyte. Any form of promising finding were slow until research intensively propelled in the late nineteen seventies. Notable accomplishments include cloning of mammals in 1997 and development of the first stem cell culture in 1998. The virtually simultaneous discovery of cloning and stem cells were the building block of forthcoming research. Stem cells cultured in today's laboratories are removed from the blastocyst early in differentiation and frozen in their state until they are differentiated into the cells required for a specific medical therapy (Shanthly et. al. 2006). Research is ongoing and promising; the biomedical community has only scratched the surface of the untapped potential of stem cells. There are various issues and setbacks that are limiting stem cell potential (Stem Cell Information, n.d.).

Stem cell research raised sharp scientific, technological, ethical, and political controversies. The United States federal and state governments enforced laws to enhance and restrict actual stem cell research. States such as Florida and New York agree to provide state funding while other states such as Arkansas and Michigan have placed bans on the creation and use of ESC for biotech research (Vestal 2008). Some forms of stem cell research and experimentation are more disputed than others; stem cells derived from oocytes are more controversial than induced adult cells. For example, in 2006 the Senate passed a law allowing federal money to benefit the specific research of iPSC, umbilical blood and bone marrow treatments but not ESC. However, in 2011 President Obama removed all bans that restricted federal funding from being assigned to new lines of embryonic stem cells. Regardless of the origin of the cell, human stem cells research raises ethical sensitivities. The ethical policies are continuously evolving and the biomedical community is attempting to overcome the challenges to ensure that research is carried out in an appropriate manner (Levin et. al. 2013).

In addition to legal constraints the anatomical components that comprise stem cells are intrinsically a hindrance. The same factors, enzymes, and transcription that insure the cell's ability for self-renewal cause biomedical complications (Li et. al. 2015).

## Differentiations

Some mammalian tissues, such as the skin and liver, have the ability to regenerate yet others cannot. The capability of bioengineering and sustaining all organs, regardless of their regeneration factor, will be invaluable; this can ultimately circumvent the transplant system ensuring that patients don't die waiting for an organ. Regeneration capability is reliant on stem cells. For the purpose of this paper stem cells are divided into two categories, embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC). In more recent years, researchers have developed methods of directly reprogramming differentiated cells to produce iPSC. iPSCs are rare and found in only some differentiated tissues throughout the human body. A common use for iPSC is bone marrow transplants to fight leukemia. Although only one in every one hundred thousand cells is stem cell, advanced techniques allow for harvesting and collecting those cells in concentration to be donated by injecting the donor with colony-stimulating factor 3 (CSF 3). This glycoprotein stimulated the bone marrow to produce more stem cells; a necessary tool to collecting these otherwise dispersed cells. Such techniques can be used to collect adult stem cells but the process is tedious and does not allow for the unlimited potential hESC offer (Shanthly et. al. 2006). Although iPSC involves complications, such as the complexity of collection, it also provides therapeutic potential because these cells are harvested from a patient's own cells. This can alleviate tissue and cell rejection complications.

Since adult stem cells cannot give rise to all cells and tissues in the body, the ideal regenerative cell is embryonic stem cell. There are three different forms of embryonic stem cells used for medical research. The foremost method, true embryonic stem cells, is made from fertilized eggs generally donated by patients undergoing in vitro fertilization. Somatic cell nuclear transfer (ntES cell) is the process of creating an embryonic cell by inserting the nucleus of a general human cell into an egg cell. Although this technology has not been tested on higher mammals, it can be of great use because the nucleus of the cell will be a genetic match to the patient. The third form of ESC, not ready for clinical use, is called parthenogenetic embryonic stem cells (pESC). Through the process of parthenogenesis, an unfertilized egg is stimulated into differentiating as an embryonic cell would (Shanthly et. al. 2006).

## Stem Cell Links to Tumorigenic Cells

Regardless of the types of ESC, these stem cells are derived from the core of mammalian blastocysts and can multiply infinitely

and differentiate into three germ layers: mesoderm, endoderm and ectoderm. However, there are stem cells that have been documented to have significantly more genetic mutations as they grow in vitro. Research performed on zebra fish indicates that nodal signaling and pathways are found in both metastasized cancerous cells and embryonic stem cells yet not in healthy skin cells (Topczewska et. al. 2006). Nodal proteins are growth factors important to pattern formations needed for the embryonic differentiation, specifically as it relates to the formation of the mesoderm and endoderm (Shen 2007). Activation of the nodal pathways is tightly regulated and prompts self-gene inscription with positive and negative feedbacks (Cheng, Sun, 2014). The similarities between ESC and tumorigenic cells may lead to discoveries that can limit the association between stem cells and cancer. Nodal signaling pathways are not the only unique link between stem cells and metastasized tumor cell; telomerase is an enzyme that facilitates growth and development of DNA in ESC but also found in upwards of fifteen times more often in cancer cells than in healthy cells (Hiyama, Hiyama, 2007).

Nonsense-mediated mRNA decay (NMD) is been known to clean RNA in order to protect from early termination. At the PTC site, the location where early termination would commence, the NMD complex is assembled. This complex balances and regulates the proteins and is essential for homeostasis and overall survival of not only the embryonic cell, but organisms as a whole. One factor of the NMD complex is Smg5, this factor is involved in telomere and telomerase maintenance. Thus this NMD factor and Telomerase protein are both essential components that regulate cell viability. They may cause cell fatality or paradoxically, cancer (Li et. al. 2015).

Understanding the similarities between these cells help with limiting ESC. This process is called clinical transitions; scientific knowledge is gathered, understood and then turned into medical treatments. Regardless of these setbacks, the utility of these cells should not be undermined; simple laboratory practices such as freezing can assist with excess cell growth. Chemicals have been tested to determine if ESC can be treated to induce certain forms of apoptosis. Apoptosis is the shrinking of a complete cell and its membrane. Apoptosis differs from the generic form of cell death by the fact that it does not leave residue. Excess cell components that did not shrivel; undergo phagocytosis. The purpose of these chemicals would be to generate cell death and on the specific component of the cell that may lead to complications, while leaving the remainder of the stem cell unharmed. Research performed on these compounds and chemical were published in 2012 and these findings claim to identify compounds that altered the viability of ESC (Conesa et. al. 2012).

### Research In Vivo/ In Vitro

A ten-year research program beginning in the late 1980's studied soft tissue engineering using totipotent stem cells. The experiment used mouse embryonic stem cells to attempt to build lymphatic tissue, among other structures. Prior to commencement the researchers set out to establish what would be an ideal environment for embryogenesis. Building tissues in vivo requires an environment that mimics all aspects of uterus or in vitro development; components such as nutrient and culture conditions are key. However, the physical creation of such an environment is quixotic, at best (Chen et. al. 1997, Shanthly et. al. 2006). A later study characterizes the complexity of stem cells by explaining the conditions for maintaining undifferentiated status. This study dictates that FGF2 and conditioned medium (CM) support hESC in an undifferentiated state. In 2005 undifferentiated hESC were maintained with either FGF2 or CM. These stem cells remained in undifferentiated colonies apposed to the hESC that were maintained without these factors. Maintenance of the undifferentiated cells is an extremely delicate process, indicating that the steps from that point forward, toward proper differentiation, is that much more complex (Sumi et. al. 2007, Umar et. Al. 2014).

Development of any tissue, regardless of its origin requires three steps: cell differentiation, complex tissue formation, and the building of a three-dimensional, three-layered germ celled organ (Chen et. al. 1997). The most complex portion of complete in vitro embryogenesis is the maintenance of a live culture throughout this process. As it is with a fetus, tissues, and organs can only function if the fetus is alive. Considerable research has been completed, and is ongoing, on many different forms of tissue growth. To better understand the processes of building cells and tissues with stem cells, this paper will only be discussing two different tissues developed in culture, cardiac myocytes and lymphoid tissues. The decision to use these specific examples is based on varied dates of the research and the difference in tissue complexity, due both to function and development of each cell.

### Cardiac Myocytes

The differentiation of stem cells to cardiac myocytes is a rather complex. Cardiac muscle is a prime example of a complex tissue due to the various forms of specific muscular tissues and their functions as it relates to an efficiently operating heart. Cardiac tissue is made up of interlocking, single nuclei, striated, muscle fibers. The unique and complex aspect of this tissue includes the intercalated disc, which assist in conduction speed, and autorhythmicity, the ability to set its own contractions. Research published in April of 2011 suggests promising potential of differentiation of cardiac myocytes from hESC. By inhibiting Bone Morphogenic Proteins (BMP), a growth factor,

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during cardiac differentiation, cardiogenesis is initiated. This step is far from the only one that is needed to allow for ESC to be transplanted for therapeutic uses. Additional research is required such as the study of the regulation of retinoid sig-

**Table I**

ESC:	iPSC:	SSC:
Derived from inner mass of blastocyst	Derived from somatic cells	Isolated from postnatal adult tissue
Allogenic material	Autologous or Allogenic material	Autologous or Allogenic material
Pluripotent	Pluripotent	Multipotent
Can differentiate into all three germ cells	Can differentiate into all three germ cells	Can differentiate to limited cell types.
Self-renewal	Self-renewal	Limited Self-renewal
Indefinite Growth	Indefinite Growth	Limited Lifespan
Significant Teratoma Risk	Significant Teratoma Risk	No Teratoma Risk
Serious Ethical Issues	Serious Ethical Issues	Serious Ethical Issues
Immuno-privileged	Not Immuno-privileged	Unpredictable

(Prendergast 1999)

naling, the key that defines the difference between ventricular and arterial myocytes (Zhang et. al. 2011, Hartman et. al. 2014).

### Lymphatic Tissue

The microenvironment needed for embryogenesis is delicate. Being that lymphoid cells develop toward the end of this process, it was difficult to assume that the microenvironment need for ES cultures could be sustained until that point. Initiation of the research began with mice. Through the use of viral vectors, oncogenes were inserted and developed in vivo to produce B and T cells. Many attempts and adjustment were made. The generation of B and T cells from lymphatic tissue was rare at the time publication of this journal but at the end of the research it was confirmed that lymphoid precursor cells could be derived from ESC. These years of research, experiments, and analysis of data only begins to explain the complexity of building tissues, regardless of their origin (Chen et. al. 1997).

### Complications

Regardless of the difficulty of building tissues from stem cells and the improvements to cell cultures explained in cardiomyocytes formation, there are other complications that require attention. In fact, some stem cell therapies such as bone marrow transplants with somatic stem cell, do not involve the intricate complexities that tissue growth requires. Yet even these therapies must be addressed as it relates to the other risk factors involved in such a therapy. Risk factors begin in procurement and linger through the many phases involved, beginning with culturing and injection. Subsequent to any initial success, long-term affects must be analyzed. There is deficient clinical experience

in embryonic and adult stem cells. This lack of experience is an inevitable aspect of relatively new, current, and ongoing research and results in a variety of complications, after stem cell injection (Herberts et. al. 2011).

General observations of the three most common forms of stem cells are summarized in Table I. Improved comprehension of the differences and characteristics of these cells will assist in establishing their risk analysis.

An example of a consequence is tumor formation. This is a known complication that is being investigated from different standpoints. Understanding cell growth and death is crucial to such an analysis. The Myc gene family encompassed both factors. It is for that reason that this paper uses c-Myc as an example of a protein that may help limit tumor genesis. In addition fifty percent of tumor formation found in mouse research of iPSC is attributed to c-Myc.

### Tumor Formation

The basic and universal self-renewal factors that assist in feedback and feed forward loops is Oct4, Sox2 and Noanog. However, the Myc gene family is additional self-renewal proteins and factors that affect intrinsic stem cell existence and apoptosis. Research published in 2007 investigated the function of c-Myc in stem cells regulation, differentiation, and apoptosis. Initially the tests were performed on mouse stem cells and later applied to hESC. It was widely known that that c-Myc is required to regulate normal cell development. However, the application of this factor in hESC regulation was confirmed and verified as being expressed as three different factors of the same family (Myc, Mycn, MycL1). Each of these Myc proteins were tested and found in the mRNA of hESC. Furthermore, just as c-Myc was present in regulation of mice cells, it was traced in undifferentiated human stem cells. Testing of this protein suggests that c-Myc is a factor of self-renewal. This research further investigated if c-Myc would also affect apoptosis and differentiation (Sumi et. al. 2007).

The properties of the c-Myc protein are best explained when looking at cancer cells. The protein factor c-Myc is found to be uncontrolled in end-stage cancers. This overexpression in tumorigenic cells helps explain the role it plays in normal cell growth, stem cell proliferation, differentiation, and apoptosis. Even prior to a complete discovery of the potential of c-Myc, it was known that increased levels of c-MYC indicated the initiation of cell death. There are various pathways in which apoptosis is initiated. The first is by the regulator p53 ultimately activating BAX. The second is activation of PI3'K-AKT. The third and final pathway to cell apoptosis is through cell ligations that activate c-Myc. The activation and increase of this protein then caused apoptosis (Prendergast 1999).

Being that this factor plays a role both in the differentiation of stem cells and the death of tumor cells, it must be assumed that investigations on regulation of this protein may be vital to advancements in stem cell therapy. With better control of this protein a clinical laboratory scientist may be able to use it in either furthering the differentiation of cells or leaving them in an undifferentiated state when deemed necessary. The link between the Myc gene family and oncogenic cells will additionally assist with the considerable complications facing stem cell therapy, tumorigenesis.

Aside for the risk of essentially causing tumor formation, stem cell therapy may also affect the growth and formation of existing tumor cells. If, for example, a patient has a dormant tumor of cancerous cells, stem cell therapy may activate it. This has not been proven but is under investigation with the use of mouse stem cells.

### Immune Response

Any donation requires proper human leukocyte antigen matching (HLA); stem cell therapy is no different. Taking care to ensure that the donor and recipient match decreases the risks of Graft Versus Host Disease (GVHD). As detailed in table one, some forms of stem cell therapy are allogeneic. Therapies that are not recognized by the body risk immune rejection. Even those that are immune privileged (hESC) may be subject to rejection and/or an inflammatory response due to the increase in response stimulus, caused by the actual stem cell therapy induction. For that reason, stem cell therapy may be administered while the patient is on immune suppressants. Placing a patient on immune suppressants runs the risk of adverse drug reactions. There are ongoing studies investigating different methods of stem cells growth in vivo that will limit the risk of rejections and GVHD. Such studies include introduction of mesenchymal stem cells in vivo. Data from such research indicated that these stem cells tranquilize the immune system and regulate its responses (Herberts et. al. 2011).

### Teratoma

A teratoma is a benign mass composed of a hazardous mixture of tissues, generally resembling remnants of all three germ layers. It is established in vivo and assumed to be congenital. Being that pluripotent stem cells can create all three layers, this therapy is susceptible to such a risk. In fact the same characteristics that defines stem cell, pluripotency, are the same trait that creates teratomas. Research produced in 2011 details the comparative affects of tumor formation on both hESC and iPSC. The research indicates that because iPSC is a stem cell engineered in vitro and thus a more controlled cell than hESC, iPSC is less susceptible to tumor genesis. In fact the reason that teratomas are found less frequently in iPSC is

because the heterogeneity of this cell causes slower growth rate (Zhang et. al. 2012).

In addition to cell type effects on teratomas, injection sites may also influence distinctive increase or decrease levels of formation. Although not enough clinical research and analysis can confidently support this claim, it is reasonable (Zhang et. al. 2012). More research has been performed on mouse stem cells to detect the correlation of the environment in which the stem cells are injected into and malignant tumors. Although tumor growth differs from teratomas, these discoveries may lead to additional exploration into the plausible affect of increased teratomas based off of site administration (Herberts et. al. 2011).

### Success

Success is a complicated term to define when associating it with ongoing scientific research. Success can be attributed to a research breakthrough or a clinical accomplishment; it's simply relative. Longevity is fundamental determinate of success. Pluripotent haematopoietic stem-cell transplantation for cancer therapy has made significant stride since initial investigations over sixty years ago. It progressed from research that was plagued with complications and hurdles to a standard form of treatment. This blood forming stem cell is the only therapy approved for standard use because of sufficient understanding of the cells, possible complications, and long term affects. Expansion of this therapy for use other than replacement of blood and immune cells causes similar complications mentioned throughout this document. There are many trials taking place and success may be recognized within each trial but a complete understanding of overcoming challenges and complications can only be determined by continual research and the test of time (Stem Cell Information, n.d.).

### Conclusion

Research, along with case studies and accounts show there are far more complications with stem cells than would appear at first glance; they require numerous forms of adjustments to insure an innocuous treatment. Limiting stem cell therapies is a promising prospect, and in some therapies it has been successful. However, there is insufficient research completed at this time to form a definite universal response for limiting any form of stem cell for every therapy application.

Researchers are investigating the factors that cause the risks involved in various therapies and consequently exploring remedies and suggestive solutions. However, stem cell therapies involve a multitude of complexities, some yet to be ascertained. The reason for these uncertainties appears to be based on the varied potential administrations, forms of stem cells (hESC, iPSC, ntES), their associated risks, and the limitless therapies they can

produce. This research is a relatively new and revolutionary field of study. With increase research, funding, and integration of continual findings, selectively listed in this paper, scientists are advancing to the goal of utilizing all that stem cells have to offer. This includes the capability to understand its potential, related limitations, and safely developing the two in an effort to advance medicine. Stem cell therapy is the promise for infinite preventative care and treatment options.

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# Active Immunotherapy and Adoptive Cell Transfer as an Effective Cancer Treatment

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## Abstract

There are many ways to fight cancer using the body's own immune system. Some methods include the administration of vaccines while others involve stimulatory factors injected near tumors. One promising method is enlisting the help of T cells. To fight cancer effectively, T cells must be able to recognize cancerous antigens and the environment in which these T cells reside must be conducive to their function, survival, and proliferation. This paper discusses a method of providing such an environment called adoptive cell transfer, as well as the elements that effect this protocol and the ways in which the environment can be manipulated to increase the effectiveness of adoptive cell transfer.

Many factors contribute to the observation that the effectiveness of adoptive cell transfer increases as immunodepletion increases, namely, the depletion of regulatory T cells. Additionally, the existence of natural killer cells during adoptive cell transfer has been shown to decrease its effectiveness. Also, increased levels of cytokines IL-7 and IL-15 enhance the function, survival and proliferation of transferred T cells which would increase their effectiveness. Moreover, the results of adoptive cell transfer are more positive when patients' own T cells are used.

These findings show that T cells can be used through adoptive cell transfer as an effective treatment for metastatic melanoma patients, and that there is potential for adoptive cell transfer to be adopted as a widespread effective treatment for cancer.

## Introduction

Cancer is a disease characterized by the presence of mutated cells that continue to divide uncontrollably. Cancerous, or malignant, tumors, can spread to other parts of the body especially via the lymph system which can act like a cancer highway, and new tumors can then form in places far from the original tumor source (What is Cancer 2015). These tumors can have negative health effects and in many cases can be life threatening.

According to the World Health Organization more than 14 million new cancer cases were diagnosed in 2012 and there were more than 8 million deaths attributed to cancer. Additionally, the American Cancer Society projects the number of new cancer cases in the United States in 2015 to exceed 1.5 million and the number of deaths to exceed a half million.

Radiotherapy, a common cancer treatment, works by directing high energy beams, including x-rays and gamma rays, at cancer cells to disrupt their DNA and ultimately result in cell death ("Radiation Therapy"). Although radiation therapy can be successful at eradicating cancerous cells, relapse is a common problem because a few cells, or even just one cell, left behind can continue to divide and pose a serious health risk (Wayteck et. al., 2014).

To combat the problem of relapse, chemotherapy is commonly prescribed to ensure that all cancerous cells are eradicated. Chemotherapy works by killing all rapidly dividing cells ("Chemotherapy"). However, chemotherapy has many unwanted side effects as it does not differentiate between healthy rapidly dividing cells and cancerous rapidly dividing cells. This effects many different systems within the body and can cause anemia, hair loss, infection and other unhealthy and unwanted symptoms.

Another form of treatment, immunotherapy, works by using immune elements to target cancerous cells through recognition of cancer antigens. There are two different ways of recognizing cancerous cells through antigens. One way is through tumor specific antigens which are expressed solely in cancerous tumor cells, while the other involves overexpressed antigens which more abundant in cancerous tumor cells than in healthy cells. This method of treatment aims to avoid the side effects of chemotherapy by targeting only cancerous cells and to also thwart the problem of relapse seen in radiation therapy (Wayteck et. al., 2014).

Passive immunotherapy uses stimulatory factors such IL-2 which is injected into the tumor area to stimulate anti-tumor T cells to proliferate, activate, and increase effector functions (Wayteck et. al., 2014). Active immunotherapy, on the other hand, involves CD 8 and CD4 T cells that are primed to recognize specific cancerous antigens and thereby direct immune cells to target and kill these cancerous cells. This report discusses the effectiveness of active immunotherapy in general and, specifically, a branch of active immunotherapy called adoptive cell transfer.

## Methods

To research the effectiveness of active immunotherapy and specifically adoptive cell transfer, relevant information was gathered from many databases and journals. Those databases included: the Touro College Library database, Proquest Science Journals, Pub MEDLINE (EBSCO), and Oxford Journals. The information gathered was narrowed further and analyzed to glean an understanding of the effectiveness of these treatment protocols.

## Active Immunotherapy and Adoptive Cell Transfer

### Discussion

A recent study indicates that active immunotherapy can be an effective treatment for cancerous tumors (Raez, et. al. 2003). This study assessed the impact and response of CD8 T cells to tumor-cell-based allogeneic vaccines in patients with advanced non-small-cell carcinoma, commonly known as lung cancer. However, the selection of patients with advanced stages of non-small-cell carcinoma may have resulted in reduced effectiveness of treatment, as cancerous cells may have been too numerous for the body's immune system to handle. Similarly, as only patients previously unresponsive to conventional treatments like chemotherapy and radiation therapy were studied, the results may have been lower than they might be had the body's ability to fight off harmful cells not already been undermined.

Vaccinations were delivered in three courses. After completion of the first course, only patients with stable disease progression or positive response against non-small-cell carcinoma as determined by CT scan, coupled with low toxicity, continued to a second course of vaccination. Again, patients with no tumor progression and non-life threatening levels of toxicity continued on to a third course of vaccination (Table 1). The patients were evaluated at the beginning and end of each course to study clinical effects and to determine toxicity levels (Raez, et. al. 2003).

The results of the above study, as shown in Table 2, indicate an increase in CD8 T cell response in all but one patient. Clinically, however, the results were less profound. Only 27% of the patients

**Table 1**

	Course 1					Course 2				Course 3			
Study entry	1	2	4	6	7	8	10	12	13	14	16	18	19
Weeks on Study													
Pre-entry-evaluation	x												
Immunization		1	2	3		4	5	6		7	8	9	

**Table 2, Clinical and Immunological response**

Patient # HLA	Response	Fold titer increase	Previous	Survival (months)	Time to progression (months)	Preimmune	First course	Second course	Third course
1005 A1	PD	190	C+R	10	-	0	190	ND	ND
1012 A1	NE	NE	C	15	-	0.2	ND	ND	ND
1001 A2	PD	25	C+S	18	-	0	25	ND	ND
1002 A2	PD	1.6	C+S	22	-	41	65	ND	ND
1009 A2	PD	6.5	C	3	-	2	13	ND	ND
1010 A2	PR	41	S	27+	3	3.8	46	88	157
1011 A2	PD	19	C	11	-	3	30	57	ND
1013 A2	PD	34	C+R+S	2	-	5.2	164	178	ND
1014 A2	SD	19	C+S	13+	3	1.6	30	30	25
1015 A2	PD	0	C+R	7	-	0	0	ND	ND
1003 non	SD	134	S	31+	26+	1	134	113	84
1004 non	SD	424	C+R	23	11	0	424	232	>450
1006 non	PD	9.3	C+S	30+	-	16	150	ND	ND
1007 non	SD	14	C+R+S	29+	23+	1.2	2.8	0.8	0/17
1008 non	PD	32	C	6	-	5.6	178	ND	ND

PD=progressive disease; PR=partial response; SD=stable disease; ND=not determined; C=chemotherapy; R=radiation therapy; S=supportive care; NE=not evaluable

showed stable disease progression while another 7% showed a partial response. This total clinical response of 33% suggests that immunotherapy may be a viable option but requires further study to assess clinical effectiveness. (Raez, et. al. 2003)

The vaccine used in this study was developed by Dr. N. Savaraj through modification of a cell line harnessed from a patient in 1994. The cells were rendered incapable of colonizing ensuring they would not cause any harm to study participants. However, perhaps a more effective albeit more expensive method, demonstrated at the Johns Hopkins School of Medicine (Perica, et.al. 2015), may be to extract a patient's own CD8 T cells from the vicinity of the tumor and subsequently culture them in vitro. This would ensure that the T cells would be reactive to the patient's own cancerous cells. Using pharmaceuticals to regulate the patient's regulatory T cells, the CD8T cells harnessed in vitro can be reinserted with free rein to target cancerous cells (Perica, et.al. 2015).

Adoptive cells transfer is a treatment in which immune cells harnessed in vitro are transferred to a patient to give him specific immune functions. To treat cancer patients using adoptive cell transfer, a piece of a tumor is removed from the body, so that T cells can be removed from the tumor, and stimulated to grow rapidly in vitro. These T cells are then infused back into the patient to target and kill cancer cells (Figure 1).

When attempted on patients with metastatic melanoma, adoptive cell transfer showed potential as an effective treatment although admittedly it had limited clinical results. A trial of 20 metastatic melanoma patients were treated with adoptive cell transfer and given IL-2 to stimulate the transferred T cells. The results revealed tumor regression in multiple sites in 11 of the 20 patients studied (Phan, Rosenberg, 2013). However, it should be noted that although IL-2 promotes the growth and function of transferred T cells, its addition may also help regulatory T cell

**Figure 1**

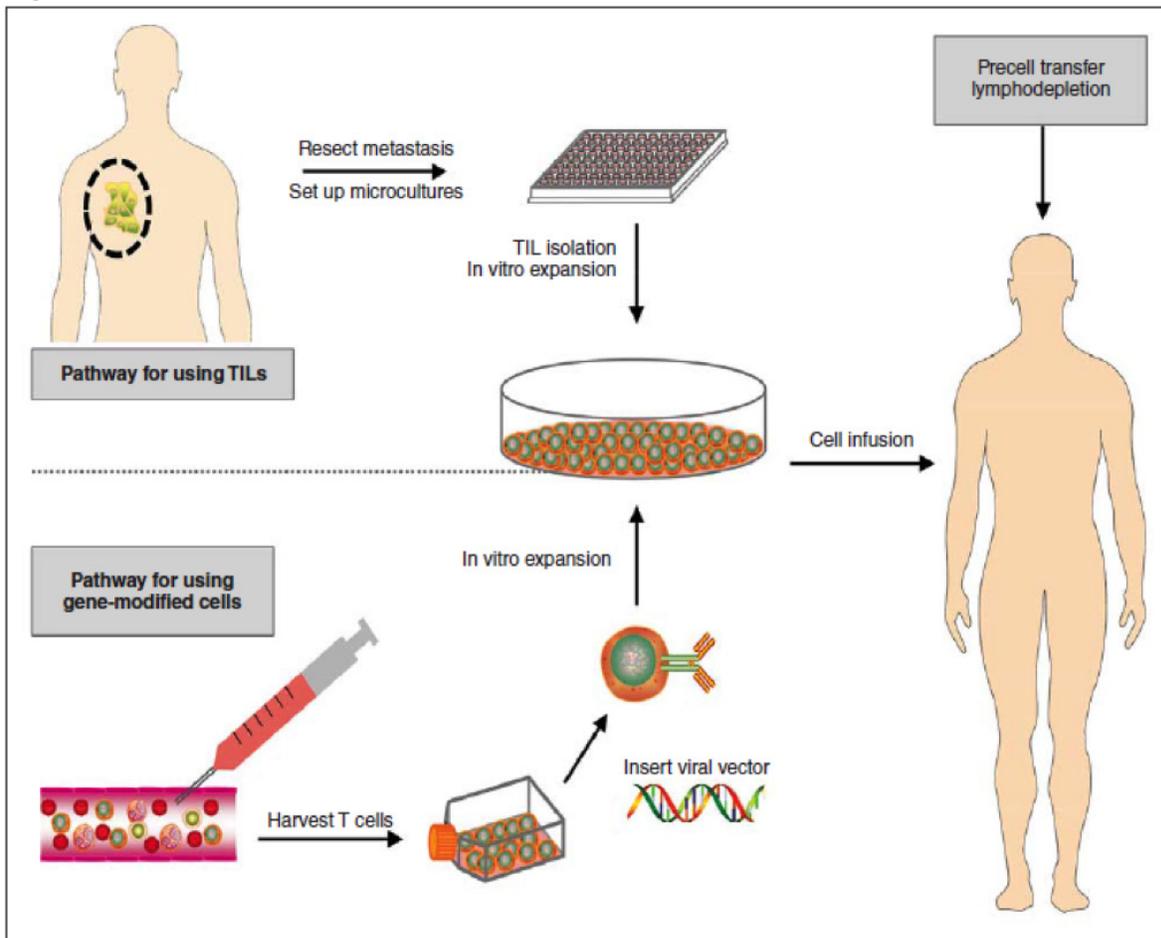


Figure. — Adoptive cell transfer therapy using either autologous tumor-infiltrating lymphocytes (TILs; upper panel) from excised tumors or autologous peripheral blood lymphocytes gene-modified to express engineered T-cell receptors or chimeric antigen receptors (lower panel). Patients undergo a preparative lymphodepleting regimen prior to cell infusion.

Phan, Rosenberg, 2013

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suppress these transferred T cells thus countering the intended effect and perhaps limiting the overall effectiveness of the treatment. This indicates that adoptive cell transfer may have a place in the pursuit of a cure for cancer but it does require some further fine tuning.

Depletion of immune cells and other immune elements before adoptive cell transfer of CD 8 T cells in mice has been shown to increase the effectiveness of transferred T cells (Gattinoni et. al., 2005). It has been proposed that depleting the immune system of its natural elements keeps regulatory T cells from turning off anti-cancer CD 8 T cells transferred during adoptive cell transfer. Additionally, adoptive cell transfer helps lower the immune system's tolerance of the cancerous "self-antigens" by selecting and activating highly specific T cells, mostly CD 8 T cells, and by changing the body's internal environment to one that is more receptive to these cells.

A recent study looked at 13 metastatic melanoma patients who received immunodepleting chemotherapy specifically targeted to regulatory T cells. The patients were then injected with in vitro cultured T cells as well as IL-2. Because the patients first received immunodepleting chemotherapy, in this study the addition of IL-2 avoided the adverse effect of activating regulatory T cells. This allowed for highly favorable results (Table 3) that were significantly more efficient than the results of the study involving non-small-cell carcinoma patients. Of the 13 patients, 6 showed positive clinical responses, and 4 showed mixed responses consisting of considerable shrinkage of at least one tumor. Although the study was relatively small and was limited to melanoma patients, it nevertheless demonstrates the potential of adoptive cell transfer in treating cancer patients (Dudley et. al., 2002).

In a similar trial, metastatic melanoma patients were treated with different levels of immunodepletive therapy prior to adoptive cell

**Table 3**

Patient Demographics, Treatments Received, and Clinical Outcomes.								
Patient	Age/Sex	Treatment				Sites of evaluable Metastases	Response duration (months)	Autoimmunity
		Cells infused (10 <sup>8</sup> -10 <sup>10</sup> )	CD8/CD4 phenotype (%)	Antigen specificity	IL-2 (doses)			
1	18/M	2.3	11/39	Other	9	Lymph(axillary nodes mesenteric pelvic)	PR (24+)	None
2	30/F	3.5	83/15	MART-I gp100	8	Cutaneous, subcutaneous	PR (8)	Vitiligo
3	43/F	4.0	44/58	gp100	5	Brain, cutaneous, liver, lung	NR	None
4	57/F	3.4	56/52	gp100	9	Cutaneous, subcutaneous	PR (2)	None
5	53/M	3.0	16/85	Other	7	Brain, lung, lymph nodes	NR-mixed	None
6	37/F	9.2	65/35	Other	6	Lung, intraperitoneal, subcutaneous	PR (15+)	None
7	44/M	12.3	61/41	MART-I	7	Lymph nodes, subcutaneous	NR-mixed	Vitiligo
8	48/M	9.5	48/52	gp100	12	Subcutaneous	NR	None
9	57/M	9.6	84/43	MART-I	10	Cutaneous, subcutaneous	PR (10+)	Vitiligo
10	55/M	10.7	96/2	MART-I	12	Lymph nodes, cutaneous, subcutaneous	PR (9+)	Uveitis
11	29/M	13.0	96/3	MART-I	12	Liver, pericardial, subcutaneous	NR-mixed	Vitiligo
12	37/F	13.7	72/74	MART-I	11	Liver, lung, gallbladder, lymph nodes	NR-mixed	None
13	41/F	7.7	92/8	MART-I	11	Subcutaneous	NR	None

PR = Partial Response, NR = No Response

Dudley et. al., 2002

transfer in order to establish a level of immunodepletion that would best enhance this treatment protocol (Rosenberg et. al., 2011). One group of 43 patients received a nonmyeloablative preparative regimen, a less toxic method of immunodepletion. A second and third group of 25 patients each, received a more toxic total body irradiation of 2 Gy and 12 Gy (Dept. of Homeland Security 15), respectively, in addition to a non-myeloablative preparative regimen. All three groups were subsequently treated with adoptive cell transfer (Rosenberg et. al., 2011).

The results of this study (Table 4) showed that at higher levels of immunodepletion positive clinical outcomes following adoptive cell transfer increased. In the group that received only a non-myeloablative preparative regimen, 49% of subjects showed an overall response while 5% showed complete response. The group that received 2 Gy of total body irradiation exhibited slightly higher incidence of overall response at 52%, but showed a large increase in complete response at 20%. The group that received 12 Gy of total body irradiation showed a marked overall response of 72% with complete response at 40%, indicating that the greater the immunodepletion the greater the clinical outcome of adoptive cell transfer (Rosenberg et. al., 2011).

**Table 4**

Regimen	No. of Patients	Partial Response n (%)	Complete Response n (%)	Overall Response n (%)
No TBI	43	16 (37)	5 (12)	21 (49)
2 GY TBI	25	8 (32)	5 (20)	13 (52)
12 GY TBI	25	8 (32)	10 (40)	18 (72)
Total	93	32 (34)	20 (22)	52 (56)

TBI=total body irradiation, TIL=tumor-infiltrating lymphocyte

*Phan, Rosenberg, 2013*

One possible method to increase the overall effectiveness of adoptive cell transfer and such positive results is to harness a patient's own CD 8 T cells to ensure that the T cells are as specific to the cancer cell antigens as possible to. However, this is likely a complicated task as it would require development of a "new drug" for every patient. Another method that may enhance the results of adoptive cell transfer is to take a sample of the patients own cancer cells and test CD 8 T cell reactivity to the cancer antigens thereby ensuring a highly reactive T cell response. In another experiment, 20 metastatic melanoma patients received a less intense form of immunodepletion called nonmyeloablative preparative regimen. However, no patient received total body irradiation. In this trial, however, T cells were taken directly from the actual patients' tumor and grown quickly in vitro, as opposed to using previously developed anti-tumor T cells. This may have helped ensure T cells would be highly specific and highly reactive to the patients' own cancerous cells.

Of the 20 patients treated, 10 had an overall response, of which 2 had a complete remission and 8 had a partial remission. Additionally, 4 patients had stable disease, and 6 had progressive disease for an overall response of 70% (Besser et. al., 2010). The results show that nonmyeloablative preparative regimen, coupled with adoptive cell transfer that uses cells harnessed from patients own tumors can be an effective treatment. Furthermore, analysis of the results may show that the results are more positive than what is seen on the surface. Of the 6 patients with progressive disease after treatment, all 6 started with stage M1c melanoma, which represents the stage in which the tumor has traveled to vital organs (excluding the lungs) or when the tumor has traveled to other areas and the patient shows elevated levels of low-density lipoprotein ("How is Melanoma Staged"). At this stage the cancer cells may have been too numerous for the transferred T cells. However, all patients with stages below M1c melanoma and even some patients with stage M1c melanoma showed at least some response to adoptive cell transfer, either complete response, partial response, or stable disease. This indicates that adoptive cell transfer using patient's own T cells, combined with nonmyeloablative preparative regimen for immunodepletion is an effective treatment for early stages of metastatic melanoma patients.

This trial, in which patients received only nonmyeloablative preparative regimen for immunodepletion prior to adoptive cell transfer, significantly outperformed the previously mentioned trial in which patients received only nonmyeloablative preparative regimen for immunodepletion prior to adoptive cell transfer. One possible explanation may be that this trial used T cells taken from removed portions of the patients own tumor, ensuring that the T cells used were highly reactive to the patients cancerous cells.

As previously stated, immunodepletion prior to adoptive cell transfer increases the effectiveness of the treatment. However, immunodepletion effects the entire immune system, which can have life threatening side effects. Therefore further study to determine key components that effect the effectiveness of adoptive cell transfer and how to precisely block them may be helpful.

The most obvious immune element that would reduce the effectiveness of adoptive cell transfer is regulatory T cells. When developing T cells and B cells, the body has mechanisms through which it ensures that no immune cell is released that reacts to self-antigens; however, the system is not foolproof. Regulatory T cells restrain the few immune cells that get through those mechanisms and suppress them ensuring that no self-cells are targeted by the immune system.

## Active Immunotherapy and Adoptive Cell Transfer

It has been shown that regulatory T cells suppress anti-cancer CD 8 T cells in vitro (Antony et. al., 2005). It would be logical to hypothesize that regulatory T cells would also effect the T cells transferred during adoptive cell transfer. To test for a correlation between regulatory T cells and adoptive cell transfer, tumor bearing mice were treated with adoptive cell transfer. Some mice were then injected with anti-Thy-1.2 antibody and complement to suppress function of regulatory T cells, while others were given active regulatory T cells. The results showed that when regulatory T cell function was turned off the transferred T cells destroyed the cancerous cells. However, when active regulatory T cells were added the size of the tumors continued growing exponentially (North, 1982). This shows that regulatory T cells have a large impact on the effectiveness of adoptive cell transfer.

Although regulatory T cells decrease the effectiveness of adoptive cell transfer, simply turning them off while leaving the rest of the immune system intact would allow surviving immune cells that recognize self-antigens to attack patients' healthy cells causing autoimmune disease.

One method to reduce the effect of regulatory T cells on adoptive cell transfer without effecting the entire immune system may be to induce apoptosis only in regulatory T cells in close proximity to the tumor. This would allow the transferred T cells to operate in an environment conducive to their function, while allowing regulatory T cells in other areas of the body to operate freely avoiding autoimmune disease.

Recently, it was suggested that the protein FasL-Fc can be used to deplete regulatory T cells located only in tumors (Chen et. al., 2007). To confine the protein FasL-Fc to the tumor, a protein is incorporated into cell membranes and acts as a trap for the Fc portion of FasL-Fc, not allowing it to escape the confines of the tumor (Chen, Zheng, Tykocinski, 2000). To test this suggestion, tumor bearing mice were injected with FasL-Fc in the tumor region. The results showed a significant increase in apoptosis of regulatory T cells in the tumors treated with FasL-Fc, indicating this may be a viable option to decrease the regulatory T cell effect on adoptive cell transfer without effecting the entire immune system (Chen et. al., 2007).

To test the actual effect of the protein FasL-Fc on adoptive cell transfer, tumor bearing mice were treated with both FasL-Fc and adoptive cell transfer. The results were a significant retardation of tumor growth in a large portion of mice. Additionally, complete tumor regression was seen in 53% of the mice (Chen et. al., 2007). This indicates that using the protein FasL-Fc may be a viable option to eliminate the effect of regulatory T cells on adoptive cell transfer while allowing the immune system to

operate regularly and without the risk of autoimmune effects.

To determine the impact of other immune elements on adoptive cell transfer, tumor bearing mice were tested and the results analyzed. In many studies, after irradiation of cancer bearing mice lacking regulatory T cells, effectiveness of adoptive cell transfer increased. This indicates that regulatory T cells are not the only elements responsible for decreased effectiveness of adoptive cell transfer (Gattinoni et. al., 2005).

One hypothesis is that Natural Killer cells act as sinks for cytokines responsible for survival, proliferation, and function of transferred CD 8 T cells (Gattinoni et. al., 2005). Host Natural Killer cells, also in need of cytokines, compete for the same cytokines necessary to support the transferred T cells leading to a limited amount of cytokines available to support the transferred T cells. To test this hypothesis, tumor bearing mice lacking endogenous B cells and T cells, including regulatory T cells, were treated with anti-NK1.1 antibody to decrease the number of Natural Killer cells to the number found after irradiation of the entire immune system. They were then treated with adoptive cell transfer and compared to mice not given the anti-NK1.1 antibody and treated with adoptive cell transfer. The results showed that removal of Natural Killer cells increased the effectiveness of adoptive cell transfer, indicating that Natural Killer cells play an important role in the function of transferred CD 8 T cells (Gattinoni et. al., 2005).

One way to avoid the effects of cytokine sinks may be to precisely determine which cytokines the Natural Killer cells sink, and stimulate an increase of their production giving the transferred T cells access to those cytokines.

To determine the effects of cytokines on adoptive cell transfer, different cytokines were removed and added to tumor bearing mice treated with adoptive cell transfer. Results indicated that the cytokine IL-7 was needed to maintain survival and continued growth of transferred T cells, but was not necessary to maintain function of the T cells. Conversely, IL-15 was necessary to maintain function, but did not play a part in survival and proliferation of transferred T cells. This shows that increasing both IL-7 and IL-15 at the same time increases the transferred T cells effectiveness against tumors, while decreasing both IL-7 and IL-15 at the same time would decrease the transferred T cells effectiveness against tumors (Gattinoni et. al., 2005).

These findings indicate that there may be a path by which regulatory T cells can be eliminated from the area of the tumor (Chen et. al., 2007), and transferred CD 8 T cells can be stimulated to proliferate and enhance their effector functions without effecting the entire immune system (Gattinoni et. al., 2005).

### Conclusion

In conclusion, increasing CD 8 T cell count alone is not the most effective way to use T cells to fight cancer. Instead immunodepletion together with an increase in anti-cancer T cells delivered through adoptive cell transfer is an effective approach that has been shown to be effective in humans with metastatic melanoma. Other factors besides immunodepletion also effect the results of adoptive cell transfer such as cytokines, and the origin of the T cells injected. The key to adoptive cell transfer success with limited side effects, is the balance of all elements involved which is a large task and requires further study but does seem possible in the future.

However, for now, adoptive cell transfer is still a relatively new method of treatment and still requires immunodepletion for its effectiveness. This can bring with it its own problems, such as an increased risk of infection and a limited ability to fight infection. Additionally, most trials of adoptive cell transfer on humans have been limited to melanoma patients, making further research necessary for it to be used to fight other cancers. To that effect, further studies are currently underway to broaden this treatment to fight other cancers including, lymphoma, leukemia, and neuroblastoma (Deng et. al., 2014) giving hope to cancer patients all over the world.

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# Assessment of Three Acute Responses to Traumatic Brain Injury

By Shoshana Fireworker

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## Abstract

Traumatic brain injury has a devastating effect on millions worldwide each year. As yet, there are no methods which have been proven to improve recovery from the trauma. Current treatment protocols revolve around reducing secondary insult, such as hypoxia, hypotension, and cerebral edema, which raises intracranial pressure. The purpose of this study is to assess the efficacy of three responses to traumatic brain injury. Two of them, the administration of hypertonic saline and the administration of progesterone, are pharmacologic, while the third, the performance of a decompressive craniectomy, is surgically invasive. A number of original studies have been analyzed to develop an understanding of the topic. It was concluded that hypertonic saline should only be given to patients in whom surgery is indicated, while progesterone should be a widespread acute response. For relatively young patients suffering from uncontrollable intracranial pressure, decompressive craniectomy should be considered as an immediate response as well.

## Introduction

Traumatic brain injury serves as a major cause of death and disability. Over one million people are treated for traumatic brain injury in emergency rooms in America each year. Fifty thousand die as a result, while seventy to ninety thousand are left with debilitating long term neurological impairment. Among men under the age of 35, traumatic brain injury as a result of vehicular accident is the leading cause of death and disability (Shear, et al. 2002). Besides for the direct effects of the primary injury, outcomes are affected by the onset of secondary insult. Such insult includes the onset of hypoxia, hypotension, and an increase in intracranial pressure, which is often due to cranial edema. Hypotension has been correlated with a mortality rate which is double that of patients without the condition (Cooper, et al. 2004; Cooper, et al. 2012; Shackford, et al. 1998). As yet, no pharmacologic agent has been proven to improve the outcome of traumatic brain injury, notwithstanding the intense efforts of many researchers. To date, the management of traumatic brain injury consists of preventing further neurologic insults, managing intracranial pressure, and instituting surgical procedures to minimize damage (Shear, et al. 2002; Wright, et al. 2007; Xiao, et al. 2008).

The severity of a traumatic brain injury is universally measured according to the Glasgow Coma Scale, which assesses eye, verbal and motor condition following the injury. The scale which is most often used is the Extended Glasgow Coma Scale, which ranges from 3 to 15, awarding points for various functions which can be achieved. A score on the Glasgow Coma Scale of 3 indicates death or a deep coma, while a 15 indicates complete consciousness.

The outcome of traumatic brain injury is universally assessed according to the Extended Glasgow Outcome Scale, which is highly sensitive. It consists of eight diagnoses, which are: Dead, Vegetative State, Lower Severe Disability, Upper Severe Disability, Lower Moderate Disability, Upper Moderate Disability, Lower Good Recovery, and Upper Good Recovery. This study aims to analyze and assess the effectiveness of three of the current acute

## Extended Glasgow Outcome Scale

Eye Opening Response	Spontaneous-open with blinking at baseline	4 Points
	Opens to verbal command, speech, or shout	3 Points
	Open to pain, not applied to face	2 Points
	None	1 Point
Verbal Response	Oriented	5 Points
	Confused conversation, but able to answer questions	4 Points
	Inappropriate responses, words discernable	3 Points
	Incomprehensible speech	2 Points
	None	1 Point
Motor Response	Obeys commands for movement	6 Points
	Purposeful movement to painful stimulus	5 Points
	withdraws from pain	4 Points
	Abnormal (spastic) flexion, decorticate posture	3 Points
	Exterior (rigid) response, decerebrate posture	2 Points
	None	1 Point

responses to traumatic brain injury, including both chemical and surgical options. Methods used to decrease intracranial pressure include hypertonic saline resuscitation and the administration of progesterone, which has been touted as a neuroprotector. The performance of decompressive craniectomy has also been factored into this study.

## Hypertonic Saline

Hypertonic saline is administered intravenously, often en route to the hospital. The underlying claim to the administration of hypertonic saline is that it restores systemic blood pressure and cardiac output to manageable levels with less volume being necessary than when using the standard Lactated Ringer's solution. The hypertonic saline extracts water from the intracellular space in order to restore intravascular losses and it has a positive inotropic effect on cardiac output. In addition, hypertonic saline has been shown to improve oxygen transport, mesenteric and

coronary blood flow, myocardial contractility, and redistribution of extracellular and interstitial fluid (Mattox, et al. 1991; Shackford, et al. 1998).

### Progesterone

Progesterone is administered in a similar fashion to hypertonic saline. The concept of using progesterone to alleviate problems caused by traumatic brain injury was discovered when researchers noticed a significant sex difference in trauma outcome in correlation to hormonal cycling. Female rats with elevated levels of progesterone showed reduced tissue damage and improved behavioral recovery (Shear, et al. 2002). As progesterone has been used safely on humans for years, it was hoped that it would have a positive effect on people with traumatic brain injury (Wright, et al. 2007). While the mechanism is unknown as of yet, progesterone has been shown to reduce cerebral edema, prevent neuronal loss, improve functional outcome, and inhibit oxidative damage in the central nervous system. It might also promote peripheral remyelination of axons following injury. Progesterone is lipid soluble, so it rapidly crosses the blood-brain barrier to reach equilibrium within an hour of administration (Shear, et al. 2002; Wright, et al. 2007; Xiao, et al. 2008).

### Decompressive Craniectomy

Decompressive craniectomy is a surgical measure which is taken when patients present with dangerously high intracranial pressure. Part of the skull is removed to allow room for the brain to swell. Proponents of decompressive craniectomy state that it can relieve uncontrollable intracranial pressure by increasing the volumetric capacity of the intracranial cavity. Craniectomies have been performed since the early nineteenth century, with varying degrees of success (Akyuz, et al. 2010; Morgalla, et al. 2008).

### Methods

This study was performed through analyzation of published original studies on the effects of hypertonic saline, progesterone, and decompressive craniectomy on traumatic brain injury. Sources include TouroLib databases, PubMed databases, and the archives of Lancet.

### Discussion

Hypertonic saline was found in all cases to be safe for use. Shackford, et al. (1998) do caution that solutions that are too hypertonic are liable to reversibly open the blood-brain barrier, cause cerebral edema, and increase intracranial pressure. However, great care is taken to assure that proper proportions are used. Solutions that are 7.5% hypertonic saline have been shown to be both safe and effective (Bulger, et al. 2010; Cooper, et al. 2004; Mattox, et al. 1991). Administration of hypertonic saline causes a slight increase in intracranial compliance, as shown by a decrease in mean intracranial pressure in patients receiving

hypertonic saline, as compared to a positive trend in the intracranial pressure of patients receiving Lactated Ringer's solution. This data was observed on the second day following trauma (Shackford, et al. 1998).

When administering hypertonic saline, fluid input must be increased, as the saline decreases the fluid balance. No cases of renal failure or neurological complications due to hypernatremia or hyperosmolarity have been found, but precautions should be taken all the same (Shackford, et al. 1998).

Patients receiving hypertonic saline showed a higher rate of nosocomial infection due to the increased rate of the bloodstream. The increased amount of fluid output also led to a higher incidence of urinary tract infections. In addition, administration of hypertonic saline might cause regulation of the A3 receptor on neutrophils, which would cause an increase in susceptibility to infection (Bulger, et al. 2010). Patients receiving hypertonic saline required a greater number of interventions to lower intracranial pressure over the course of their hospitalization, but the difference was not significant (Shackford, et al. 1998).

While many studies have attempted to prove the superiority of hypertonic saline over other standard protocols, such as mannitol and Lactated Ringer's solution, most have been unsuccessful. Out of five studies analyzed, only one study conclusively stated that hypertonic saline was universally preferable to mannitol. In that study, Battison, et al. (2005) administered four doses each to nine patients. Two doses were of hypertonic saline and two were of mannitol, in no particular order. The minimum intracranial pressure was measured ten minutes before each treatment and was compared with the minimum intracranial pressure an hour after each treatment. While hypertonic saline reduced the intracranial pressure slightly more than mannitol did, there was no significant difference. The duration of the effect, however, was seen to be significantly longer in hypertonic saline. This study cannot be seen as conclusive, though, as it was only done on nine patients. In addition, the patients received both mannitol and hypertonic saline over a set period of time. As such, the effect could be due to the presence of both mannitol and hypertonic saline in their systems, and cannot be attributed solely to the hypertonic saline.

Hypertonic saline has been shown to work slightly faster than controls. Cooper reports that patients receiving hypertonic saline had a faster rate of decrease in intracranial pressure than those in the control group. However, the study also states that standard protocols are equally effective. All studies concur that there is no significant difference in favorable outcomes of patients who receive hypertonic saline and those who are treated with standard measures (Battison, et al. 2005; Bulger, et al. 2010;

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Cooper, et al. 2004; Mattox, et al. 1991; Shackford, et al. 1998). While it may improve physiologic parameters, the long term benefit of administering hypertonic saline is negligible. Patients who received 7.5% hypertonic saline and those who received Lactated Ringer's solution showed identical neurologic function after six months. The percentage of patients who survived to hospital discharge was similar. Studies found no significant difference in intracranial pressure for the duration of hospital stay, and there was no discernible difference in Glasgow Outcome Scale values. (Bulger, et al. 2010; Cooper, et al. 2004; Mattox, et al. 1991; Shackford, et al. 1998).

Patients who were to undergo surgery showed a far better outcome when given hypertonic saline in dextran (Mattox, et al. 1991). However, at this time, hypertonic saline in dextran is quite expensive, as compared to the cost of standard treatments, such as Lactated Ringer's solution and mannitol (Battison, et al. 2005). Therefore, rather than staying as a first tier response to traumatic brain injury, perhaps hypertonic saline should be relegated to a measure which is used only when surgery is indicated.

### Progesterone

Aside from its long history of safe use, progesterone has been proven to be completely safe for use in humans. A study was performed with the express purpose of determining the safety of progesterone as a response to traumatic brain injury (Wright, et al. 2007). It was found that administration of progesterone was in no way harmful to patients, and it was indicated that it might be beneficial to their recovery. Furthermore, endogenously released progesterone causes a 1° F increase in core body temperature. This deviation from standard basal temperature might hinder neurologic outcome in a patient with traumatic brain injury. However, the study found that no such increase in body temperature manifested in relation to administered progesterone.

Multiple studies have found immense short term and long term benefits from the use of progesterone as an acute response to traumatic brain injury. Patients receiving progesterone had significantly fewer deaths due to neurologic causes. At 30 days postinjury, the mortality rate of patients receiving progesterone was less than half of that of the control group (Wright, et al. 2007). A significant difference in neurologic outcome was evident up to six months post-treatment, with the experimental group having a 58% favorable outcome as compared to the control's 42% (Xiao, et al. 2008). In addition, treated patients showed a decrease in cerebral edema, necrotic cavity formation, and neuronal loss as compared to control groups, which presented with neuronal and glial shrinkage and neutrophil infiltration. For example, necrotic cavity formation decreased by approximately 20% in treated patients. A decrease in apoptosis

was noted, as well as an increase in peripheral remyelination of axons, an inhibition of oxidative damage in the central nervous system, and an overall enhanced recovery from cortical, cerebral, and spinal cord injury (Pan, et al. 2007; Shear, et al. 2002; Wright, et al. 2007; Xiao, et al. 2008).

There has been much speculation as to the mechanism behind progesterone. It has been ascertained that it works, but not how it works. Progesterone down-regulates the inflammatory cytokine cascade, which can increase the damage caused by trauma. Trauma causes a release of amino acids, which cause neuronal excitotoxicity. Progesterone might act at the GABA receptor to diminish that excitotoxicity (Shear, et al. 2002). The study by Pan, et al. (2007) posits that progesterone acts as a sigma-1 receptor antagonist, which can initiate the opioid-like capabilities of the receptor. In addition, progesterone inhibits nuclear factor kappa B, which is known to be a pro-inflammatory transcription factor. Nuclear factor kappa B is the activator of numerous inflammatory cytokines, including tumor necrosis factor alpha, interleukin-1 beta, C3, and glial fibrillary acidic protein. Thus, inhibition of nuclear factor kappa B decreases system-wide inflammation (Pan, et al. 2007).

The timing and duration of administration are key to the effectiveness of progesterone. Most studies found a correlation between speedy dispensation of progesterone and favorable outcome. Wright et al. (2007) reports that the greatest benefit is gleaned when progesterone is administered within two hours of injury, but that there is still great advantage when it is given within 24 hours. Pan et al. (2007) and Shear et al. (2002) both started treatment within an hour of injury. It was noted that there is a consistent reduction in formation of cerebral edema when progesterone is given within 24 hours of injury (Shear, et al. 2002). There is a consensus that much of the success of progesterone is dependent upon the timing of its initiation.

The duration of administration is slightly more controversial. One study, citing the contradictory effects of inflammatory cytokines, limited the duration of progesterone to 24 hours. The researchers claimed that while immediately following an injury, inflammatory cytokines are neurotoxic, they are later neuroprotective. Seeking to block the initial harmful effect of inflammatory cytokines yet capitalize on their latent neuroprotective abilities, the study administered progesterone from 30 minutes to 24 hours following injury, at which time they ceased administration of the treatment (Pan, et al. 2007). However, other studies noted that the greatest benefit was attained when progesterone was administered for five days (Shear, et al. 2002; Xiao, et al. 2008). In the experiment carried out by Shear, et al. (2002) on rodents, one group received progesterone for three days, while the other received it for five. It was concluded that five days of progesterone

are necessary to achieve the desired results, namely an alleviation of the neuropathological and behavioral abnormalities which are caused by traumatic brain injury. This finding seems to contradict that of Pan's. However, as harmful consequences of extending the progesterone treatment have not been found, it might be advantageous to forfeit the possible neuroprotective capabilities of inflammatory cytokines in favor of the documented benefits of an extended progesterone treatment.

### **Decompressive Craniectomy**

Craniectomy has long been used as a last resort in the treatment of traumatic brain injury. It is only when indicators of incredibly high intracranial pressure manifest that craniectomy is considered. Such indicators include compression of cortical gyri and basal cisterns, signs of immediate herniation, and cerebral swelling (Morgalla, et al. 2008). In the majority of studies analyzed, craniectomy was only used after standard protocols had failed to lower intracranial pressure sufficiently. As such, it is difficult to assess the efficacy of craniectomy, as it is generally used only on patients with severe injuries who have already undergone other treatments. Usually, by the time a craniectomy is performed, there has been a lapse of time between injury and the surgery (Morgalla, et al. 2008; Olivecrona, et al. 2007), limiting its benefit. Another hindrance to the study of decompressive craniectomy is that due to the surgical nature of the procedure, a blinded study is impossible.

While one study analyzed found decompressive craniectomy to lower intracranial pressure but hinder favorable outcome (Cooper, et al. 2012), four other studies found that long term results justify the surgery (Akyuz, et al. 2010; Morgalla, et al. 2008; Olivecrona, et al. 2007; Qiu, et al. 2009). Cooper, et al.'s 2012 study reports that patients who received a craniectomy along with standard treatment showed a less favorable outcome after six months. However, the mortality rate was the same in patients who had undergone the procedure and those who had not. In contrast, Olivecrona, et al. (2007) found a 10% difference in outcome, with those who received a decompressive craniectomy having the more favorable outcomes when compared to patients who received only standard care.

Patients who had undergone a decompressive craniectomy needed fewer interventions to lower intracranial pressure. They required a shorter period of time on mechanical ventilation, and they spent a shorter amount of time in the intensive care unit (Cooper, et al. 2012). There was a significant reduction in intracranial pressure directly after the surgery, which later stabilized to manageable levels (Olivecrona, et al. 2007). One study found that 40% of patients who were otherwise likely to die had favorable results after undergoing a decompressive craniectomy (Morgalla, et al. 2008).

There are a number of caveats in regard to craniectomies. Firstly, patients who underwent the surgery had a higher incidence of hydrocephalus (Cooper, et al. 2012), which necessitates additional medical or surgical care. Secondly, unilateral craniectomies have been linked to delayed intracranial hematomas and subdural effusion, both of which must be treated through surgical intervention (Qiu, et al. 2009). However, success has been seen in bilateral craniectomies. A problem with all decompressive craniectomies seems to be an increase in transcapillary leakage. The point of a craniectomy is to allow room for swelling. The loss of resistance in the brain leads to transcapillary leakage due to an increase in the transcapillary hydrostatic pressure gradient. In order to counteract this effect of a craniectomy, the transcapillary pressure must be lowered by preventing an increase in arterial pressure and infusing albumin and packed red blood cells (Olivecrona, et al. 2007). These issues are all collateral damage of decompressive craniectomies, but the benefit of the surgery seems to outweigh the detriment.

An important factor to consider is the timing of the procedure. While most craniectomies are performed after standard protocol has been tried and found unsuccessful, thus lengthening the amount of time between the injury and the surgery, this might not be the most advantageous use of craniectomies. Akyuz, et al. (2010) compared the results of craniectomies on patients who received the surgery following standard procedure to those who received the surgery immediately. The first group had their craniectomies around 35.7 hours after trauma, as compared to the second group, who underwent the procedure approximately 4 hours after trauma. It was found that early decompressive craniectomy resulted in a 48% decrease in intracranial pressure. The group which underwent standard protocols before receiving craniectomies yielded a 27.8% favorable outcome as compared to the other group, which had a 50% favorable outcome after 12 months. While the best results were found when the procedure was performed within 4 hours of injury, performing the craniectomy within 48 hours still provides benefit (Qiu, et al. 2009). Using craniectomy as a second tier response hinders the positive effects of the procedure, as an extended amount of time with high intracranial pressure is detrimental to functional outcome.

The size of the decompressive craniectomy is also significant. If the craniectomy is too small, it will not allow the brain enough room to expand. The brain will then swell through the incision, causing external herniation. Bilateral craniectomies have the advantage of providing more space for the brain to expand (Akyuz, et al. 2010).

Age is also an integral consideration when determining the outcome of decompressive craniectomy. In fact, the study done by

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Cooper, et al. (2012), which found the least favorable results, was the study which included the oldest patients. It seems that the highest recommended age upon which a craniectomy for traumatic brain injury should be performed is 40, and that it is detrimental to those over 60 (Akyuz, et al. 2010; Morgalla, et al. 2008; Olivecrona, et al. 2007). The greatest benefit is to younger patients. This might be due to a stronger immune system or to an elevated recovery ability in younger patients.

### Conclusion

These three responses to traumatic brain injury are still being researched. From the studies analyzed, it is apparent that due to the negligible benefit and immense cost of using hypertonic saline, it should be reserved for patients with injuries that indicate surgery. The status of hypertonic saline as a first tier response should be reevaluated. Progesterone, on the other hand, should be initiated into standard protocol. It shows no discernable harm and has been proven to do much good. It is essential that it be included in first tier response, as the immediacy of administration bears effect on the amount of good it can do for the patient. Decompressive craniectomy should be considered immediately in a case of uncontrollable intracranial pressure in a relatively young patient, so as to capitalize on the efficacy of a rapid craniectomy. In such a case, the gains outweigh potential damage. However, all assessments must be made on an individual basis, as each patient presents a unique set of properties.

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# The Rehabilitative Potential of Auditory to Visual Sensory Substitution Devices for the Blind

By Naomi Perl

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## Abstract

Living with a sensory impairment is challenging, and those who have lost the use of one sensory modality need to find ways to deal with numerous problems encountered in daily life. When vision is lost, these challenges include navigation through space, finding objects, recognizing people or surroundings, reading or even communicating without access to nonverbal signs provided by others such as eye gaze or facial expressions. Nevertheless, the blind manage to function efficiently in their environment, often to a surprisingly high degree. The key to this amazing phenomenon lies in the plasticity of the brain and the connections it makes after loss of a sensory modality. Based off this theory is the idea that the brain's plasticity allows for the effective use of sensory substitution devices (SSD). Sensory substitution refers to the transformation of the characteristics of one sensory modality into the stimuli of another modality. Primarily, this paper will attempt to answer the question of whether or not auditory to visual sensory substitution devices have the potential to be incorporated into long term rehabilitation efforts for the blind. In order to conclusively answer this question, this paper will discuss how effective these devices are in recreating the lost sense, in terms of acuity, pattern recognition, depth perception, SSD based movement, and sensory perceptions acquired from long term use of SSD's by blind patients.

## Introduction: Neuroplasticity following blindness

Neuroplasticity describes the brain's ability to change its structure and function throughout the course of a lifetime. The largely differing conditions in the brain following early onset sensory impairments such as congenital blindness or deafness, allow for large scale changes that promote a full reorganization of the brain. This may result in a functional network remarkably different from the one seen in healthy individuals. Accordingly, in a congenitally blind person, despite the lack of visual input to the brain, the visual cortex does not degenerate, but rather it receives input from non-visual functions such as touch and audition (Bubic, et. al., 2010). Functional neuroimaging methods validate these findings by showing that the occipital cortex functionally engages in perceptions such as audition (Gougoux, et al., 2005; Kujala, et al., 2005) and tactile Braille readings (Gizewski, et al., 2003). Studies in which auditory (Collingnon, et al., 2006) and tactile processing (Merabet, et al., 2004) were disrupted via transcranial magnetic stimulation to the occipital cortex confirm the necessity of occipital engagement in these non-visual functions. Recently, anatomical studies in primates indicated the existence of projections from the auditory to the visual cortex (Chabot, et. al., 2007) Furthermore, it is important to realize that the involvement of unimodal brain regions (occipital cortex) in cross modal perception (auditory and tactile stimuli processed by the occipital cortex) is not only limited to individuals with sensory impairments, but can under specific circumstances be identified in the general population. The difference is that the cross modal involvement is much more pronounced in those with sensory impairments, perhaps due to increased neuroplasticity, as sensory areas deprived from their customary sensory input become integrated into other neural circuits, affecting the entire system as a whole (Bubic, et. al., 2010).

## Sensory substitution devices/ the human-machine interface:

Sensory substitution refers to the transformation of the characteristics of one sensory modality into the stimuli of another modality. For example, it is possible to replace vision with touch or audition, and to replace audition or vestibular sense, with touch. This paper will focus on sensory substitution devices (SSD's) which replace vision with audition, otherwise known as auditory to visual sensory substitution devices. In general, auditory to visual SSD's capture visual information via a video camera, which then transforms the images into auditory input that is conveyed to the user using headphones or an earpiece (Bubic, et. al., 2010). (Figure 1)

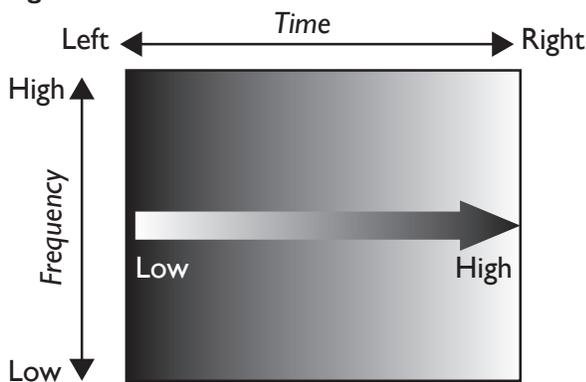
Figure 1



The general concept of a sensory substitution device (SSD) and a typical visual to auditory setup. SSD's typically include a visual capturing device, a computational device transforming the visual input into auditory input, and an output device, transforming this information to the user (Bubic, et. al., 2010).

Different auditory to visual SSD's primarily differ in the conversion algorithm the computer utilizes to transform the visual information into auditory input. To formulate an answer regarding the rehabilitative value of auditory to visual sensory substitution this paper takes into account the following selection of auditory to visual SSD's which are used in the various studies cited later on. The first auditory SSD developed is the vOICe system. The capital letters conveniently stand for "Oh I See". The vOICe utilizes a conversion algorithm in which for every image picked up by the camera, the vertical axis is represented by frequency, the horizontal axis is represented by time and stereo panning, and the brightness of the image is encoded by the amplitude of the sound (loudness). The resulting sound encoding the image is termed a "soundscape" (Bubic, et. al., 2010). (Figure 2) The voice can theoretically generate a resolution up to 25,344 pixels (Striem- Amit, et al., 2012).

**Figure 2**



*A schematic summary of the vOICe algorithm. Time and stereo panning constitute the horizontal axis in the sound representation of an image, tone frequency makes up the vertical axis, and loudness corresponds to pixel brightness (Bubic, et. al., 2010).*

Another more recently developed device is the Prosthesis for Substitution of Vision by Audition (PSVA). The algorithm is similar to vOICe, except the PSVA uses a frequency mapping to map horizontal position as well as vertical position. Thus, the frequency associated to each pixel increases from left to right and from bottom to top. Furthermore, in order to enhance the similarity with the human visual system, the receptor field of the PSVA has a higher resolution in the center of the picture to simulate the human fovea (Hanneton, et al., 2010) In contrast, the PSVA has a maximal theoretical resolution of only 124 pixels (Striem- Amit, et al., 2012). Another device known as the Vibe converts a video stream into a stereophonic sound stream. It uses a virtual retina composed of two levels of "cells"; sensors and receptors. Each sensor corresponds to a particular pixel. The activity of the sensor is a function of the coded components of the captured pixel. A receptor has a receptive field

determined by a set of sensors. So, a receptor is concerned by a particular area of the captured video frames. Each receptor produces a signal that can be interpreted as a sound. The signals of all the receptors are mixed together to produce a stereo audio output that can be adequately adapted for human perception (Hanneton, et al., 2010). Lastly, Israeli Scientists have come up with a new device called EyeMusic which uses an algorithm that is similar to vOICe algorithm in most respects, except that it also conveys color. It distinguishes color by using different musical instruments for each of the four colors: white, blue, red, and green. Black is represented by silence. In order to increase the pleasantness of the sound the device was created with a relatively low resolution of 24 by 40 pixels, is (Abboud, et. al., 2014)

### **Occipital Activation through the use of Sensory Substitution Devices**

Recent neuroimaging studies have investigated the neural bases of sensory substitution, raising questions about the nature of sensory substitution in blind versus blindfolded individuals. (Poirier, et al., 2007a) Studies show Occipital activation in both blind and blindfolded sighted subjects while using auditory SSD's. Using Positron Emission Tomography (PET), Arno and colleagues have shown that pattern recognition using the PSVA induced the recruitment of extra-striate occipital areas (BA 18 and 19) in early blinded subjects, and to a lesser degree in blindfolded sighted control subjects (Arno, et. al., 2001). In another study PET was used to show activation of the visual cortex during depth perception using an auditory SSD. In this study blindfolded sighted volunteers used the PSVA to determine depth based on the relative target size, the proximity of the target to the horizon, and the linear perspective. The exercise was found to involve the extra-striate area BA 19 (Renier, et. al., 2005). A study using functional Magnetic Resonance Imaging (fMRI) has shown that pattern recognition through an auditory to visual device can induce the recruitment of striate (BA 17) and extra-striate (BA 18 and 19) areas in blindfolded sighted subjects (Poirier et al., 2007b). These studies show similar occipital activation in the blind and sighted when using these devices, however the basis of the visual activation in each subject group is debated. In general, the use of SSD's seems to induce visual brain areas via two processes: mental imagery and cross modality. Though cross modality, a function of the brain's neuroplasticity, seems to be the primary basis of SSD use, mental imagery may play an important part in the process, especially for blindfolded sighted users. Both blind and sighted people can perform mental imagery. The phenomenon of cross modality, though more significant in the blind, occurs in the sighted too. So, both processes can be performed by either group. In addition, both mental imagery and cross modality are known to activate the visual area of the brain. After reviewing the data a study by Poirier et al proposes that perhaps mental imagery is predominant in sighted subjects, and

## Auditory to Visual Sensory Substitution Devices for the Blind

cross-modality is predominant in blind subjects (Poirier, et. al., 2007a). Both populations may fare better in different respects following this proposal. The sighted or for practical purposes late onset blind, who predominantly utilize mental imagery, will be able to better associate the cross-modal input to the properties of vision as they knew it. On the other hand, the early blind who lack such understandings of the visual world, and instead primarily utilize the cross modality of the brain, due to more highly developed cross modal networks and plasticity, seemingly have a larger potential in the realm of sensory substitution.( Bubic, et. al., 2010) In general, this paper will value this proposal, and when analyzing various studies which utilize blind and/or blindfolded participants, it will acknowledge the discrepancy in their use of SSDs, and the effect that these differing modes of use have on the results when applicable, while still focusing on the broader picture. This approach will help when trying to determine an answer regarding the rehabilitation potential of these devices for the blind, as this paper is less concerned with the effects and potential of these devices in regard to sighted individuals.

### Methods:

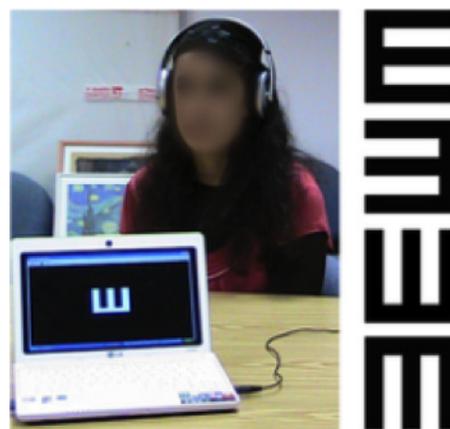
The information in this paper was obtained from Touro College's online database and from various online medical journals. All of the data is experiment based, collected and analyzed to best answer the question of how effective SSD's are and to analyze their potential to be incorporated into rehabilitation efforts for the blind.

### Discussion: Acuity

An important factor regarding the usefulness of SSD's is the amount of detail resolvable to the user. To measure this, a study was conducted using the Snellen E- chart visual acuity test. This test, generally used by ophthalmologists, was adapted into an auditory version via the vOICE technology, and used to test the visual acuity of a group of eight congenitally blind, and one early onset blind individual. Each participant was trained for several months in a two hour weekly training session, by a single trainer, on a one by one basis. The training program was composed of two features. One part focused on structured two dimensional training, in which the participants were taught how to process two dimensional static images such as letters, numbers, faces and houses using the vOICE. The second part, live view training, focused on visual depth- perception and training in hand "eye" coordination using the vOICE. The test was conducted by playing soundscape stimuli in a pseudo randomized order of E directions. Patients had to state the rotation of the letter E (up, down, left, right). (Figure 3)

The results, analyzed on both a group level and on an individual basis, showed that group performance differed statistically from

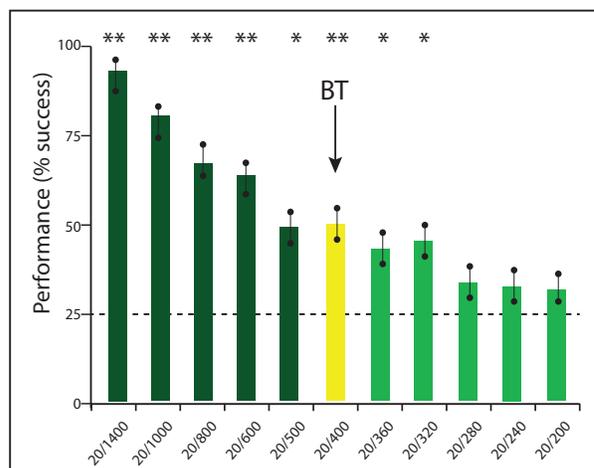
Figure 3



A blind participant perceiving an image of a large Snellen E and identifying its direction (Striem-Amit et. al., 2012).

chance level, and individual visual acuity scores varied between 20/200 and 20/600. Five of the nine participants had visual acuity that exceeded the visual acuity threshold for blindness of 20/400 as defined by the World Health Organization (WHO). (Figure 4)

Figure 4



The group performance on the Snellen acuity test, also displayed is the WHO Blindness Threshold (BT) (Striem-Amit et. al., 2012).

The study also showed that participants were able to identify and mimic the body posture of a person standing a few meters away and navigate in crowded corridors while avoiding obstacles. One of the participants was able to identify live three dimensional emotional facial expressions. Capabilities such as these provide a basis for the device to be used in natural settings, which are sufficiently more complex than those present in the study. (Striem-Amit et. al., 2012) Another study also investigated the acuity of users of the vOICE via the Snellen E

test. This time, however, all the participants were blindfolded sighted vOICe users who received no training prior to experimentation. In this way the study aimed to provide a benchmark measure of acuity, because in the previous study it is unclear whether the acuity levels achieved are due primarily to the resolution of the device, or rather the compensatory neural plasticity of the blind participants, combined with their expertise in using the device. The participants included 26 adults all of whom reported normal vision. The participants completed two experimental procedures using the Snellen E test. In the first test scores of 20/2464 and 20/4682 (in comparison with normal vision: 20/20) were achieved by the highest number of participants. In the second test a score of 20/2464 was achieved by the highest number of participants, showing a marked improvement from the first test. In general, the acuity results from this study are much lower than those of the previous. However, the participants in this study received less than 1% of the training received by participants of the previous study. In addition, since all the participants of the previous study were blind this was probably a factor in their high performance. Nonetheless, these results are significant, because they show that very little training or explanation is required to carry out this task using an SSD (Haigh, et. al., 2013). This study is important because it helps to define the minimum capability of the device. The participants were both blindfolded and received no training and still managed to extrapolate results, showing the even greater potential of the device with blind users who will use the device after many hours, days or even years of practice, and who may also have developed compensatory neuroplasticity. In addition, these studies of acuity along with future studies of acuity using auditory SSD's, are important in providing a standard measure of acuity for comparing sensory substitution algorithms, and individual differences in sensory substitution acquisition. Furthermore, a standard measure of acuity might be helpful to test the resolution and precision of synesthetic experiences described by long term users of SSD's as described below.

### Pattern Recognition

One of the most important developments in information technology is the graphic user interface (GUI). However, the GUI has presented a new challenge for blind people given its inherent visual nature, with icons, multi windows, and mouse-based command structure. Similarly, in the current world of communication, graphics play an increasingly important role. Numerous graphs, charts, diagrams and other forms of visual communication are included in documents intended to be read by sighted people. Thus, blind people remain at a great disadvantage for graphical information access. In a study by conducted pattern recognition in a computer environment was investigated in six early blind and six blindfolded sighted subjects using the PSVA. By comparing performance of both groups, the study aimed to

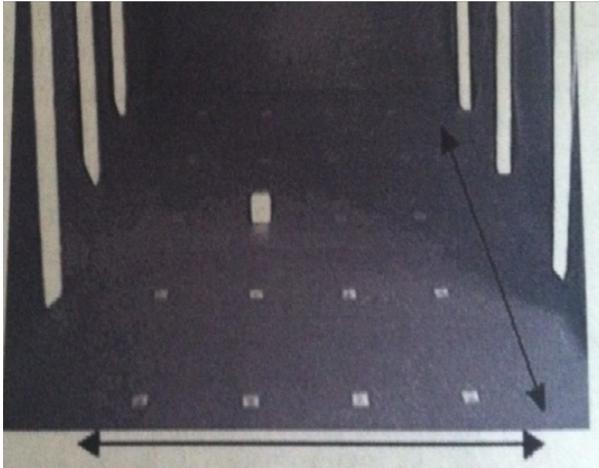
investigate the effect of early visual deprivation on recognition of visual patterns. Subjects were trained during twelve, one hour sessions. During training the subjects learned how to recognize patterns displayed on a PC screen by exploring the graphics tablet with a pen. Subjects heard sounds related to their hand movements and had to recreate the pattern in a frame using aluminum dots and strips. At the end of training, learning was tested by having the subjects recreate patterns which they had encountered during training. Performance was evaluated on the basis of response accuracy and processing time needed to answer. Accuracy was assessed by finding the number of common points between the subject's recreated pattern and the actual pattern. The results showed that early blind and blindfolded sighted subjects are able to recognize patterns from auditory feedback related to hand movements, but the early blind scored better in both accuracy and processing time. This suggests that mental imagery is not a prerequisite for the development of these representations. The results are encouraging from a rehabilitation point of view by showing that pattern recognition in a computer environment is possible using a vision to audition coding scheme without previous visual experience. (Arno, et. al., 2001)

### Depth Perception

Sighted people use depth perception for many applications such as obstacle avoidance, navigation, object localization, and grasping. A study investigated how early blind subjects interpret visual depth cues and use them to locate objects using the PSVA SSD, and how sensory substitution can contribute to the development of depth perception and visual perspective in early blind subjects through interactions with the environment. The participants included twenty blindfolded sighted volunteers and ten early blind individuals. All received training in recognizing 2D shapes with the device. None of the participants had previously used the SSD to localize objects, or to explore a 3D environment. The experiment was divided into a pre-test, a practicing session, and a post test. Results from the pre and post-test were compared to see the effect the practicing session had on each of the subjects groups. During the pre-test subjects explored a three dimensional set up via a head mounted camera. The set up consisted of a black table surrounded with six white poles with twenty preselected positions on the table for a white cube. (Figure 5).

The cube was placed pseudo randomly at different positions during the experiment. After exploring a specific set up, the PSVA was turned off, the cube was removed, and the subjects had to replace it by hand in its initial location. Scores were calculated by finding the difference between the correct position and the position the subject placed it in. Each participant completed 20 set ups. In sighted subjects the mean error score was

**Figure 5**



*View of the 3D display used in the experiment. The perceived size, height in the field view and the geometrical perspective induced by the poles can be used as depth cues to estimate the egocentric distance of the white cube. The arrows indicate the width and depth axes of the scene. (Renier, et. al., 2010).*

about 11 cm for the depth and about 5.5 cm for the width. In early blind subjects, the error score was about 19 cm for the depth and 5 cm for the width. These results show better performance in sighted subjects for the depth and little difference for the width. These results are explained due to the fact that sighted subjects used their knowledge about visual depth to perform the task with the PSVA, in other words using a form of mental imagery, while the early blind subjects were affected by their lack of visual experience. Then, during three 45 minute practicing sessions, subjects got to practice with the PSVA in the 3D display. All subjects had the same number of trials to practice with the PSVA. The post-test following the practicing session consisted of the same procedure as the pre-test. When comparing the scores, significant improvement was seen in the early blind subjects for determining depth, while on a whole there was no significant improvement in the sighted subjects. Only those in the sighted group who were the least accurate in the pre-test showed an improvement in the post test, seemingly representing a ceiling level for accuracy in sighted subjects. However, the improvement seen in the early blind was probably due to enhanced tactile and auditory abilities which contributed to an optimization of the learning process, thus enabling them to learn how to correctly use visual depth cues. With a longer practice period it is likely that early blind subjects would outperform the sighted subjects. These results suggest that visual experiences via an SSD can help blind people learn visual perspective and pictorial depth cues both of which are key for depth perception. (Renier, et. al., 2010)

## **Fast accurate reaching movements with a visual to auditory SSD:**

Previously this paper has discussed acuity, pattern recognition, and depth perception using auditory SSD's. A further study discusses SSD's in regard to sensorimotor integration, which is critical in the effort to make SSD's relevant for everyday tasks, including making accurate reaching movements toward objects, and interacting with people and objects. The purpose of the study was to test the use of auditory SSD's to guide a fast reaching movement. This study utilized the EyeMusic SSD device, and involved 18 sighted participants who were naïve to the use of SSD's. The experiment consisted of a familiarization session and a test session. During the test session participants used a pen shaped stylus to perform 2D reaching movements with their dominant hand on top of a digitizing tablet. Movements were made from a center location to one of four 2 cm radius targets, represented by a white square located 6 cm from the center. In the testing session, participants performed two blocks of trials, which differed by the type of feedback provided: either auditory (SSD) or visual (VIS). During the SSD block participants were blindfolded, while during the VIS block, the participants' arm was placed under an opaque cover, such that they did not have direct visual feedback of their hand. (Figure 6)

**Figure 6**



*The experimental setup: A participant performing the SSD block blindfolded (on left). A participant performing the VIS block, with his forearm hidden from view by an opaque cover (right) (Levy-Tzedek et. al., 2012).*

Participants did not receive feedback on their movement path, but received feedback on the location of their hand at the end of each trial. If the endpoint location was within 2 cm of the center of the target the trial was considered successful and only feedback on the location of the endpoint was given in the form of a blue square. However, if the end location of the hand was farther than 2 cm away from the center of the target, feedback on the location of both the target square (white), and endpoint (blue) was given, such that participants could use their end position relative to the target to correct future movements. During the SSD block the participants did not see the squares when receiving feedback, but could distinguish between the

target and the endpoint square because of special eye music algorithm which allows for color incorporation, piano for white and marimba for blue. The following measures were used to characterize participants reaching movements under the two feedback conditions; movement time: the time elapsed from the movement onset to termination, peak speed: the maximal hand speed during the movement, path length: the total displacement of the hand from the beginning to the end of the movement, and endpoint error: the distance between the hand's final position and the target. Surprisingly, there were no significant differences between movements performed with SSD feedback compared to those performed with visual feedback in term of movement time, peak speed and path length. Average endpoint error in both types of movements was smaller than 0.5 cm. It is likely that with further practice participants will be able to perform movements with an even smaller endpoint error using the EyeMusic SSD. The main limit on the study is the fact that it did not include any blind subjects. As previously explored, because the subjects were sighted it is possible that rather than using auditory information directly to control movement, they "translated" it into a visual image of the target location, and acted based on this image. However, the study is strengthened by the fact that there was no visual information given of the target directly before or during the testing block, so there was no possibility to perform any vision based calibration between trials, which could help to improve subsequent trials (Levy-Tzedek et. al., 2012) Furthermore, the use of sighted subjects is auxiliary, because it allows for the movements using an SSD to be compared to the movements of the same individuals using sight. This helps to better define this capability using the device. With blind subjects there would be no comparison point, because the subjects can only reach out blindly. Nonetheless, a future experiment with blind individuals would reveal the ability the ability of blind subjects to create a spatial representation, and act on it, without the possibility of mediation by visual imagery. Still these results are important since those obtained from the SSD block can be used in a future study as a comparison point for blind individuals using an SSD. Furthermore, if the blind can replicate the accuracy level reached in this study by the sighted subjects, then performing daily tasks with an SSD is feasible, and thus the prospects of the rehabilitative use of SSD's are broadened.

### **Novel SSD's what they offer to the world of rehabilitative techniques: Eye Music and The Vibe**

Thus far, various experiments have demonstrated the ability of blind users to use SSD's on various levels. However, most auditory SSD's generate unpleasant sounds and also lack color information. For some this provides for a bland and somewhat irritating experience at times. Eyemusic was created to address these issues by using natural instruments to convey visual information in a pleasant manner, while also conveying color

information. Different instruments represent different colors and the ceiling frequency was limited to 1568 Hz, because high frequency ranges have been linked with unpleasantness. A study was conducted to see if the device accomplished these goals. The study included twelve blind participants, and ten sighted blindfolded controls. Part of the study included a survey which asked the participants to compare the pleasantness of EyeMusic with the pleasantness of the vOICE, the leading algorithm for many auditory SSD's. This was done by generating two second soundscapes using the vOICEe SSD and EyeMusic in their default modes with a two second break between them. All but two participants scored the soundscapes generated by the EyeMusic as more pleasant on average. Of these two participants one scored the vOICEe soundscapes as equally pleasant on average, and the other found the vOICEe soundscapes to be slightly more pleasant on average. On a whole these results are promising suggesting that EyeMusic could be a step forward in terms of user experience. Notwithstanding, the increased pleasantness and potential for prolonged use come at the expense of image resolution. Therefore, EyeMusic could be useful for tasks that require prolonged use or when color is valuable to the user, while other devices, such as the vOICE, that offer higher resolution could be used for tasks demanding finer detail. One device does not have to replace the other; rather, each with its specific characteristics can be utilized at different points to augment the rehabilitative process. In addition, since the sounds generated by the device are relatively pleasant this may encourage users to use the system as pleasant sounds are said to induce positive emotions ( Abboud et. al., 2014) The Vibe is another SSD developed that offers extra versatility, an important feature when trying to tailor the device for a specific user. The mechanism of the device is described above briefly. This unique format allows for several innovations. The first is pre-filtering of the video stream. A useful example of this is the application of a threshold to the captured pictures. The threshold can make a great number of receptors silent, and consequently can make the resulting audio signal less complicated and more comfortable for the user. A second example of pre-filtering is the use of a filter that computes as input to the Vibe a time difference of successive captured frames. With this kind of filtering, instead of producing a continuous and complex audio stream, the Vibe will generate sounds only if there are changes in the video stream. This functioning can also be more comfortable and less tiresome for the user than the static solution that continuously produces a complex sound. One can imagine building filters that combine thresholds and time differences for an optimal user experience. Another adaption is sensor distribution which affects the pixels addressed by each sensor of each receptive field. Receptive fields can thus be large or small, identical or different, overlapping or non-overlapping. The distribution of the sensors on the 2D plane of captured pictures can affect the perceptive abilities

of the user. Thus, the sensor distribution can be modified for specific users and in specific situations when different effects are wanted. Finally, the Vibe also offers the possibility to enhance the binaural perception and differentiation by the listener of the sound by adding inter-aural disparity cues to the sound like interaural time differences. This can be done by adding delays to the transfer functions of the receptors (Hanneton, et. al., 2010) The versatility of the Vibe, allows for extra capabilities, which enhance the user experience and customization, allowing it in these respects to serve as a better rehabilitative tool.

### Long Term visual experiences in the blind induced by SSD's

Ward and Meijer (2010) investigated the phenomenology of two late onset blind users of the vOICe system. The users both report detailed visual phenomenology that developed within months of immersive use and has continued to evolve over a period of years. In addition, their long term use of the device seems to have produced an acquired synesthesia. Synesthetic experiences are percept in nature and are elicited by a stimulus and occur involuntarily. Furthermore, the experienced sensation co-exists with the induced one rather than replacing it. So, for a synaesthete a sound is seen, but it is also heard. This effect may be due to different mechanisms of plasticity that emerge after long term use of the device. One is unmasking of existing cross modal connections, and another is a slower reorganization perhaps associated with changes in synaptic connectivity. The two subjects in the study PF and CC both became blind at the ages of 21 and 33 respectively. PF currently has a small amount of light perception in the left eye, while CC has a low visual acuity that enables her to count fingers in front of her, and notice large objects in strong contrast. Both were asked a series of questions to understand their visual experience, how it developed, and the extent to which it comprises an acquired synesthesia. Initially, both were asked about perceiving edges, contrast and acuity. PF reports visual experience in terms of a non-detailed grey scale sketch. She says, "I cannot tell fine little tiny details. Rather my vision is based upon black and white and all the little gradients in between. The best way I try to describe this to people is : take a large black sheet of paper; now take a magical piece of white chalk and sketch me here on this stage in line drawing now make me three dimensional..." CC, like PF also claims not to get enough visual detail to identify a person's sex or age with the vOICe, but can sometimes differentiate sex based on clothing. She says, "I could tell whether they had a long coat on or shorts..." Both PF and CC report being able to perceive depth, but that the ability occurred gradually, and only after having flat visual experiences of edges and shading. PF described the acquiring of depth perception as a sort of eureka moment that occurred while she was washing dishes as she looked down at the sink and realized with astonishment, "Oh

I can see down. I can see depth." In addition, both were asked about perceiving movement. The vOICe software normally converts one visual image per second into a soundscape and is not well suited for detecting fast moving objects. However, both PF and CC no longer report any subjective experience of jerkiness, nor is their experience a series of snapshots. PF describes it like using a flip book, "You don't see the different breaks between images." Likewise CC says at first it was like a very jerky movie, but now she experiences smooth movement. As far as acquired synesthesia, both PF and CC claim to be able to 'see sounds' when not using the vOICe. Their brains have internalized the vOICe rules for mapping between hearing and vision, and the rules are applied, both, when the device is worn, and when it is not. CC describes her synesthetic experience: "Monochrome artificially induced synesthesia, only in certain frequencies of sound. A small price to pay for very detailed vision, but the consultant's music next door sets me off as well (Bach Mass in B Minor)... It is not triggered by all sounds but by vOICe like sounds. It is almost as if you had a computer with two monitors running simultaneously different pictures... and sometimes you switched your attention between both." PF also gives visual descriptions, all monochrome, to a number of simple sounds. In addition, PF also believes her synesthetic experiences are stable, a hallmark feature of developmental synesthesia. On a whole, both the sound of human speech and the sound of most instrumental music do not elicit visual experiences. This study is enlightening, but also raises further questions. Would congenitally blind users acquire an altered sense of visual like space as a result of using the device, or is prior vision a prerequisite for these experiences that go beyond the scope of what the vOICe SSD delivers in most experiments? Furthermore, are the particular mappings used by the vOICe special in some way, or could any consistent mapping between vision and audition lead to these kinds of experiences? It is noteworthy that for both their phenomenology has developed over time. The length of time using the device may be the key to both of these questions, but further study on a larger sample size would be necessary.

### Conclusion

The data from the studies supports the use of SSD devices in the rehabilitation for the blind. Most studies comparing the use of blind versus blindfolded volunteers showed increased potential for blind users using these devices. However, more data should still be collected. The present studies are limited by the relatively small sample size of volunteers, and the lack of real data following long term use. Additionally, the testing set up of the experiments cannot parallel the real world which is fast paced and demanding, so it would have to be determined if the clear benefits as demonstrated under testing conditions could be utilized in a real world setting, when the subject has little room for error. In the long term, sensory substitution devices

seem to be both a promising and innovative addition to the rehabilitation of blind people.

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# Is the Neuraminidase Inhibitor Tamiflu Effective in the Treatment of Influenza?

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## Abstract

Influenza is a disease that has caused the deaths of tens of millions people in the last century alone. The influenza neuraminidase protein is essential in the mechanism infection. It enables the virus to leave the infected cell and proliferate. Antiviral neuraminidase inhibitor drugs can be used for treatment. The drug Tamiflu is the standard of care for both treatment and prophylaxis of influenza. The Cochrane reports of 2009 and 2014 conclude that evidence is lacking to support this. Numerous bodies disagree. Cochrane also question the accuracy and credibility of many studies and agencies in support of Tamiflu. This paper explores the issues.

## Introduction

Influenza is a viral disease that routinely causes significant morbidity and mortality. Influenza pandemics have been responsible for the deaths of tens of millions of people in the last century alone. Science has been desperately searching for any agent to mitigate the effects of this infectious disease.

Tamiflu (a neuraminidase inhibitor) was brought to market in the last 20 years with great fanfare and hope. During the 2009 influenza pandemic government agencies and hospitals spent billions of dollars stockpiling the Tamiflu. It was widely touted by health professionals and the media as an effective “silver bullet” for the disease. In 2009 The Cochrane Report created a tremendous uproar with a British Medical Journal (BMJ) report that concluded that Tamiflu was not effective. Despite the controversy, Tamiflu continues to be recommended for use at clinics and hospitals as first line defense for treatment as well as prophylaxis (Zachary 2015).

Like any medication, use of Tamiflu involves financial and other costs i.e. side effects. Given that it is not a benign drug, it behooves us to explore the controversy in detail.

## Methods

The information for this paper was obtained from many online resources. Many of the databases and journals used were accessed through the Touro library database and PubMed.gov. Much of the background information and pictures were accessed through Dr. Vincent Racaniello's (College of Physicians and Surgeons of Columbia University) “Virology 101” blog.

## Background

Influenza is a viral infection that attacks the respiratory system (the nose, throat and lungs). It is caused by one of the three types of influenza viruses A, B and C. Influenza viruses can be spread by airborne droplets (aerosols) person-to-person contact, or contact with contaminated items (fomites). Airborne spread appears to be the most important mechanism. A single sneeze can generate up to 20,000 virus containing aerosol particles. Aerosolized particles produced by these activities are of different sizes. The largest droplets fall to the ground within a few meters and will transmit an infection only to those in the

immediate vicinity. Smaller droplets can travel long distances determined by their size.

Onset of symptoms ranges from 1 to 4 days with an average of about 48 hours. Symptoms include sudden onset of chills, fever, cough, and generalized aches and pains. Severe Headache is common. In mild cases, many symptoms are like those of a common cold e.g. sore throat, runny nose, and mild conjunctivitis may also occur. After 2 to 3 days, acute symptoms rapidly subside, although fever may last up to 5 days. Cough, weakness, sweating, and fatigue may persist for several days or occasionally for weeks.

Influenza-related pneumonia is an important cause of increased morbidity or mortality in high-risk patients. Encephalitis, myocarditis, and myoglobinuria, sometimes with renal failure, develop infrequently after influenza A or B infection. Patients at higher risk are those with: underlying illness, acute respiratory distress syndrome, primary influenza pneumonia, or secondary bacterial pneumonia. These include: Children under the age of 4 years; adults over the age of 65 years; people with chronic medical disorders (e.g., cardiopulmonary disease, diabetes mellitus, renal or hepatic insufficiency, hemoglobinopathies, immunodeficiency); women in the 2nd or 3rd trimester of pregnancy and patients with disorders that impair handling of respiratory secretions (e.g., cognitive dysfunction, neuromuscular disorders, stroke, seizure disorders).

Aside from the use of antivirals such as Tamiflu treatment is symptomatic. This includes rest, hydration, and antipyretics as needed. Appropriate antibiotics are necessary for treating complicating bacterial infections. Antiviral drugs given within 1 to 2 days of symptom onset may decrease the duration of fever, severity of symptoms, and time to return to normal activity. The two main drug types are the neuraminidase inhibitors Oseltamivir and Zanamivir, and the adamantane drugs, Amantadine and Rimantadine. Neuraminidase inhibitors interfere with release of influenza virus from infected cells thereby halting the spread of infection. These will be discussed at length in this paper. Adamantanes block the M2 ion channel thereby interfering with viral uncoating inside the cell. They are effective only against influenza A viruses (influenza B viruses lack the M2

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protein). Choice of antiviral drug is complicated by resistance of different influenza types and subtypes to different drugs (Pringle, 2014).

The influenza virus is unique in that it completely experiences changes in the characteristic of the antigens on its surface. Typically, small changes occur from one year to the next. This is known as “antigenic drift”. Less frequently, there are significant changes in the surface antigens. This is known as “antigenic shift.” Hosts who have been previously exposed to influenza generally have some residual immunity against viruses that have drifted antigenically. In contrast, there is little/no preexisting immunity against viruses that have shifted antigenically. The former is responsible for epidemics which tend to be milder in severity. The latter situation results in influenza pandemics, with high mortality e.g. The Spanish influenza of 1918 is thought to have caused 30-50 million deaths worldwide.

Influenza Type A can cause pandemics and epidemics and is our main concern. Type B can only cause epidemics. Type C can cause mild illness but does not cause pandemics nor epidemics. Influenza is often confused with “Influenza-like- illness” which may be caused by other factors but has similar symptoms.

Influenza is most common in the winter months. This is because the winter conditions are optimal for the spread of influenza. The transmission of infection is most effective at a humidity level of 20-35 degrees and colder temperatures. At these conditions virion particles are more stable and can travel further distances in droplets (Mubareka et al., 2009). Increasing levels of humidity of indoor air during the winter may be an effective way of decreasing the spread of influenza.

### Pathophysiology

#### Influenza types

As noted above There are three types of Influenza A, B and C. Type A and type B cause the same spectrums of disease but type B can only infect humans and seals and therefore limits the reassortments in contrast to type A which has numerous hosts and numerous reassortments.

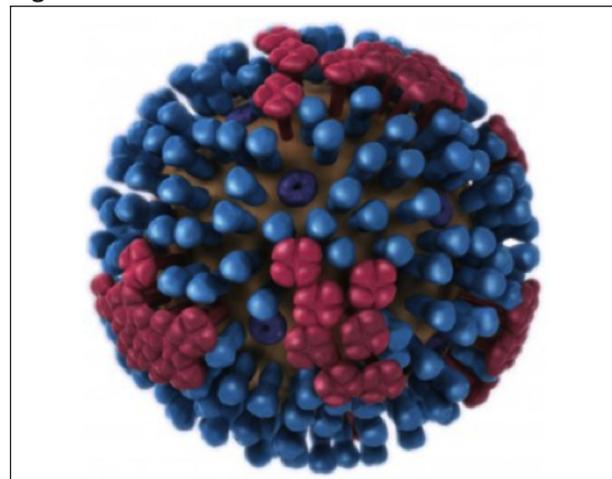
#### Influenza A Subtypes

Type A has 3 different membrane proteins, surface proteins hemagglutinin (HA), neuraminidase (NA). Matrix protein 2 (M2) traverses the membrane. As shown in figure 1. (Type B has the HA and NA proteins but does not have the M2 protein). (Tscherné and Garcia-Satre, 2011). The subtypes of influenza A are classified based on the HA and NA proteins. There are 18 HA types and 11 NA types. This means there are 198 possible combinations. The nomenclature of the virus describes the HA and NA subtypes for example a virus with HA type H1 and NA

type 7 is called Influenza H1N7. Only a few types of influenza are pathogenic to humans. This depends on their ability to bind to Human sialic acid, as discussed later.

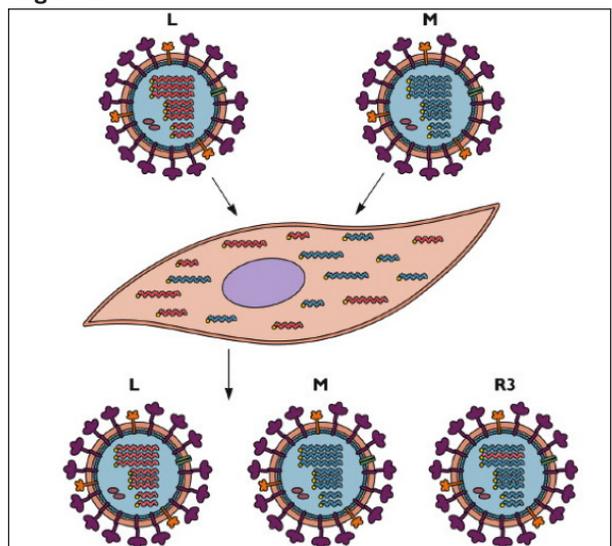
Antigenic drifts are minor mutations in preexisting HA and NA combinations resulting in new strains. This decreases the effectiveness of antibodies to the Influenza. Antigenic Shifts are new combinations of HA and NA proteins. Such as a change from H1N1 to H1N2. Antibodies produced against previous influenza strains are generally ineffective, thereby increasing its ability to infect and cause illness. These shifts often occur when two different influenza types infect one cell. When the viruses replicate the RNA can then combine and form a new “reassortant” type (see picture 3 below (Ranciello, 2013)).

Figure 1



HA (blue), NA (red), and M2 (purple) proteins (Ranciello 2013)

Figure 2



Influenza reassortment (Ranciello, 2013)

### NA Structure

NA is a tetramer of four identical polypeptides each containing approximately 470 Amino Acids. It has four sections: A cytoplasmic sequence, a transmembrane sequence, a stalk and a globular head. There are two phylogenetic groups of NA, NA group 1 and NA group 2. Group 1 contains N1, N4, N5 and N8. Group 2 contains N2, N3, N6, N7 and N9. Group 1 has an additional cavity "150 loop" that is not present in group 2 (Air, 2012).

### Cell Invasion

Animal respiratory cell membranes contain Sialic acid molecules. These molecules are the binding site receptors of the influenza virus. (Gamblin, and Skehel, 2010). When viral HA proteins bind to sialic acid receptors on the host cells a sequence of events is set in motion resulting in the virus entering the cell.

### Types of Sialic Acid

Sialic acid is a glycoprotein. It is typically linked to a galactose molecule. As stated, Sialic acid is linked to galactose. There are 2 main types of such linkages: 1. Alpha2, 3 linkage (pictured above), in which the carbon 2 of the acid is linked to the carbon 3 of galactose. 2. Alpha 2, 6 linkage, in which the carbon 2 of the acid is linked to carbon 6 of galactose. Humans primarily have  $\alpha$ 2, 6 receptors. This means that viruses targeting this structure are more likely to infect these cells. The results of studies of early influenza virus isolates from the 1918, 1957, and 1968 pandemics suggest that these viruses preferentially recognized alpha (2, 6) linked sialic acids. In contrast, viruses targeting the  $\alpha$ 2, 3-linked structure are not very pathogenic for humans. In humans, these linkages are only found in ciliated epithelial cells, which are a minor population within the human respiratory tract. Nevertheless, these are the preferred receptors for the avian flu (Matrosovich et al., 2000).

### Mechanism of infection

First the viral HA protein binds to the sialic acid receptors on the cell membrane. The virus is then engulfed by the cell through endocytosis. This causes the pH of the endosomal vesicle to drop to 5 which activates a conformation change on the HA protein which exposes a fusion peptide. The fusion peptide enables the viral envelope to fuse with the vesicle membrane wall. The viral RNA is then released into the cytoplasm. Viral RNA travels to the nucleus to initiate protein synthesis and assemble new viruses. The newly formed viruses travel back to the cell membrane. The virus interacts with the cell membrane causing it to bud off. The virus is released to infect other cells.

The mechanism of the virus leaving involves a potential problem. One would expect the viral HA protein to bind to the sialic acid

receptors on the cell membrane as it leaves. This would result in the virus becoming trapped on the host cell. This is prevented by the NA protein which cleaves the sialic acid to allow the virus to escape (Wagner et al, 2002). NA also cleaves sialic acid molecules in mucus in the human respiratory tract (Cohen, et al., 2013). This increases viral infectivity.

### Neuraminidase inhibitor drugs

In order to prevent viral infection the NA protein has been exploited by developing drugs that act as sialic acid analogs which bind to the NA active site. These are called NA inhibitors. These drugs disable the NA protein from cleaving sialic acid, leaving the virus trapped on the cell (Russel et al., 2006). Tamiflu (Oseltamivir) and Relenza (Zanamivir) are the two drugs which dominate the market. These have been stockpiled by governments and public health agencies (Jefferson et al., 2009) for the treatment of Flu and are recommended by the CDC.

### Oseltamivir resistant strains

There have emerged strains of flu that are resistant to oseltamivir. This is due to a point mutation of histidine being switched for tyrosine (H274Y) in NA. This leads to decreased binding of the drug. Nevertheless this change is also detrimental for the virus as it leads to a decrease of surface NA reducing the virus replicating abilities and infectivity.

During the 2008-09 flu season oseltamivir resistant influenza H1N1 viruses with the H274Y change became more prevalent, and within a year they were found in most seasonal isolates. Two amino acid changes were identified that even in the presence of H274Y restore surface levels of NA. These are V234M and R222Q. These secondary mutations seem to balance the deleterious effects of the H274Y mutation, thus enabling it to spread (Bloom et al., 2010).

### Discussion- The Tamiflu Controversy Cochrane Report

At the height of the 2009 H1N1 influenza pandemic, the British Medical Journal (BMJ) released a Cochrane update (of a 2005 Cochrane meta-review) which shook the medical world. The report cast serious doubt on the usefulness of neuraminidase inhibitors (Nis) in influenza. Specifically concluding that, at best, NIs reduce symptoms by approximately one day- a moderate benefit.

They reviewed 1416 titles for neuraminidase inhibitors mostly on oseltamivir. They discarded all but 20 due to various problems. These included, variously, insufficient information, inaccessibility to data, poor description of methods, and issues of reliability. For example, some studies used a mixed population of healthy adults and those with comorbid complications. This

meant the studies weren't effective in properly determining outcome in healthy populations. They noted that, even this data may not be accurate as up to 80 percent of the studies may have not been "pure" influenza, rather "influenza like illness". This is because influenza was unconfirmed by laboratory tests.

Most importantly, according to Cochrane, there is insufficient data as to whether NIs are effective in reducing complications of lower respiratory tract infection as indicated by antibiotic use or hospital admissions. They also note that there is a significant risk of toxicity, especially of psychosis, in prophylactic treatment. They concluded "because of the moderate effectiveness of neuraminidase inhibitors, we believe they should not be used in routine control of seasonal influenza."

Kaiser et al (2003) published a meta-analysis of 10 studies of oseltamivir. They concluded that Oseltamivir was effective in reducing lower respiratory tract infections (LRTIs). The Cochrane 2005 report, which relied on this study also concluded that it was effective in reducing LRTIs.

Hayashi (Jefferson, et al, 2009) pointed out that the 2005 Cochrane report was flawed in that they didn't actually review the data of the individual studies themselves. In addition, he questioned the reliability of the Kaiser report as only 2 of these studies were actually published in peer reviewed journals (JAMA and Lancet). The other eight were not and were not available for review. He also notes a conflict of interest in that the authors of the Kaiser report included four employees and one paid consultant of F. Hoffman-La Roche (the manufacturer of oseltamivir). Hayashi also noted that in the two published studies, there was no significant difference between Oseltamivir and placebo in the incidence of LRTIs. He suggests that the only way to rely on the report is by a rigid appraisal of the other eight trials-which were not released by the drug company La Roche.

Cochrane was unable to obtain the data of the 8 unpublished studies for further analysis. This led Cochrane to conclude in 2009 that "paucity of good data has undermined previous findings for oseltamivir's prevention of complications from influenza. Independent randomized trials to resolve these uncertainties are needed." The question of a publication bias was also raised by Cochrane due to the fact that these results weren't published.

Doshi (2009), (a Cochrane author) goes so far as to suggest that oseltamivir is no better than NSAIDs, such as aspirin, in the treatment of influenza. He also notes the numerous contradictory reports in the clinical studies on the effectiveness of oseltamivir which casts doubts upon the reliability of the methods and information used

to generate these reports. He criticizes the fact that government organizations have spent so much money on a drug before ascertaining the reliability of the data of its effectiveness. He notes that the Centers for Disease Control and Prevention (CDC) based their recommendations on the problematic Kaiser report.

The US government also partly based its national pandemic preparedness strategy on similar assumptions. As a result billions of dollars were spent building drug stockpiles, and oseltamivir was elevated to the status of a public health drug.

In 2014, Cochrane (published in BMJ 2014) released an update of a 5 year campaign it launched against Roche to obtain all the clinical study reports (CSRs)- which are extensive summaries of RCTs, on Tamiflu. Cochrane managed to pressure Roche into releasing all their data. They note that this is the first time all clinical study reports of trials in a manufacturer's program have been made available to readers without any restriction. The importance of this is the ability of researchers to assess the clinical trials for reliability, adherence to protocol, clarity of definitions and avoided reliance on conclusions of researchers who may have published biased material. It also allows for considerable more information on potential harms.

Cochrane notes that many discrepancies, problems and biases have been found in these trials. These include: Lack of clear definitions. For example there are eight definitions for laboratory confirmed influenza and no clear definition for influenza-like illness. Lack of reliability of the placebo. In many cases the placebo capsule had a different colored cap than that of the active capsule. This was not remarked on in the report. Many of the placebo capsules in oseltamivir trials contained dehydrocholic acid and dibasic calcium phosphate dihydrate. Both can cause gastrointestinal symptoms. Although the substances seemed to be in low doses, no discussion of their potential effects in people with fever was reported. Missing documents. This includes missing study protocols and amendments, study manuals of procedures and minutes of safety data monitoring committee meetings. Ghost authorship and lack of accountability. Authorship and accountability for the writing of many of the clinical study reports remains unclear. Some names on studies were redacted and no one seemed to claim responsibility for assembling and writing the reports. Missing data on duration and durability of symptom relief. Data on relapse after the five day treatment period were not reported in the clinical study reports.

There was also a mix-up with follow-up cards in a few of the trials which was not even reported in CSRs and only came to light from the FDA Summary Basis of Approval papers. There is ambiguity as to whether treatment of the flu shown through reduction of antibody titers was due to effectiveness of the viral

fighting activity of the drug or the immunosuppressant activity of the drug.

Cochrane 2014 concludes that oseltamivir is helpful for prophylaxis and modestly helpful in treatment (though they downplay its usefulness). In contrast to the assertion of various organizations Cochrane concludes that there is little evidence of significant benefits with regards to complications caused by influenza and viral transmission. Due to the above as well as the side effects, oseltamivir should be used cautiously. Due to all the discrepancies and poor quality of the studies, they question governmental sole reliance on this data to stockpile oseltamivir, its inclusion on the WHO list of essential drugs, and its use in clinical practice as an anti-influenza drug. This is especially true due to lack of sizeable benefits, and concerns of toxicity (Jefferson et al, 2014).

### Cochrane study results

For Adults in Treatment trials: oseltamivir reduced the time to first alleviation of symptoms by 16.8 hours—from seven days to 6.3 days. In treatment trials there was no difference in admissions to hospital.

For Adults in prophylaxis pre-exposure: oseltamivir reduced symptomatic influenza in participants by 55%. There was no significant effect on asymptomatic influenza (increase in antibody titers without symptoms). In post-exposure there are two components to interrupting transmissions of the virus. The reduction of viral spreading from nasal shedding and the prevention of onset of influenza in contacts.

Roche claims that it is effective, but there were serious flaws in the methods of the studies. This includes giving paracetamol which may have reduced viral shedding, and not checking antibodies in participants with potentially asymptomatic influenza. This leads to the conclusion that there is “no evidence of a reduction in transmission.”

It is important to note that these studies specifically influenced WHO's policy of recommending this drug.

Harms for Treatment: increased the risk of nausea (risk difference 3.66%, 0.90% to 7.39%; number needed to treat to harm (NNT) 28.) and vomiting (4.56%, 2.39% to 7.58%; 22, 14 to 42).

For Prophylaxis: oseltamivir increased the risk of psychiatric adverse events during the combined “on-treatment” and “off-treatment” periods (risk difference 1.06%, 0.07% to 2.76%; NNT 94, 36 to 1538) and there was a dose-response effect on psychiatric events in two “pivotal” treatment trials of oseltamivir, at 75 mg (standard dose) and 150 mg (high dose) twice daily (P=0.038).

Oseltamivir increased the risk of headaches on-treatment (risk difference 3.15%, 0.88% to 5.78%; NNT 32, 18 to 115). It increased renal events with treatment (0.67%, -0.01% to 2.93%), and increased nausea while receiving treatment (4.15%, 0.86% to 9.51%; NNT 25, 11 to 116).

For child treatment it reduced influenza for otherwise healthy children by 12 to 47 hours with a mean difference of 29 hours. There was no effect in children with asthma. There was no significant difference in admissions to hospital. There was no significant effect on bronchitis, otitis media, sinusitis unverified pneumonia or any complication classified as serious or that led to study withdrawal. There was no significant difference in prophylaxis. In treatment of children, oseltamivir induced vomiting (5.34%, 1.75% to 10.29%; 19, 10 to 57).

### Cochrane's call to governments

Compared with a placebo, taking Tamiflu led to a quicker alleviation of influenza-like symptoms of just half a day (from 7 days to 6.3 days) in adults, but the effect in children was more uncertain. There was no evidence of a reduction in hospitalizations or serious influenza complications; confirmed pneumonia, bronchitis, sinusitis or ear infection in either adults or children. Although when used as a preventative treatment, the drug can reduce the risk of people suffering symptomatic influenza, it is unproven that it can stop people carrying the influenza virus from spreading it to others.

Evidence from treatment trials confirms increased risk of suffering from nausea and vomiting. And when Tamiflu was used in prevention trials, there was an increased risk of headaches, psychiatric disturbances, and renal events.

Evidence also suggests that Tamiflu prevented some people from producing sufficient numbers of their own antibodies to fight infection.

Claims about the effectiveness of Tamiflu against complications were a key factor in decisions made by governments around the world to stockpile these drugs in case of a pandemic. The US has spent more than \$1.3 billion buying a strategic reserve of antivirals, while in the UK the government has spent almost £424 million for a stockpile of about 40 million doses\*.

It was initially believed that it would reduce hospital admissions and complications of influenza, such as pneumonia, during influenza pandemics. However, the original evidence presented to government agencies around the world was incomplete.

Along with the evidence of harms from the medication, it raises the question of whether global stockpiling of the drugs is still justifiable given the lack of reliable evidence to support the original

claims of its benefits. The BMJ and Cochrane issued a joint call to government and health policy decision makers the world over asking, in light of the latest findings from the Cochrane Review, would you make the same recommendations today, choosing to stockpile Tamiflu? (Breeze and Burns 2014).

### Criticisms against the Cochrane reports

There are many that criticized the BMJ/Cochrane reports. The study was done in collaboration with channel 4TV station in Britain. This may have led to a conflict of interests in overdramatizing a story for viewers. As Revere notes (Revere 2009) “Doing this in tandem with a media outlet whose objectives are not science but snagging viewers is unseemly at best and borders on the unethical”.

An article in Nature (Noorden 2014) notes that it seems as if Cochrane, in their overzealousness to confront the big Pharmaceutical agencies have been “cherry-picking” the results to make them look worse for antivirals. Some of their statements have contributed to media misinterpretation that the drugs are ‘ineffective’ or ‘useless.’ For example: Cochrane writes “(oseltamivir) reduces symptoms of influenza by half a day” instead of the more precisely stating 18 hours for adults and 29 hours in children. In the 2009 report they also neglect to mention the prophylactic benefits. The Cochrane article obscures the therapeutic effect on decreased risk of diarrhea and cardiac system events by sandwiching them among the harms of the drug. A Cochrane author takes the extreme approach-not supported by evidence- of suggesting that Oseltamivir is no more effective than paracetamol.

It is important to note that Cochrane hasn’t shown that antivirals didn’t work for healthy people who got flu and were given a neuraminidase inhibitor to avoid more serious complications like pneumonia. It just alleged there was no “Cochrane-required-level-of-evidence” that they did prevent complications.

Cochrane only uses Randomized Controlled Trials (RCTs). These are studies in which volunteers are randomly assigned to get a treatment or a placebo and are considered the gold standard of evidence. Observational Studies that observe outcomes in people who receive a treatment don’t make the cut because they are “unreliable for establishing treatment effects”. However, often the observational studies are better at concluding the actual facts on the ground as opposed to the theory. For example, an observational study (Muthuri, 2014) of 30,000 people hospitalized during the 2009–10 swine-flu pandemic reported that neuraminidase inhibitors reduced mortality by 25%.

Although RCTs are considered the gold standard in of establishing effectiveness of a drug, they lack sufficient statistical power to allow reliable conclusions to be drawn about the effects on flu

complications and hospitalizations- which are the key outcomes of interest during a flu pandemic. This is especially true noting that these small clinical trials were carried out to gain regulatory approval for Tamiflu as treatment and prophylaxis for seasonal flu. The trials were not designed to test for the severe outcomes that are most relevant to pandemics.

Additionally, The Metro (Branswell 2009) notes, sometimes it isn’t possible to conduct randomized controlled trials. Sometimes observational data is the best evidence available.

For instance, it’s unlikely any researchers could get permission to test Tamiflu against a placebo in people severely ill with H1N1. It would be considered unethical to withhold the drug from severely ill H1N1 patients if observational data suggested the drug might help.

Researchers worry that the Cochranes recommendation “we believe they should not be used in routine control of seasonal influenza” and the ensuing media storm of overly negative publicity risks undermining public confidence in this class of drug which will increase illness. Tamiflu is prescribed as the main treatment for serious cases of flu. Many experts say based on the observational data that these drugs work and have beneficial effects on severity of illness and preventing death. They are worried that we risk losing one of the few weapons we have. This is especially true based on the timing of the release and the lack of conclusive evidence that it doesn’t work. “So here we are in the middle of a pandemic and the Cochrane folks, aided and abetted by the BMJ and television producers, are saying, “How do you know for sure that they will prevent pneumonia in an otherwise healthy person who gets flu?” There is evidence about this, even if the Cochrane zealots don’t recognize it”.

### Evidence supporting the efficacy of Oseltamivir

It is hard to say that Oseltamivir is completely ineffective. One of the first human studies on the effectiveness of Tamiflu was published approximately fifteen years ago. In a 1999 study (Hayden et al) 117 healthy adult volunteers were inoculated with the influenza A virus. Two studies were conducted, one on prophylaxis where volunteers were given the drug 26 hours before inoculation and one on therapeutic treatment where they were given the drug 26 hours after inoculation. Treatment was continued for 5 days. In these trials, prophylaxis and early treatment with oral oseltamivir were both associated with significant antiviral and clinical benefits in experimental human influenza.

### Prophylactic

Among the 12 evaluable placebo recipients, 8 (67%) had laboratory-confirmed infection and 6 (50%) had recovery of the virus from nasal washings. In contrast, of the 21 receiving oseltamivir

## Is Tamiflu Effective in Influenza Treatment?

only 8 (38%) had laboratory confirmed infection and none of the 21 evaluable oseltamivir recipients had virus isolated.

The total symptom score area under the curve value was lower in the combined oseltamivir groups (n=21) compared with placebo (n=12); P=.02. Fourteen symptoms related to influenza were included in the score.

### Therapeutic

Of the 69 with laboratory confirmed illness At 24 and 36 hours after initiating treatment, the median time to cessation of viral shedding was reduced from 107 hours in the placebo group to 58 hours in the combined oseltamivir group as shown in the graph. No virus was detected by 60 hours after infection. Effect of Oral Oseltamivir Treatment on Illness Following Experimental Influenza A/Texas/36/91(H1N1) Infection The total symptom score area under the curve value was lower in the combined oseltamivir groups (n=56) compared with placebo (n=13);

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### Dobson et al, 2015

In 2015 the Lancet published a study by Dobson et al. which shows that oseltamivir in adults with influenza accelerates time to clinical symptom alleviation, reduces risk of lower respiratory tract complications, and admittance to hospital.

The Cochrane reviews were based on meta-analysis of clinical trial study reports alone and not on individual patient data. In the Lancet study, Dobson et al did a meta-analysis of all available randomized treatment trials of oseltamivir-which includes both published and unpublished data (thereby overcoming previous concerns regarding potential publication bias). This analysis is first of its kind that reviewed individual patient data.

Nine trial studies were done between 1997 and 2001 involving 4328 participants. Participants were labeled as laboratory confirmed influenza-infected ("intention to treat infected") and Influenza-like ("intention to treat non- infected"). Participants were within 36 hours of feeling unwell- with a fever and at least two other influenza symptoms. They received 5 day regimens of Tamiflu with a 21 day follow up.

Limitations of these studies are that they weren't set up to test for relieving of respiratory complications As such, specific diagnostic tests were not used. Instead, in order to enhance reliable reporting of complications they incorporated "antibiotic use" in the definition of LTRIs. These tests were also not set up to determine prophylactic effects.

### Effectiveness

Included are data from nine trials including 4328 patients. In the Influenza confirmed population (intention-to-treat infected population),

Alleviation of all symptoms for oseltamivir versus placebo recipients-21% shorter time of 25.2 h,

(The median times to alleviation were 97.5 h for oseltamivir and 122.7 h for placebo)

An estimated 44% reduction in risk lower respiratory tract complications requiring antibiotics more than 48 h after receiving oseltamivir (65 of 1544 participants given oseltamivir and 110 of 1263 participants given placebo. Components were 56 versus 87 bronchitis, nine versus 21 pneumonia, and one versus four lower respiratory tract infections, respectively)

An estimated 63% risk reduction of admittances to hospital for any cause In the intention-to-treat (nine of 1591 participants had to be admitted to hospital for any cause versus 22 of 1302 participants given placebo). Participants given Oseltamivir had significantly less diarrhea, infections and infestations, and respiratory, thoracic and mediastinal disorders and fewer cardiac disorders. Regarding safety, increased the risk of nausea (9.9% oseltamivir vs 6.2% placebo), increased vomiting (8.0% oseltamivir vs 3.3% placebo). No recorded effect on neurological or psychiatric disorders (although slightly higher with 150 mg). No serious adverse events.

Non-confirmed influenza (intention-to-treat non- infected population)

Oseltamivir was ineffective in non-confirmed influenza. This is consistent with the fact that Oseltamivir is an anti-viral. In some studies some researchers haven't distinguished between confirmed and non-confirmed influenza population. This will skew the results of efficacy of the drug being that it is not meant to treat non-confirmed influenza. Nevertheless, these results will more clearly resemble the effectiveness in real world situations where there is mix of these populations.

### Conflict of interests

Dobson reveals that funding for his study was provided by the Multiparty Group for Advice on Science (MUGAS). They received an unrestricted grant from Roche but stipulated that Roche would not be involved in the actual review process in any way other than providing the requested data dictionaries and datasets. The results were not shared with Roche until the analysis was completed.

The author ASM reports fees from Biocryst and Roche outside of the submitted work. The author RJW reports fees as a board member of Gilead Sciences, funding for travel from Roche to attend an Influenza Resistance Committee meeting, and fees as Associate Editor of the Journal of Infectious Diseases.

### **CDC rejection of Cochrane Report**

The CDC (CDC 2015) continues to promote antivirals such as Oseltamivir for treatment of the flu. The CDC promotes the “Take 3” campaign to fight the flu. Step 1 is to get vaccinated. Step 2 is taking preventative action to stop the spread of germs. Step 3 of the campaign encourages people to “take flu antiviral drugs if your doctor prescribes them.”

In CDC (2014) an article entitled ““Have You Heard? CDC Recommendations for Influenza Antiviral Medications Remain Unchanged.” The CDC addresses their recommendations and the Cochrane’s criticism. Based on the observational studies published, the CDC says “treatment with a neuraminidase inhibitor antiviral drug was associated with a 25% reduction in the likelihood of death compared to no antiviral treatment. Early treatment with neuraminidase inhibitor antiviral drugs (i.e., within 48 hours of development of influenza illness) halved the risk of death compared to no antiviral treatment. This confirms findings from previous observational studies in hospitalized influenza patients that the clinical benefit of neuraminidase inhibitor antiviral treatment is greatest when started within two days of influenza illness onset.”

The CDC states that their disregard for the Cochrane findings is because Cochrane did not consider any data from observational studies of oral oseltamivir. CDC adds that observational studies of antiviral treatment of seasonal influenza or influenza A (H1N1) pdm09 (2009 H1N1) have been conducted among hospitalized patients, including critically ill children and adults. These have consistently found that early oseltamivir treatment of influenza patients reduces the duration of hospitalization and risk of severe outcomes such as intensive care unit admission or death. CDC states that Cochrane RCT reviews of data on outpatients with clinically mild influenza-like illness is limited in by the narrow scope of participants. It is statistically underpowered and not designed to assess the effects of the medications on more severe influenza illness outcomes, such as hospitalizations, intensive care unit admissions, or deaths. RCT data is unavailable for those at highest risk for developing severe complications from influenza: hospitalized patients with severe influenza illness, the elderly, young children, pregnant women, and persons with underlying medical conditions such as chronic obstructive pulmonary disease (COPD), asthma, congestive heart failure and diabetes.

They conclude “available evidence for seasonal influenza and 2009 pandemic H1N1 virus infections consistently indicates that antiviral treatment, when initiated as soon as possible, can have clinical and public health benefits in reducing severe outcomes of influenza. Therefore, neuraminidase inhibitor antiviral medications continue to be recommended for treatment of influenza.”

### **BMJ/Cochrane response to CDC and Lancet**

In a response article written by the BMJ’s Jeanne Lenzer “Why aren’t the US Centers for Disease Control and Food and Drug Administration speaking with one voice on flu? ” (BMJ 2015) Lenzer raises allegations regarding the reliability of CDC. She accuses them of being “more emotional than scientific” and notes that they are at odds with the FDA who have said oseltamivir “has not been proven to have a positive impact on the potential consequences (such as hospitalizations, mortality, or economic impact) of seasonal, avian, or pandemic influenza.” She considers the CDC’s reliance on the Lancet review as unreliable. This is because the authors have not made their study protocol available for critique nor have they released an appraisal of the methodological quality of each study. She concludes by raising the concerns that the policies of the CDC and Lancet review have been influenced by funding from the Pharmaceutical companies. The CDC Foundation confirmed to The BMJ that the CDC received a directed donation from Roche for the campaign, stating, “Roche provided a grant of \$198 000 to CDC Foundation [which] has an administrative fee of 13.5%, so \$174 800 was provided to [the CDC to] support qualitative research into influenza prevention and treatment messaging.” The CDC Foundation also receives funding from the pharmaceutical industry. A spokesperson said that over the past three years the foundation has received an average of around \$6.3m annually from the industry, 21% of the foundation’s overall funding. Some of the companies who have provided funds include Gilead, which holds the patent on oseltamivir, as well as Genentech and Roche, the drug’s manufacturers. This greatly discredits the CDC’s credibility as a nonbiased party.

### **The Lancet’s Funding**

The Lancet study itself was conducted through MUGAS which was funded by an open grant from Roche- as the study noted. Lancet asserts that neither they nor the study itself were influenced in any way by Roche. Additionally, neither MUGAS nor Roche saw the results of the study before publication.

Also two of the researchers note that they have had ties in the past to Roche. A third researcher, Stuart Pocock didn’t list any conflict of interest on the research paper, but revealed to The BMJ that he has received funding from several drug companies for cardiovascular research, including Gilead and Genentech, but that none of his funding was related to the study of antivirals for flu.

Paul Roblin in his BMJ blog notes Although the name Multiparty Group for Advice in Science ( MUGAS) might lead you to imagine an independent body bringing together representatives of a number of organizations to consider a range of issues, MUGAS is funded by Roche and is led by four scientists, three of whom are advisers to Roche. It appears to have been set up specifically as part of the attempt to counter the Cochrane's criticisms. He also notes that there is a complex set of inter-related organizations that supported the Lancet study and receive funding from Roche and Gilead. Also, one the Lancet authors and MUGAS have many more connections to the CDC, Roche and Gilead than they disclosed. One of the researchers for the Lancet article, Professor Richard J Whitley joined Gilead's board of directors in 2008 and works with/for the CDC.

### Conclusion

All parties agree about the following: Oseltamivir is effective in reducing symptoms by at least 16 hours. Oseltamivir works reasonably well for prophylaxis. Oseltamivir has significant side effects including psychosis in prophylactic patients.

The main debate is whether it reduces secondary complications, hospitalizations and spread of disease. The debate also centers on whether it is justifiable to prescribe oseltamivir in the face of significant side effects. Also questioned is the wisdom of governmental stockpiling of billions of dollars of Tamiflu. The critics of oseltamivir, most prominently Cochrane/ BMJ point to the dearth of high quality evidence. Their opponents base their opinions on observational data, which is less rigorous, but compelling nonetheless. At the present, clinical practice favors action i.e. the use of oseltamivir for both prophylaxis and treatment. The clinical decision supporting resource "UpToDate" continues to recommend treatment with the anti-virals as per the recommendation of the CDC and the IDSA. This is based on the observational study data that shows its effectiveness. It seems that the current consensus in Hospitals is to give Tamiflu as a first line defense both for prophylaxis and for treatment. Although evidence shows it may not be as effective as we once thought, this is basically our only defense against influenza. In hospitalized patients with preexisting complications we wish to take all possible precautions. This author wishes to point out that such an approach is defensible only in an environment in which cost of treatment (financial and side effect) is not prohibitive. We wonder whether this calculus would be different elsewhere.

Many things can be learned from the Tamiflu controversy. Firstly, as demonstrated by Cochrane, one may err in assuming that because a paper is published in a peer reviewed journal all the evidence is reliable. Especially when an important decision must be made the data should be reviewed for accuracy, reliability of methods and lack of bias. Secondly, as suggested by Cochrane, the

trustworthiness of decision making by government bodies should be questioned. In any situation, conflict of interest may be present. Thirdly, as suggested by their critics, was the Cochrane disingenuous in completely ignoring observational data supporting use of Tamiflu? And were the Cochrane writers sensationalists in their conclusions and media communications?

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# Which Methods of Treating Attention Deficit Hyperactive Disorder are Most Effective and Most Closely Match Patient Lifestyle?

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## Abstract

ADHD, attention deficit hyperactive disorder, is a disorder characterized by hyperactivity, inattentiveness, inability to remain on task, fidgetiness, and impulsivity. An over absorption of dopamine by the dopamine transporters leads to a lack of dopamine, the inhibiting neurotransmitter in the brain, resulting in the inability for the individual to inhibit their impulses. The disease is most likely seen in children but may continue into adulthood. The length of the disease across the individual's life span, results in expensive treatment, whether it be medication or Cognitive Behavior Therapy, CBT. Therefore, these medical costs have caused researchers to find the most efficient drugs to provide the ADHD patients with the most effective, cost effective, and beneficial results. Different individuals require different treatment options, whether it be due to sex, age of onset, socioeconomic group, and attitude toward medication. This paper will provide an overview of the different treatment options with their side effects, causes and remedies.

## Introduction to ADHD

Attention deficit hyperactivity disorder (ADHD) is a disorder most commonly seen in children and adolescents, but also may present or persist into adulthood. It is a neurocognitive behavioral disorder that presents with symptoms of maladaptive behaviors such as impulsivity and or hyperactivity, the inability to pay attention and remain on task. ADHD is also associated with other maladies such as, mood disorders and learning disabilities (Journal of Attention Disorders, 2014). ADHD is costly because it is usually diagnosed in childhood and may become a lifelong disorder that causes a wide range of symptoms. In addition, children with ADHD have been shown to be more likely to sustain major injuries and need both inpatient and outpatient emergency care. Median medical costs for children with ADHD were double those of children without the disorder when studied over a nine year period.

Because of its prevalence amongst American children and adults and its high cost to the health care system, treatment of ADHD is important. Today, many treatments are available, these may include, behavioral as well as pharmaceutical therapies. Medications available range from non-stimulant options to stimulants available in both extended release, long acting or traditional dosages (Journal of Attention Disorders, 2014). This paper will attempt to understand the causes of ADHD on a neurological level and evaluate possible treatment options, analyzing how physicians may be better able to match treatment to a patient lifestyle and disease.

## What is ADHD?

ADHD, attention deficit hyperactivity disorder, is a neurobiological developmental disorder that is characterized by the inability to remain on task, difficulty to remain focused and the tendency to be overly energetic with impulsive behaviors. It is most commonly found in school-age children but can frequently continue into adulthood. ADHD is a widespread disease, having been found in all countries surveyed with a prevalence of about 7-8%

of youths and 4-5% of adults. Furthermore, interestingly enough, ethnicity did not seem to be a factor in the frequency, however, social groups, were found to be most likely to play a role in the diagnosis (Journal of Attention Disorders, 2014).

ADHD, attention deficit hyperactivity disorder, is broken up into three different categories, ADHD-I; ADHD, Predominantly Hyperactive-Impulsive Type; and ADHD, Combined Type: Inattentive and hyperactive/impulsive. ADD, attention deficit disorder, is an alternate name for ADHD-I, the first type of ADHD. ADHD is the broad term for the hyperactive, inattentive disease and ADD is a classification of ADHD. Firstly, ADHD-I, also known as ADD or ADHD-Inattentiveness, is diagnosed by the inability to keep on task or follow and understand instructions. Furthermore, children with ADD tend to make careless mistakes and use minimal effort in accomplishing difficult tasks. Additionally, the ADHD Predominantly Hyperactive-Impulsive Type is categorized by the individual suffering with the symptoms of hyperactivity, such as, fidgeting, squirming, trouble playing quietly or interrupting constantly. Lastly, the combined type of ADHD is characterized by a pattern of inattentiveness and over activity. Meaning, an individual with the combined type of ADHD will exhibit symptoms of fidgetiness, and inability to concentrate and remain seated, in addition to, signs of constant distraction and failure to remain on task (diffen.com).

## Causes of ADHD

Attention deficit hyperactive disorder can be triggered by many matters. For instance, ADHD is mainly prompted by genetics, but, it can also be the result of the environment or specific brain injuries. Firstly, many studies have proven heritability to be the most common cause of ADHD. Genes can have the greatest impact on one's children, whether it means passing down an advantageous trait, or passing down a disease, such as ADHD (myadhd.com). Family studies were performed in order to find the relationship between ADHD and genetics. Results have shown that 25% of closely-related family members with ADHD also have the disease.

Furthermore, adoption studies and twin studies were done in order to support the idea that ADHD is genetically related. If ADHD was not genetic, then adopted children should further resemble their adopted parents than their biological. However, reports have shown that adopted children are more similar to their biological parents, further supporting the point. Twin studies, have shown similar results. Identical twins contain the same genetic materials as each other, and therefore, if one twin has the disease, the other twin does as well. The last study that was done on ADHD patients, was molecular genetic research. Molecular genetic research focuses on the specific genes related to the transmission of ADHD. DAT1 and DRD4 are two dopamine genes that were discovered to have the biggest effect on ADHD gene transmission (myadhd.com).

Additionally, exposure to toxicity can be another cause of ADHD. A relationship has been found between mothers who have smoked tobacco or intoxicated in alcohol during pregnancy, and the probability that learning disabilities and ADHD will develop in their child. Furthermore, lead exposure or poisoning in early years of childhood can have negative effects on the child's progress and advancement. The last, atypical, cause of ADHD can be a head or brain injury. Although somewhat unlikely, tumors, traumas, strokes or diseases can also result in ADHD as well (myadhd.com).

### **ADHD and the Science of the Brain**

Current research has proven that the causes of ADHD are highly related to many dysfunctions of neurotransmitters and of regions in the brain. A highly essential neurotransmitter, dopamine, has proven to have an effect in ADHD, as well as, the prefrontal cortex which requires high amounts of dopamine in order to function. Dopamine is a significant inhibiting neurotransmitter; meaning, that it plays a major role in preventing certain impulses in the brain, and ultimately, preventing the causes of ADHD (dnalc.org). Dopamine transporters are found to be responsible for the lack of dopamine in the brain since they absorb too much dopamine, disabling it to transfer from one brain cell to another, thereby causing a dopamine deficiency in the brain. The basal ganglia, a collection of nuclei located throughout the brain, also suffer from the lack of dopamine, DA, present in the brain. The basal ganglia are responsible for motion or normal voluntary movement and are excited by dopamine transmitters. Therefore, a lack of dopamine also effects the role of the basal ganglia, causing ADHD. When the levels of dopamine are low, there are high risks for attention deficit hyperactivity disorder because the main neurotransmitter which inhibits impulses is lacking (dnalc.org).

Therefore, the ADHD drugs are specially designed and intended to bind to a dopamine transporter with their nitrogen, oxygen

or carbon atom, enabling it to transfer the dopamine to the brain. The nitrogen and oxygen atoms are surrounded by at least one lone pair which assists in the formation of a hydrogen bond to the dopamine transporter. However, although the carbon atom is lacking such a lone pair, it has interestingly been discovered that it too can form a bond to the dopamine carriers (dnalc.org).

The question still remains, however, how do the ADHD drugs increase the levels of dopamine in the brain by binding to the dopamine transporters? There are three known mechanisms that ultimately inhibit the rise of dopamine in the brain. The dopamine rapidly diffuses into the synapse causing the dopamine transporters to bind to the dopamine and positioning it throughout the nerve cell membrane. Then, extracellular action of dopamine on the auto receptors of the neuron, consequently inhibit the further release of dopamine. Thus, the dopamine stimulating drugs are aimed to prevent the uptake of dopamine by the neurons so that extracellular action will not occur. Furthermore, the drugs can promote additional dopamine release. For example, either the drug can bind to the dopamine transporters hindering it from bringing the dopamine into the neuron. Or, other forms of the drug can excite the release of more dopamine (Molecular Psychiatry, 1998).

Additionally, the prefrontal cortex and its association with further brain regions, such as the striatum, cerebellum are found to be slightly smaller or less active in ADHD victims. Furthermore, the frontal lobe, basal ganglia, and the caudate nucleus, are also related to ADHD and they too play a significant role in regulating behavior. Studies of animals have shown that the prefrontal cortex has a responsibility in regulating behavior and attention, as well as, understanding representational data (sciencedaily.com). Researchers were not surprised to find a smaller prefrontal cortex in patients with ADHD due to the fact that the prefrontal cortex is critical in providing and dividing attention, and inhibiting distractions. Therefore, it was understood why the MRI's of ADHD victims exhibited significantly smaller prefrontal cortexes.

### **Treatment of ADHD: Stimulant vs. Non-Stimulant Drugs**

Treatments for attention deficit hyperactive disorder include pharmacological approaches, as well as, non-pharmacological care. When treated pharmacologically, the patients are cared for using specific prescriptions of drugs that provide them relief from their symptoms. Most often, stimulant drugs, drugs that arouse activity in the brain, are used as pharmacological treatments. However, non-stimulant medications can be used as well (Journal of Attention Disorders, 2014).

## Treating Attention Deficit Hyperactive Disorder

Psychostimulants are stimulant drugs that are psychoactive, meaning, they temporarily generate mental and physical functions in the brain such as increased awareness and attentiveness (Molecular Psychiatry, 1998). Current psychostimulants include D-amphetamine, D, L-amphetamine, Magesium pemoline, Methylphenidate, dexamethylphenidate, mixed amphetamine salts and lisdexamfetamine dimesylate (LDX). Surprisingly, the structural composition of these molecules are all different, however, share a common backbone of phenyl-ethylamine with endogenous catecholamines, such as, norepinephrine and dopamine. Uptake of the psychostimulant drugs provide the brain with significant dopamine, thereby enabling the inhibition of impulses (Molecular Psychiatry, 1998).

Methylphenidate, is an example of a short acting, central nervous system, stimulant drug for children and adults. They have an onset of approximately 30-60 minutes and remain in action for about 2-5 hours. Methylphenidate regularizes certain brain functions, specifically in the frontal lobe, prefrontal cortex, the basal ganglia and cerebellum, by stimulating the brain chemicals dopamine and norepinephrine (Molecular Psychiatry, 1998). Dopamine and norepinephrine are important neurotransmitters in the brain, and, a lack of them can cause dysfunction in brain activity. Drugs that increase the dopamine or norepinephrine levels will, therefore, provide a short-lasting relief to individuals with ADHD. Methylphenidate binds to the dopamine transporters, and blocks them from transporting to the neurons. In this way, the extracellular action in the neurons will not take place and further production of dopamine will not stop (L.E Arnold).

Methylphenidate also provides a therapeutic effect due to the slow and steady increases of the stimulants. In addition to the physical effect, methylphenidate also provides an emotional relief by increasing the child's self-esteem and bettering their interactions with others. Therefore, stimulant medications are usually given gradually until a beneficial, relaxing effect comes about (Molecular Psychiatry). Also, when treated with the sustained-release preparations, they can have longer lasting effects of 8-12 hours. Evidence to the efficacy of the drug can come from functional magnetic resonance imaging tests (fMRI) on ADHD patients, which have shown that after a dosage of methylphenidate, there was increased activations in the brain, verifying the efficacy of this drug. The medication has been continuously proven to positively affect many ADHD victims (L.E Arnold).

Ritalin, Concerta, Quillivant XR, Daytrana, Focalin, Medikinet, Equasym XL and Methylin are all derivatives of methylphenidate and all have similar effects. Firstly, the main byproduct of methylphenidate, Ritalin, is a short-lasting drug providing immediate release of dopamine and norepinephrine in the body (psychcentral.com). Hence, those patients in need of immediate relief will

benefit from Ritalin. On the other hand, Concerta, is a long acting stimulant which increases dopamine levels gradually. Thus, one may not need to take more than one dose a day, due to its all-day symptom relief. Depending on the severity, time and frequency, different patients may have a preference for either the short-acting Ritalin or the long lasting Concerta (healthline.com).

Furthermore, Quillivant XR may be an option to some ADHD patients. Quillivant XR is the only liquid long lasting medication, thereby, attracting young children who cannot swallow pills. In addition, since it is a liquid, it is easily measurable, and, therefore, those patients who only need a small dosage will tend to choose Quillivant XR. Daytrana, has an unusual advantage of existing in patch form. Meaning, a specific dosage of medication is located in a patch that is worn somewhere on the body usually the hip. Throughout the day, the Daytrana is released into the blood. Consequently, the effects on the patient will have a longer duration, usually lasting throughout the entire day. Children typically are the most common ones to use this drug, being that it makes life simpler (adhd-treatment-options 2009). The next drug, Focalin, is also a drug that offers extended-release, and is found to more effective than Concerta. Focalin has proven to offer immediate relief first thing in the morning, and then provide continuous effects throughout the day, thereby allowing children to focus during the day in school. Therefore, Focalin has been a first choice drug option for school-age children (drugs.com).

Another methylphenidate drug, Medikinet, grants immediate-relief and short lasting results. Therefore, it would need to be taken several times a day. However, Medikinet XL is a new version of the drug which splits up the dosage, releasing 50% immediately and holding back the other 50% to be released later on in the day. The latter form of the drug is most probably more beneficial to most people, not needing to retake the medication multiple times a day (netdoctor.co.uk). Similar to Medikinet XL, Equasym XL, is also a pill that releases 30% immediately and stores the other 70% to be released hourly throughout the course of the day. In this case, as well, one morning capsule is enough to last the child the whole day. Lastly, Methylin, is short-lasting pills and requires multiple doses during the course of the day. Additionally, it has many side effects so it is not the best option of an ADHD drug (netdoctor.co.uk).

The second most common type of ADHD stimulant drug, Amphetamine, is similar to Methylphenidate, except that variants of this drug are usually intermediate-acting. For example, D, L-amphetamine, has a slightly longer duration of 4-6 hours. A blend of four amphetamine salts, amphetamine aspartate monohydrate, amphetamine sulfate, dextroamphetamine sulfate and dextroamphetamine saccharate, produce Adderall, which is an equally effective drug (progressivehealth.com). Being that the

two main drugs are especially similar; differences between methylphenidate and amphetamine are not so distinct. However, the chemical activity is different. Ritalin, travels into the brain cells and prohibits them from continuous absorption of dopamine (learn.genetics). On the contrary, Adderall, not only stops the brain cells from absorbing the dopamine, but, also, forces the cells to drain the already absorbed dopamine, thereby, directly increasing the dopamine levels in the brain. Furthermore, when methylphenidate binds to the dopamine transporters, it merely inhibits the uptake of dopamine by the neurons, thereby stopping the inhibition of dopamine. Amphetamine furtherly differs from methylphenidate in that along with the immediate release formulation of the drug, it can provide extensive release as well. Therefore, less dosages per day are needed of Adderall than of methylphenidate. Furthermore, even though Adderall is more powerful than Ritalin, some people react better to Ritalin, some react better to Adderall and some react equally to both drugs (LE Arnold).

Being that stimulant ADHD drugs are similar to cocaine and morphine, many risks and side effects exist. People think that being that many children use it, the drug is harmless and mild. However, being that methylphenidate is highly addictive, many dangers are present. The FDA requires all Ritalin boxes to contain the following notification, "Ritalin is a federally controlled substance because it can be abused or lead to dependence. Keep Ritalin in a safe place to prevent misuse and abuse." The above warning states that the effects Ritalin can be abused and harmful if not used in the correct manner. The use of this drug can also lead to anxiety, agitation, trouble sleeping, decreased appetite, headaches, stomachaches etc. More serious side effects include the chance for seizures, and blurred vision (ritalin.sideeffects.net). The main concern with these stimulant drugs is its potential for physical and emotional abuse. Addiction to the drugs can be caused by increasing dosages due to a buildup of tolerance to the drugs. The higher dosages, may then cause the patient's body to become solely dependent on the drug, craving it and eventually being unable to live life without it. Withdrawal from the medication can lead to panic attacks, depression and hunger. Therefore, limitations to this drug are extremely important to prevent addiction and abuse (drugabuse.gov). Side effects of both medications include vomiting, nausea, anxiety, insomnia, skin rashes, headaches, dry mouth and agitation. Furthermore, Adderall has an additional side effect of increasing the risk of certain heart diseases, such as, heart palpitations, increased heart rates and increased blood pressure.

Although stimulant medication is usually the most effective for treatment of ADHD, these drugs are not for everyone. Whether it being the intolerable side effects, or the ineffectiveness of stimulants, some patients prefer the non-stimulant option remedy,

Strattera, Clonidine, Guanfacine, Intuniv, and Kapvay, are some of the most popular non-stimulant possibilities. Furthermore, Bupropion SR and XL, The tricyclic antidepressants, The Selective Serotonin Reuptake Inhibitors (SSRIs), and Effexor can also be used as non-stimulant relief (ncpamd.com).

The first approved ADHD non-stimulant medication for children and adults, Strattera, also called Atomoxetine, increases the amount of norepinephrine in the brain, thereby increasing a child's attention span and moderating their impulsive behavior (ncpamd.com). Strattera does not directly influence the dopamine levels as do the stimulant medications. Strattera is effective in decreasing the ADHD symptoms, however, it is not controlled and it is less likely to be abused and cause addiction. The clinical effects last throughout the day and therefore, more than one dosage a day is not necessary. Although there are some side effects, they are minimal and tolerable, such as, fatigue, dizziness and nausea (my.clevelandclinic.org).

Clonidine and Guanfacine, have been used in adults mainly to control high blood pressure but has been further used to control ADHD mainly in adults with impulsivity and aggression. Due to its painkilling and soothing characteristics, Guanfacine, is commonly used to help individuals fall asleep. However, due to the major side effects of these drugs including high blood pressure and heart rate, it is important to monitor one's blood pressure with frequent EKG's, electrocardiograms, tests that check the electrical activity of the heart. Guanfacine lasts longer than Clonidine and only one or two dosages are needed per day. Furthermore, these drugs mainly relieve over activity as opposed to inattentiveness.

Intuniv and Kapvay are other non-stimulating drugs approved by the FDA in September 2009-2010 for individuals ages 6-17. Both drugs can be used independently or simultaneously with a stimulant. They also tend to take longer than stimulants to show results. It may take up to a month to see their effects. Furthermore, just like Guanfacine and Clonidine, Kapvay may cause a rise in blood pressure and it therefore should be monitored (ncpamd.com).

Antidepressant drugs can also be used to cure ADHD that is linked to depression. Although its results are not as beneficial to helping children with attentiveness and remaining on task, children who are depressed due to their disease, commonly lead towards anti-depressants for help. The first, most popular, category of anti-depressants are The Tricyclic Antidepressants which include, Pamelor, Norpremine, Aventyl, and Tofranil. They are long-lasting so do not require frequent dosages (ncbi.nlm.nih.gov). Tricyclics, however, can cause dry mouth, blurred vision, constipation, dizziness and sedation. Other antidepressants

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such as Wellbutrin, Effexor, Effexor XR, and MAO inhibitors, are mainly used as antidepressants but can be used to treat ADHD by increasing serotonin in the brain, thereby, increasing concentration in the child. MAO inhibitors are usually a last choice for ADHD patients because of their dangerous side effects including high blood pressure when taken with certain foods. Antidepressants are sometimes mixed with stimulant drugs when the individual is suffering from symptoms of anxiety in addition to their hyperactivity and inattentiveness (Journal of Attention Disorders, 2014). In conclusion, the use of antidepressants to relieve the symptoms of ADHD may be beneficial if monitored by a health professional.

## What is Cognitive Behavioral Therapy for children or adults with ADHD?

Patients diagnosed with ADHD need to manage their symptoms of hyperactivity, impulsivity and inability to focus their attention. CBT, cognitive behavioral therapy, consists of teaching skills to control these symptoms. There are two different arms to this strategy. One is to teach ways of staying focused and organized while the other's goal is to minimize disruptive behaviors that interfere with social and academic growth. CBT is used both alone and in conjunction with pharmaceutical treatment consisting of stimulant and non-stimulant medication (Miller-Behavioral Treatments for Kids with ADHD). CBT is important as, unlike medication, it teaches life skills that can serve the patient even after medication is stopped.

For children with behavior problems that detracts from their academic progress and causes conflict both at home and in school, parent-child interaction therapy, PCIT, may be beneficial. It involves 14-17 week sessions in which parent-child interaction is viewed through a one way mirror and the parent is coached by a psychologist using an ear bud. Parents are encouraged to use positive reinforcement in conjunction with strict consequences for inappropriate behaviors. These techniques are meant to be continued at home and in school. Teacher involvement is encouraged and essential. Goals are set, such as following specific classroom rules with a prize following each positive behavior. Over time, the child learns to control behavior and therefore, have more beneficial interactions with parents, teachers, and peers (Miller, Child Mind Institute).

Another form of behavior therapy involves the attention aspect of the disorder. These include techniques to establish routines to allow the ADHD patient to organize himself, allowing him to stay on task and accomplish his goals. It also focuses on transitioning techniques to allow for less distraction. Everyday tasks such as homework or bed time are broken down into smaller achievable steps (Miller, Child Mind Institute). In general, both methods of CBT have been shown to be more effective the

younger the child is, and less benefit has been seen by those children who have reached adolescence.

## Cognitive Behavioral Therapy as a viable treatment for ADHD

Because the use of pharmacological treatments of ADHD may not be acceptable to some parents of affected children or the affected individuals themselves, cognitive behavioral therapy, CBT, has been an alternative to the use of these medications. CBT has long been used to treat ADHD both in conjunction with medication or on its own. However, not many studies are available comparing CBT applied on its own, to medication alone, to a combination of CBT and pharmaceutical options (Journal of Attention Disorders, 2014). A study by Susan Young and J. Myanthi Amarasinghe view these possible treatment options through the lifespan of the ADHD patient uncovering evidence which suggests different approaches to treatment are more effective during different periods in the patient's life.

The young preschool child, who is still cognitively immature, cannot yet put into practice skills that are taught directly. He responds best to parental training to enforce positive behavior control such as tangible consequences along with positive support to reinforce good behavior. It has been noted that preschoolers that do not meet criteria for diagnosis and therefore do not receive early treatment were further affected into their school years and beyond than those children treated early (Young and Amarasinghe). In addition, parent-child relationships are strained during these early years creating stress that lingers into adulthood. Because the focus of a child's life at this time is his home and parents, parent training is the most effective treatment yielding the most benefits throughout the patient's lifetime. Parenting techniques and interventions include teaching parents about ADHD and addressing and targeting behavioral problems associated with it. Reward and positive reinforcements for appropriate social behaviors are stressed in conjunction with consequences for negative and socially inappropriate behaviors. Parents may also be offered support groups to deal with their own reactions to their child's difficulties (Young and Amarasinghe).

Older, school age children, are cognitively capable of learning skills and techniques to aid in social and academic situations. They appreciate being involved in their care at this stage in their development. This type of behavior therapy can be developed to aid in treatment of older adolescence as well as adult patients. These patients exist in two basic environments: at home and at school, therefore treatment must be tailored to these situations. Home-school connections must be fostered and communication between the two is imperative. Parent training is similar to that of younger children as described above while teachers are

coached to set achievable academic and social goals in addition to positive reinforcement along with tangible consequences for behavioral issues or impulsivity (Miller, Child Mind Institute).

As children move past their elementary school year, a child is able to be more involved in his behavioral therapy. He is able to be involved in role playing to advance social skills and improve communication with eye contact and body language. An adolescent can be taught how to give compliments, accept constructive criticism, take turns and resolve conflicts. He can be taught to actively listen to and follow directions and monitor his own attention. All of these will improve the patient's relationships and academic achievement improving self-esteem. As an adolescent enters adulthood, all of these techniques can be furthered, setting direct treatment goals such as dealing with procrastination and developing strategies to aid in memory and impulsivity control (Miller, Child Mind Institute).

There are ongoing discussions in the medical and psychiatric communities on which avenues of treatment to choose as the optimal way to treat ADHD. Depending on the age of the child, the first line of defense may be different. The ADHD NICE Clinical Guideline advises that parent training be the initial treatment in preschool children and young school age children with behavioral issues. Parent training is not as effective in older children. Parent training has also been shown to be less effective in cases where the parents themselves exhibit symptoms of ADHD (Young and Amarasinghe).

In a Multimodal Treatment study, four treatment possibilities were compared. Firstly, medication, in the form of methylphenidate. Second, CBT including parent training, summer treatment programs and school based treatment. Third, A combined treatment including medication and behavior therapy. And, lastly, a control group of community care consisting of children, many of whom were medicated, but did not receive treatment from the researchers. Results found that the medication and combined tracks were more effective than behavioral therapy alone or the community care. The combined treatment which consisted of medication and CBT in combination was more effective for symptoms reported by parents than medication alone. In addition, CBT, with or without medication was very effective for those patients from poorer socioeconomic groups who were receiving public assistance. The combination treatment was more effective and less costly for patients with many comorbid symptoms. In addition, it is important to note that children in the combination treatment were able to control symptoms with lower doses of medication (JAMA Psychiatry).

As patients enter adolescence, boys and girls may present with different symptoms and, therefore, may need different courses

of treatment. Boys have a tendency to be more aggressive and the need for behavior control is more pressing while girls may have more social and emotional symptoms requiring social and mood altering treatments. It is for this reason that at this stage, teens must be made an integral part of their own treatments. Parent training at this stage includes the same basic premise as younger children with positive reinforcements becoming more age appropriate. For example, positive reinforcement includes more time with friends or more computer time while consequences for negative behavior may be losing privileges (Miller, Child Mind Institute). Working closely with teachers and counselors at this stage is most important as difficulties in school is a major concern. It seems that CBT and parent training alone is not as effective in the adolescent group as in younger children and a combined regimen including medication and behavior intervention is more effective than either alone (JAMA Psychiatry). More studies on adolescents need to be undertaken, however, since most assume that their problems may be successfully addressed using similar methods as those used for their younger counterparts.

### Conclusion

ADHD is a developmental and neuropsychological disease caused by low levels of dopamine release that presents with symptoms including behavioral impulsivity and inability to focus, along with the possibility of comorbid conditions of depression and behavioral defiance. It is a disease which may present at any time throughout a lifespan, but treatment is most effective when diagnosed and treated earlier in life. There are many treatment options available to the ADHD patient including stimulant and non-stimulant medications which may be administered in many modalities and doses to fit many different lifestyles. Non pharmacological approaches include cognitive behavior therapies, including parent and teacher training, student workshops and summer training programs. Individualized approaches to treatments seem to be the most effective based on a patient's age, symptoms, socio-economic group, and sex. In general, combined medication therapy with different forms of CBT tailored to patient lifestyle as he grows is the most effective treatment.

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# Do Probiotics Effectively Promote Wellbeing?

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## Abstract

Probiotics have been commonly ascribed many therapeutic powers. The aim of this review is to investigate these claims. While some of the claims are well supported by research, the effects of probiotics are very specific and depend on the strains used as well as the disease or condition targeted. Age and racial ethnicity may also be factors impacting the efficacy of probiotic strains.

## Introduction

In recent years, probiotic supplements and probiotic yogurt have become increasingly popular pharmacy and grocery items. Proponents posit that these live cultures of beneficial bacteria counteract the negative effects of antibiotics and decreased microbial exposure of modern life, while others are skeptical of these relatively new products and claims.

Every human gut contains millions of bacteria from hundreds of species which aid in digestion and promote health. These bacteria are healthy bacteria and are very important to one's wellbeing. Western, developed countries successfully controlled infectious diseases during the last century by improving sanitation and using antibiotics and vaccines. At the same time, a rise in diseases such as allergies, autoimmune disorders, and inflammatory bowel disease has been observed in both adults and children, and it is hypothesized that improvements in hygiene, decreased microbial exposure in childhood, and decreased maintenance of microflora are responsible for this increase. In 1907, the Russian zoologist Élie Metchnikoff was the first one to suggest that gut microbes may influence human health (Britannica).

Recent studies show that ingesting oral probiotics can promote health and counteract some negative effects of antibiotics, but only in specific ways. It is necessary to understand the mechanisms and limitations of probiotic supplements in order to be an informed consumer.

## Methods

This subject was researched using Touro College's online library database as well as Google Scholar. Additionally, ProQuest, EBSCO, MEDLINE, and PubMed were used to search for articles. The author had free access to these search engines through the Touro College Library. The method of research entailed reviewing published articles and studies that have been peer reviewed. Key words such as "gut bacteria", "probiotic bacteria", "health promoting bacteria", "probiotic yogurt", "human microbiome", and "microbiota" were used to find appropriate articles.

## Background: Development of gut microbiome

Babies born via vaginal births are colonized by bacteria as they pass through the birth canal. The vaginal microbial communities appear to change during pregnancy to provide newborns with beneficial microbes; at the time of delivery, the vagina is dominated by *Lactobacillus* and *Prevotella* spp (Krajmalnik-Brown et al 2012). Breast milk also populates the baby's gut with additional

health promoting bacteria from the *Bifidobacterium* family. According to very recent research, babies acquire stomach bacteria from their mothers even before birth. In healthy human fetuses, bacteria have been detected in umbilical cord blood, fetal membranes, and amniotic fluid, supporting the notion of bacterial transmission through the placental barrier. Furthermore, the meconium (ingested amniotic fluid) from newborns has been shown to be non-sterile, home to a complex community of microbes, including *Enterococci* and *Escherichia*, commonly found in the adult GI tract. Therefore, even babies born by caesarean section are born with microbiomes, despite lacking the vaginal exposure (Funkhouser and Bordenstein 2013).

## Diversity between ethnic groups

While 90% of all people's gut biome is similar, the bacteria species and proportions vary between races and ethnic groups. A study published in 2010 comparing rural children of Burkina Faso, Africa, with children in the modern and developed city of Florence, Italy, showed that *Bacteroidetes* and *Spirochaetes* were abundant in the microbiota of the African children and that the specific types that were increased were well suited to extract nutrition from their herbal-rich diet. These bacteria were from the genus *Prevotella* and *Xylanibacter* (*Bacteroidetes*) and *Treponema* (*Spirochaetes*), which are known for their skill at cellulose and xylan hydrolysis. The diet of the rural village in Burkina Faso is low in animal protein and fat but rich in starch, fiber, and plant polysaccharides from local herbs and vegetables. These bacteria were completely lacking in the European children whose diet was high in fat, animal protein, sugars, and nutrient-rich but thoroughly cleansed foods (De Filippo et al 2010).

A recent study has shown that the Japanese possess unique bacteria in their intestines which allow them to digest red algae, commonly used to wrap fish in sushi. Japanese have a seaweed-rich diet, eating on average of 14.2 grams of sushi a day. Red algae, used in sushi, contain the polysaccharide porphyran in their cell walls. It is broken down specifically by an enzyme called porphyranase. This enzymatic activity is unique to select marine bacteria such as *Zobellia galactanivorans*, the bacteria that populate the gut of the Japanese, giving them the machinery they need to digest these nutritional sea plants (Hehemann et al 2010).

A similar phenomena has been detected in the guts of rodents. Desert woodrats (*Neotoma lepida*) of the Mojave and Great

Basin deserts have special toxin-degrading gut microbes to digest the toxic creosote and juniper bushes (Kohl et al 2014).

This diversity between ethnic groups adds a dimension of complexity to understanding the workings of the microbiome. This variation may impact the manner in which the gut responds to introduced probiotic bacteria and may result in probiotic treatments being beneficial for some people but not others.

### How gut microbes affect nutrient absorption and energy regulation

In a way similar to how rhizoidal fungi allow plant roots to absorb soil nutrition, the biofilm structure of the many mucosal microbiota adhering to gut epithelium facilitates benefits for the host, including nutrient exchange and stimulation of immunity (Sonnenburg et al 2004). For example, *Bacteroides thetaiotaomicron* is a major symbiont of the human adult gut (Hayashi et al 2013). This organism makes an important contribution to human digestion by breaking down complex plant material that innate human enzymes cannot digest. The human body can produce amylase and sucrase which break down starch, glycogen, and sucrose respectively, yet it cannot independently digest complex plant material (Microbewiki). Using the plant and fungus polysaccharides ingested by the human host as its food source, *B. thetaiotaomicron* is able to create small sugars with its glycoside hydrolases which hydrolyze glycosidic bonds in complex sugars. It produces mucus that allows it to attach to the gut epithelial cells, avoiding washout from the microecosystem. The mucus sticks to epithelial lining as well as to undigested food particles. These aggregations serve to promote the assembly of more microbes and allow their syntrophic relationship with the gut.. Other gut bacterial species such as harmless strains of *E. Coli* benefit from the small sugars provided by *B. thetaiotaomicron*, which the *E. Coli* cannot produce independently. Methanogens, another family of the microbiome, use the short-chain fatty acid products of the fermentation of carbohydrates. These symbiotic relationships add productivity to the human gut (Sonnenburg et al 2004). It is clear that the many interactions between gut bacteria are extremely complex and to date only a fraction of this organization is understood. Much research is needed to fully understand the way in which introduced probiotic bacteria interrelate and network with the host gut microbiome.

### Vitamins

Vitamin B12 is required for metabolism in cells and for the formation of red blood cells. The human body cannot produce vitamin B12. Only bacteria and archaea are capable of manufacturing this vitamin. Species of *Pseudomonas* and *Klebsiella*, normal residents of the human small intestine, have been shown to produce vitamin B12 (Albert et al 1980). *E. coli* in the large intestine produce Vitamin K2 (Bentley and Meganathan 1982).

Biotin (vitamin B7), a vitamin required for production of fatty acids and cell growth, is synthesized in significant quantities by the intestinal flora (Scheinfeld et al 2015)..

### Gut bacteria help prevent pathogenic infections

Lactic acid bacteria are found naturally in acidic foods such as pickles, olives, and yogurt, preventing spoilage by maintaining a low pH. In the same way, many pathogenic bacteria are deterred from growing in the human intestines due to the unfriendly acidic environment created by members of lactic acid bacteria family, *Lactobacillales*. Furthermore, by merely colonizing the gut, the healthy bacteria leave little real estate for pathogenic bacteria to occupy (Nester).

Having established the presence and functions of gut microbes, a question of concern is how the common use of antibiotics affects this microbiome and the life functions dependent upon it.

### What happens to the bacteria when antibiotics are taken?

Studies on isolated cultured colonies of gut bacteria in a laboratory setting have shown how antibiotics impact them. In one study, 14 common gut bacterial species from the *Clostridium*, *Bifidobacterium*, and *Bacteroides* genera were treated in vitro with ampicillin and metronidazole, clinically prescribed antibiotics. Effects on bacterial physiology and metabolism were monitored over a 48 hour period. *Bacteroides* and some *Clostridium* species were substantially reduced by Ampicillin. On bifidobacterial species ampicillin only had a bacteriostatic effect. Metronidazole strongly affected *Bacteroides* communities, reduced some *Clostridium* species, but had no effect on bifidobacterial communities. This study showed that the antibiotics ampicillin and metronidazole have a real inhibitory effect on some intestinal bacteria species but not others (Newton F et al 2013).

Modeling tests have been conducted on mice as well to better understand the relationship between antibiotics and intestinal bacteria. Mice have similar gut bacterial composition to humans. Oral intake of antibiotics, streptomycin and vancomycin, was used to agitate the intestinal microbiota of the mice. Thereafter, the mice were infected with *Salmonella enterica* serovar Typhimurium to gauge the results of antibiotic treatment. Analysis showed that the number of intestinal bacteria was not altered significantly by the antibiotic regimen, but the microbiota composition was affected. Both vancomycin and streptomycin treatments significantly decreased *Lactobacilli* and *Enterococci*/group D *Streptococci* populations and promoted the overgrowth of *Enterobacteriaceae*. These perturbations in the microbiota caused the mice to be more susceptible to *Salmonella* serovar Typhimurium intestinal colonization and infection than

before antibiotic treatment. This study demonstrates the importance of a healthy microbiota in limiting susceptibility to enteric pathogens. This study showed that the antibiotics vancomycin and streptomycin can have a detrimental effect on intestinal bacteria species, limiting host immunity (Sekirov et al 2008).

A leading expert in this field, David Relman M.D., investigated the gut bacterial communities of three healthy humans before and after treatment with ciprofloxacin, a commonly prescribed broad spectrum antibiotic of the fluoroquinolone family, by comparing stool samples before and after treatment. He monitored them for a period of ten months. Ciprofloxacin treatment eliminated about a third of the bacterial taxa in the gut, decreasing the diversity of the community. *Faecalibacterium*, *Lachnospiraceae*, *Bacteroides* and *Alistipes* are names of several genera that were reduced by ciprofloxacin. The magnitude of this effect varied to some degree between individuals. However, by 4 weeks after the end of treatment, the makeup of the community closely resembled its pretreatment state in all three individuals with the exception of several taxa which failed to recover even 6 months later. During the four week recovery period, the participants reported normal intestinal function. The rapid resurgence of the pretreatment community indicates strong bacterial community resilience. However, the fact that several bacterial species did not recover tells that even a short course of antibiotics may cause minor permanent changes to gut community flora. Though the participants did not experience any immediate obvious stomach problems from the medication, this cannot predict the possibility of long term impacts such as increased susceptibility to allergies or skin irritations (Modi S et al 2014). Relman found that a second exposure to ciprofloxacin a half year after the first treatment causes similar effects but was accompanied by incomplete recovery (Dethlefsen and Relman 2010).

Similar studies using clindamycin showed that the gut *Bacteroides* community never returned to its original composition even two years after antibiotic treatment (Jernberg et al 2010). Clearly antibiotics impact the bacterial gut population with possible effects on host wellness.

### **Which diseases result from antibiotic use?**

Campylobacteriosis (stomach ulcer) has been linked with intake of antibiotics up to one year before the onset of disease (Folkhälsomyndigheten 2014). *Candida glabrata*, an opportunistic pathogen of the urogenital tract and bloodstream in immunocompromised persons, has been associated with taking of specific antibiotics (Ben-Ami 2012). In one large study, fluoroquinolones were found responsible for over 55% of cases of *Clostridium difficile*-associated diarrhea, an often fatal disease (Pepin et al 2005). Studies have demonstrated using mouse

models that the two antibiotic-associated pathogens, *Salmonella typhimurium* and *Clostridium difficile*, infect their host by catabolizing mucosal carbohydrates liberated from microbiota by the antibiotic streptomycin (Katharine et al 2013). Use of antibiotics in children was significantly associated with Crohn's disease even if 6 months had elapsed between diagnosis and the latest intake of antibiotics (Virta et al 2012).

Aside from the infection of *Salmonella typhimurium* and *Clostridium difficile*, the exact biological mechanism for most of these epidemiological associations is currently unknown. However, it is logical to assume that killing off good bacteria is causative because we don't know of any other lasting effects of antibiotics, but we did see from the studies of Modi et al (2014) and Dethlefsen and Relman (2010) that some of the good bacteria decrease and don't ever reestablish themselves.

### **Results: Probiotic theory**

To counter the adverse effects of antibiotics many have considered consuming probiotics. The theory behind probiotics is that swallowing new live bacteria will replenish the lost bacterial communities diminished by the antibiotics. Because a healthy gut microbiome is believed to promote and maintain health, probiotic yogurt and dietary supplements have been suggested even for individuals who have not taken antibiotics. First, though, it must be determined that probiotic bacteria are alive and can reach the gut when consumed orally.

### **Do probiotic bacteria die in the acidic pH of the stomach before they reach the intestine?**

Critics of probiotics question whether the majority of the bacterial communities housed in a probiotic pill survive the acidic environment of the stomach on their journey to the intestine. Research has been done to determine the effects of the stomach's acidic environment on probiotic bacteria. It is worth noting that *Lactobacilli*, which are native gut bacteria and are included in most probiotic formulations, are acidophilic and are not adversely affected by stomach acid (Tannock 2004).

De Campo et al (2005) performed a double-blind study with 114 healthy volunteers. After 15 days of yogurt consumption, the participants' feces were analyzed by culture, specific PCR, and DNA hybridization for presence of the yogurt organisms *L. delbrueckii* and *Streptococcus thermophilus*. Detection of yogurt lactic acid bacteria in total fecal DNA by bacterial culture and PCR assay was consistently negative indicating that the strong acidic environment of the stomach killed microbes. However, Marina Elli et al (2006) showed that the De Campo et al's analytical detection methods were poorly set up. Elli's studies in turn confirmed that yogurt bacteria, especially *L. delbrueckii* subsp. *bulgaricus*, can be retrieved from feces of healthy

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individuals after a few days of ingestion of commercial yogurt. Thus, Marina Elli's research established that many strains of probiotic bacteria do indeed survive transit through the gastrointestinal tract. It still is a possibility that though the low pH of the stomach does not destroy the entire probiotic population, it may limit the number of live bacteria that reach the intestine, reducing the effectiveness of the probiotic administration.

To overcome the possibility that a portion of the probiotic bacteria die in the stomach acid, new delivery technology has been developed to protect the strains from stomach acid. Scientists have constructed a coating for susceptible probiotics to avoid this possibility. *Bifidobacterium breve*, a model probiotic, was encapsulated into a multilayer alternating alginate-chitosan coating. This construction improved the endurance of *B. breve* during contact with stomach acid. During exposure to *in vitro* gastric conditions, a tremendous increase in viability from that seen in free cells was demonstrated (Cook et al 2013). Other studies have shown that capsules made of alginate, xanthan gum, and carrageenan gum increased the survival of probiotic bacteria in acidic stomach conditions (Ding and Shah 2009). Many probiotic supplements sold today include protective coatings. Though probiotic bacteria in yogurt has no such coating, the study of Marina Elli et al (2006) confirms that much of the bacteria do survive the acidic stomach environment.

Ancillary support for the survival of probiotic bacteria comes from the animal world. Coprophagy is the norm in an overwhelming number of vertebrates. Rodents, rabbits, pigs, foals and gorillas and chimpanzees all eat their feces regularly (Soave and Brand 1991). The young of elephants, pandas and hippos eat their maternal feces and thereby obtain the bacteria required to properly digest and obtain nutrition from vegetation in their diet. They are born without these necessary bacteria in their intestines (Zilber-Rosenberg 2013). The idea of sharing gut microbiota via feces has made its way to human medicine. Fecal bacteriotherapy or stool transplants have a well-entrenched place in the history of medicine of many ancient cultures. Recent studies have shown that *C. difficile* can be effectively treated with fecal transplants (Shultz 2014). The idea behind the consumption of feces is very similar to the reason to eat probiotics. It replenishes the microbiome with friendly bacteria.

### Viability of organisms in capsules

Skeptics have questioned whether probiotic bacteria are alive and viable in their capsules or whether they die during production or storage. Many studies have been conducted proving that the bacteria are indeed alive. This is the general manufacturing process of probiotic pills as described by the Lallemand Health Solutions probiotic producer (2015): Chosen bacteria are inoculated onto a culture media and multiply. Live bacteria

are then separated from the culture medium by centrifugation; they are mixed with a cryoprotectant, to help them survive the freeze-drying process. Alternatively, they are vacuum dried or spray dried. The dried bacteria form a solid cake which is milled into a fine homogeneous powder, each grain of which contains approximately 1 billion bacteria. Bacteria powders are blended with other carrier and diluent ingredients for the desired bacterial concentrations and then encapsulated and packaged.

Studies into the storage stability of spray-dried (Ananta E et al 2005), and freeze dried (Kurtmann L et al 2009) probiotics are positive. The bacteria remain alive, although dormant, and start to grow again after they reach the moist gut environment. Even higher storage stability has been found for vacuum-dried probiotic cells. Vacuum-dried cells show much higher stabilities than the freeze-dried cells (Foerst P et al 2012).

### Effects of temperature on probiotic quality

Temperature has been shown to play a role in the stability of probiotics. Warmth and moisture are the ideal growing conditions for probiotic and gut bacteria. The growth and reactivation of these dormant organisms is inhibited in the presence of cold air, which holds less moisture and is not in the ideal temperature range for these bacteria to thrive. Cold air is therefore optimal for storage of probiotics as it keeps the bacteria from activating and hence dying before they have a chance to reach the human body. High heat can also degrade the viability of these organisms and care should be taken to keep them away from high temperatures. A thorough study on the effects of temperature on vacuum-dried probiotic bacteria revealed that after three months of storage at 4°C cells remained stable with a survival rate of 70%. At non-refrigerated temperatures (~37°C) only 54% of the cells survived (Foerst P et al 2012). Similarly, a study of freeze-dried common probiotic lactobacteria strains showed that stored at 4°C for three months, the survival rate was 76%, while storage at 23°C for the same length of time had only a 37% survival rate (Jalali M et al 2012). Similar effects of temperature on probiotic bacteria were determined for cells produced by spray-drying (Corcoran BM et al 2004). Based on this it would be best to store probiotics at refrigerated temperatures. Ideally, they should be kept at such temperatures in warehouses, shipment, and stores as well, though this is not currently standard.

It should be noted that as an extra measure, many manufacturers add an overage of bacteria to their probiotic products to compensate for the expected decline in numbers over time (NowFoods).

### Probiotics and health

To date there has been insufficient research to prove whether probiotics counteract the many adverse effects of antibiotics

mentioned above, besides for treating antibiotic-associated diarrhea. However, a large number of studies have demonstrated the benefits of prophylactic probiotic treatment for general health benefits, preventing a variety of ailments, and curing other diseases not associated with antibiotics.

### **Probiotics as treatment for antibiotic-associated diarrhea**

In 2012, researchers (Hempel S et al 2012) conducted an analytical search of hundreds of earlier studies and reviews about the effects of probiotics on antibiotic-associated diarrhea. This systematic review found that using the lactic acid-producing bacteria such as *Lactobacillus rhamnosus*, or *L. casei* as well as *Saccharomyces boulardii* [*cerevisiae*] may be helpful in preventing and curing antibiotic-associated diarrhea. The number-needed-to-treat value (NNT) was found to be 13. This means that statistically on average out of thirteen susceptible patients being treated with probiotics for antibiotic-associated diarrhea, one will benefit. Due to overall poor documentation of the probiotic strains, it was not clear if the efficacy of treatment was strain specific. Furthermore, the studies were spread over a vast population so more research needs to be performed to determine whether the elderly, middle-aged, or children would benefit most from adjunct probiotics therapy. Another question that requires clarity is which specific antibiotics are more likely to cause diarrhea and which probiotic strains best combat those particular antibiotics. However, the studies analyzed included patients taking a variety of antibiotics or did not specify the antibiotics used, limiting any conclusive correlation (Hempel S). Because the overall results from these studies are promising, many medical experts see no reason not to suggest probiotics when prescribing antibiotics (Kligler and Cochrane 2008).

A more recent (2013) large study targeting 2,941 inpatients over 65 years of age exposed to broad-spectrum antibiotics found no supporting evidence that multi-strain lactobacilli or bifidobacteria probiotics were effective in prevention of antibiotic associated diarrhea in that group (Allen S et al 2013).

Studies on the effects of probiotics on the particularly dangerous condition, *Clostridium difficile* colitis, often caused by an impaired microbiome due to antibiotics, provide insufficient evidence to recommend probiotic therapy even merely as an adjunct treatment (Pillai A and Nelson R 2008).

### **Probiotics may shorten the duration of infectious diarrhea**

Infectious diarrhea is often caused by shigella, *E. coli*, salmonella, and clostridium bacteria. A meta-analysis of almost 2000 patients from 23 studies of infectious diarrhea in both adults and children indicates that the duration of symptoms may be shortened by

the use of probiotics by a mean of 30 hours (Allen SJ et al 2004). The majority of the probiotics tested in these studies were lactic acid bacteria; two studies used *Saccharomyces boulardii*. Though infectious diarrhea is not necessarily a result of antibiotic treatment, infectious diarrhea from Salmonella often is associated with antibiotic use. Thus, in this way probiotics may be considered to be counteracting the effects of antibiotics.

### **Bifidobacterium infantis 35624 reduced symptoms of irritable bowel syndrome (IBS)**

In a review analyzing 16 randomized clinical trials of irritable bowel syndrome (IBS) patients who received either placebo or probiotic supplements, *Bifidobacterium infantis* 35624 was effective in reducing irritable bowel syndrome symptoms, such as intestinal gas, abdominal pain, bloating, and bowel function (Brenner DM et al 2009). There was no evidence of adverse results. No other probiotic, including isolated *Lactobacillus* species, showed significant improvement in IBS symptoms in appropriately designed randomized clinical trials.

Another systematic review of the literature revealed that probiotics succeeded in reducing irritable bowel syndrome symptoms with a number needed to treat (NNT) of 4 (Moayyedi P et al 2010). Almost all probiotic combinations contained *Bifidobacteria* species (Verna 2010).

### **Probiotics for constipation**

Dr. Mary Morgan, an immunologist and researcher for probiotics companies, conducted a meta-analysis to uncover which probiotics have the best results for constipation. She found the following five are best: *Bifidobacterium lactis* DN-173 010, VSL#3 formula (a probiotic mix), *Bifidobacterium lactis* Bb-12, *Lactobacillus casei* Shirota, and *Bifidobacterium longum* (Morgan 2013).

### **Probiotics may prevent hepatic encephalopathy development from cirrhosis**

A recent study shows that probiotics are effective in preventing the progression of liver cirrhosis to hepatic encephalopathy (Lunia MK et al 2013). Cirrhosis is an advanced liver disease characterized by replacement of healthy liver tissue with scar tissue, leading to progressive loss of liver function. It is usually caused by alcoholism, Hepatitis A or Hepatitis B. As cirrhosis is irreversible, treatment of cirrhosis focuses on preventing progression. In a progressed state, cirrhosis may lead to hepatic encephalopathy, in which an over accumulation of toxic ammonia in the blood due to loss of liver function brings the patient to an altered level of consciousness or coma. Natural members of gut flora such as *Eubacterium aerofaciens*, *E. lentum*, and *Peptostreptococcus productus* produce urease, which hydrolyzes urea into ammonia (Suzuki K et al 1979). In a healthy body,

this ammonia is absorbed from the intestine into the bloodstream then filtered out by a functioning healthy liver. Hepatic encephalopathy, the result of poor liver function, is commonly treated with the sugar, lactulose, which reduces the absorption of ammonia from the gut into the bloodstream. The usefulness of lactulose is limited by side effects such as diarrhea, bloating, and flatulence. The Lunia MK, et al study found that intake of a specific set of probiotics will alter the intestinal microbiota, preventing development of hepatic encephalopathy in patients with cirrhosis. By recolonizing the gut with non-urease producing bacteria such as those in the supplements used in this study, a buildup of ammonia is avoided, thus preventing hepatic coma.

The 86 patients in the treatment group received a regimen of probiotics which contained *Bifidobacterium breve*, *B. longum*, *B. infantis*, *Lactobacillus acidophilus*, *L. plantarum*, *L. paracasei*, *L. bulgaricus*, and *Streptococcus thermophilus*, 110 billion colony-forming units in all, in one capsule 3 times per day. The control group included 74 patients. Patients were told to refrain from eating any commercial probiotic yogurt. The patients were followed up on each month for six months to ascertain that they were keeping to their probiotic treatment plan and to track the development of any hepatic encephalopathy symptoms. The researchers observed that the incidence of hepatic encephalopathy was significantly lower in patients treated with probiotics. No adverse effects were detected from the probiotics. These results offer a more comfortable and better-tolerated alternative to current lactulose treatments (Lunia MK).

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### **Probiotics as bowel movement and nutrition enhancer in the elderly**

Hilla Zaharoni et al (2011) conducted a study to determine the preventive impact of probiotics on problems with bowel movement and malnutrition in the elderly (Zaharoni H et al 2011). The study included 215 elderly patients at an orthopedic rehabilitation center (107 as control, 108 as probiotic recipients), age 65 and over. Each participant received a daily dose of commercially sold probiotic bacteria. The dosage consisted of four strains of *Lactobacillus* – *L. plantarum*, *L. paracasei*, *L. bulgaricus*, *L. acidophilus*; three strains of *Bifidobacterium* – *B. breve*, *B. longum*, *B. infantis*; and one strain of *Streptococcus*, *S. thermophilus*. The control group received a daily placebo packet looking exactly like the probiotic complement. After being fed daily probiotics or placebo pills for 45 consecutive days in a randomized, double-blind, placebo-controlled trial, the patients were monitored for 45 follow-up days. The incidences of diarrhea were significantly lower among the study group (HR=0.42, p=0.04) with a more significant difference among participants age 80 and older (HR=0.32, p=0.026). The necessity to use laxatives, an indicator of constipation, also showed significant decrease in the probiotic group compared with the control group (HR=0.74, p=0.032).

Additionally, healthier levels of transthyretin, reflecting a positive nutritional intake status, and increased serum albumin levels, necessary for proper distribution of body fluids, were detected in the octogenarians of the treatment group compared with the control group (P=0.047, p=0.07, p=0.03 respectively) but not in the younger age group (between 65 and 80 years

of age). This study strongly indicates that the studied bacterial strains in commercial probiotics have a positive effect on the bowel movements of elderly patients in orthopedic rehabilitation (Zaharoni H et al 2011).

**Bifidobacterium lactis (BB-12), Lactobacillus reuteri prevents occurrence of infantile fever and diarrhea, though not respiratory illness**

An important study led by Weitzman Z et al (2005) shows excellent results for probiotics' prophylactic effect against fever and diarrhea in infants. A placebo-controlled, double-blind trial was conducted at 14 child care centers on healthy infants four to ten months old. Infants were assigned randomly to either no probiotics or baby formula supplemented with Bifidobacterium lactis (BB-12) and Lactobacillus reuteri 55730 for a period of 12 weeks. The infants were not breastfed prior to the study, and were fed only the assigned formula. The parents agreed not to administer any other probiotic or prebiotic supplements. The number of episodes of fever (>100.5 degrees F), diarrhea, and respiratory illness and the days of duration were measured for both groups.

Of 201 participating infants who were similar in gestational age, birth weight, and prior breastfeeding, 60 infants were controls, 73 were fed B. lactis, and 68 were fed L. reuteri. Febrile outbreaks and diarrheal episodes were nearly double in the control group compared to the recipients of B. lactis. The duration of diarrhea was also protracted in the control group. Results from the 68 infants of the L. reuteri group were even more encouraging. Compared with the controls and even B. lactis infants, the L. reuteri group had a substantial decrease in number of days with fever, doctor visits, nursery absences, and antibiotic medication prescriptions. The probiotic supplements showed no effect on the incidents of respiratory illnesses between the groups.

**Bifidobacteria infantis, Streptococcus thermophilus, and Bifidobacteria bifidus for necrotizing enterocolitis**

Prophylactic use of the probiotics, Bifidobacteria infantis, Streptococcus thermophilus, and Bifidobacteria bifidus, were tested by Alona Bin-Nun et al (2005) to determine their effect on the incidence and severity of necrotizing enterocolitis. Necrotizing Eterocolitis is a postnatal medical condition where portions of the bowel undergo necrosis. Primarily seen in premature infants, it is among the most common causes of mortality in premature infants. Neonates were randomized to either receive a daily feeding supplementation with a probiotic mix of 109 colony-forming units per day or to receive no probiotic supplements.

For 72 study and 73 control infants, respectively, birth weight, gestational age, and time to reach full feeds were nearly equal. The incidence of necrotizing enterocolitis was reduced in the study group (4% vs 16.4%). Additionally, necrotizing enterocolitis was less severe in the probiotic-supplemented infants. Three of 15 babies who developed necrotizing enterocolitis died, all three from the control group.

**Discussion and future study**

Over the past two decades there has been significant advancement in the field of probiotics since it has been first suggested in 1907 by Elie Metchnikoff. These studies have used random undirected criteria for choosing which bacteria from amongst thousands of species and strains to test for potential health benefits. They often chose strains occurring in various fermenting foods. Each trial has been like a shot in the dark hoping to find a cure. The author suggests that future studies focus on the strains unique to the gut of residents of the rural village in Burkina Faso in the De Filippo et al (2010) study and of other remote non-westernized locales for their potential probiotic qualities. Epidemiological evidence tells that the improved sanitation, sterile industrial foods, and antibiotics of westernized civilization has controlled infectious diseases but also has increased the incidence of allergic, autoimmune disorders, and inflammatory bowel disease (IBD). By selecting gut bacteria unique to isolated populations living with an environment and diet of the pre-modern era, we may discover probiotic beneficial bacteria which can restore and implant the health benefits of those primeval and rustic lifestyles into the modern urbane world. The De Filippo et al study identified bacteria from the genus Prevotella and Xylanibacter (Bacteroidetes) and Treponema (Spirochaetes) exclusive to the Burkina Faso population. These results should be verified and similar investigations should be made into the gut bacteria of other traditional societies such as remote Tibetan villages and Northern Okinawa farm towns. New randomized clinical trials for probiotic efficacy should be designed utilizing the bacterial findings of these remote searches.

**Practical consumer implications; Considerations for choosing probiotic supplements**

There are hundreds of probiotic supplements available on the market. Many of these products have no claim to any supporting studies. As probiotics are considered dietary supplements and not medications, they do not require certification from the Federal Department of Agriculture (FDA). There are great variations in the strains of bacteria they contain. It is best to buy products that are backed by clinical research. The wise consumer will examine the labels of the product to be certain that it contains the particular research-backed strains he or she desires. If one hopes for a particular cure or health benefit, the supplement one chooses should contain strains shown to be

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Table 1

Condition	Probiotic Bacterial Species	Population*
antibiotic-associated diarrhea	Lactobacillus rhamnosus, L. casei, Saccharomyces boulardii [cerevisiae]	people younger than 65 years
infectious diarrhea caused by shigella, E. coli, and salmonella	Lactic acid bacteria species, Saccharomyces boulardii	adults and children
irritable bowel syndrome (IBS)	Bifidobacterium infantis 35624	adults
promoting bowel movements and better nutrition intake	Lactobacillus planturum, L. paracasei, L. bulgaris, L. acidophilus, Bifidobacterium breve, B. longum, B. infantis, Streptococcus thermophiles	individuals of 65 years and older
fever and diarrhea (prophylactic treatment)	Bifidobacterium lactis (BB-12), Lactobacillus reuteri 55730	infants
constipation	Bifidobacterium lactis DN-173 010, VSL#3® formula (a probiotic mix), Bifidobacterium lactis BB-12, Lactobacillus casei Shirota, Bifidobacterium longum	adults
necrotizing enterocolitis	Bifidobacteria infantis, Streptococcus thermophilus, Bifidobacteria bifidus	infants
hepatic encephalopathy (prophylactic treatment)	Bifidobacterium breve, B. longum, B. infantis, Lactobacillus acidophilus, L. plantarum, L. paracasei, L. bulgaricus, Streptococcus thermophilus	cirrhosis patients

\*These particular populations were studied. Effects on other populations are not included unless indicated.

Table 2

Yogurt Brands	Bacteria Strains
Yoplait	Lactobacillus bulgaricus Streptococcus thermophilus sometimes: Lactobacillus acidophilus
Chobani	Lactobacillus acidophilus Bifidobacterium bifidum Lactobacillus casei
Stonyfield Farms	Lactobacillus bulgaricus Streptococcus thermophilus Lactobacillus acidophilus Bifidobacterium bifidum sometimes Lactobacillus rhamnosus
Yakult	Lactobacillus casei Shirota
Dannon	Lactobacillus bulgaricus Streptococcus thermophilus sometimes: Lactobacillus acidophilus Bifidobacterium lactis DN-173 010 in Activia® Lactobacillus casei DN-114-001 in DanActive®
La Yogurt	Lactobacillus bulgaricus Streptococcus thermophilus Lactobacillus acidophilus Bifidobacterium bifidum Lactobacillus casei Bifidobacterium animalis BB12

beneficial to that end. Table 1 summarizes researched bacterial strains and the conditions they benefit.

### **Probiotic Yogurt**

Many yogurt products claim to be probiotic. Again, these claims are not backed by the FDA. Some bacterial strains in yogurt have more scientific support than others. According to the Dairy Reporter (August 2, 2013), Dannon, Yoplait, Chobani, and Stonyfield rank among the most popular brands in the United States. Table 2 lists the strains included in commonly available yogurts.

### **Starter bacteria**

Most of the brands use *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as yogurt culture starters to turn milk into yogurt. The only researched health benefits of these two species is that they boost the immune system in anorexia patients (Nova E et al 2006) and help lactose absorption those who are lactose-intolerant (Riskalla SW et al 2000).

### **Other added strains**

#### **Bifidobacterium animalis lactis BB-12 (LaYogurt)**

The precise bacteria combination found in La Yogurt hasn't been scientifically researched. However, there have been many trials using the strain *Bifidobacterium animalis lactis* BB-12, which all La Yogurt probiotic products contain (\*personal communication). Studies have shown BB-12 reduces incidences of fever and need for antibiotic treatment in infants (Weitzman Z et al 2005). Also, it has been demonstrated that negative immune-related effects of non-breastfed infants can be significantly reduced by including *B. animalis lactis* BB-12 in their diet (Holscher HD et al 2012).

#### **Bifidobacterium lactis DN-173 010 (Activia®)**

On the yogurt ingredient panel this strain is known as *Bifidus regularis*. This strain is well suited to treating intestinal inflammations such as colitis (Veiga P et al 2010). Studies showed that it can reduce symptoms of irritable bowel syndrome (Agrawal A et al 2009) and improve digestive comfort and symptom experience of adults from the general population (Guyonnet D et al 2009) and well as help improve constipation in children (Tabbers MM et al 2009).

#### **Lactobacillus casei DN-114 001 (DanActive®)**

*L. casei* DN-114 001, also known as *L. casei* immunitas, used in DanActive®, has great study results. Daily consumption of a product containing *Lactobacillus casei* DN-114 001 has been shown to reduce the risk of gastrointestinal and respiratory common infectious diseases in shift workers (Guillemaud E et al 2010).

#### **Lactobacillus casei Shirota (Yakult)**

The yogurt drink Yakult's live *Lactobacillus casei* Shirota has been

tested for effect on constipation with excellent results. Chronic constipation patients experienced a sharp reduction in symptoms after only two weeks of drinking Yakult (Koebnick C et al 2003) (Cassani E et al 2011).

### **Yogurt Certification**

In the USA, a 'Live Active Culture Seal' (image 1) was introduced by the National Yogurt Association to identify refrigerated or frozen yoghurt products which contained at least 108 or 107 viable bacteria per gram at the time of manufacture (AboutYogurt.com). In heat-treated yogurt, a process done to prolong shelf life or decrease yogurt's natural tartness, these cultures are killed during heating after fermentation.

However, because these counts do not differentiate between true scientifically proven probiotic strains and mere starter cultures, the National Yoghurt Association's certification emblem is still not reflective of true probiotic value (Senok AC et al 2005). One must also check that the strains inside the yogurt are the well-researched bacteria discussed above.

### **Conclusions**

While it is true that many strains of bacteria confer positive health benefits when taken orally, these bacterial strains demonstrate great specificity. Precise subspecies and strains have been shown to cure certain ailments but not others, to benefit only a particular age group, and to provide these advantages only when taken on a regular basis. The research in this paper is important because it shows the specific ways in which probiotic bacteria are effective. The widely spread beliefs about benefits of probiotic products are not ensured by any governing body. Research indicates that the functions and mechanisms of gut bacteria are very complex and therefore there is no reason to assume that random "probiotic" bacteria will offer any health benefit. In addition, considerations for form and storage of probiotics were discussed: namely that alginate coating may aid in probiotic colonization and that probiotic supplements should be stored at or below 4°C. Consumers should note that the positive results in the studies were generally observed after 2-4 weeks or more of daily probiotic consumption. Additionally, suggestions for future studies utilizing gut bacteria unique to non-modernized traditional societies have been made. This paper aids the general population by providing the necessary knowledge needed in order to be an educated consumer of probiotic products.

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# Biological Therapy in the Treatment of Ulcerative Colitis

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## Abstract

Ulcerative colitis (UC), a subdivision of inflammatory bowel disease, is a chronic disease of the large intestines. Ulcerative Colitis is normally a lifelong chronic illness with times of intense flairs and remission. During a flare, the lining of the colon becomes inflamed, and develops small ulcers causing patients to experience rectal bleeding, vomiting, anemia and diarrhea. The treatment options available to treat colitis are very small, causing many patients to need a total colectomy within the first five years of their diagnosis. However, recent advancement in bio-technology has led to the development of a large array of new therapeutic agents intended to target the exact site in the multifaceted cascade of cytokine and chemokine effector molecules involved in UC pathogenesis. This article discusses the introduction of the chimeric monoclonal antibody to TNF $\alpha$  that has deeply affected the clinical treatment of moderate to severe ulcerative colitis, opening the door to a new era in the treatment of this disease. Studies discussed in this paper prove the effectiveness of both Remicade and Humira, two different biologics, given to patients with an active state of this disease.

## Introduction

Acronyms: UC-ulcerative colitis IBD-inflammatory bowel disease  
Ulcerative colitis (UC), a subdivision of inflammatory bowel disease (IBD), affects 1 to 2 million people in the United States each year. The reason for ulcerative colitis is not quite understood. However, the condition appears to be related to a combination of genetic and environmental factors. Minor symptoms include unformed stools, abdominal cramping, and diarrhea (Lichteger et al., 1994). As the severity of the disease increases, patients experience fatigue, loss of appetite, weight loss mucus present in the stool, intense rectal bleeding in addition to fever and anemia. UC can ensue at any time yet it is usually diagnosed in a person's teenage years. Roughly around 20% of people with UC have a close relative with IBD. (CCFA.org, 2015).

UC is normally a lifelong chronic illness with times of intense flairs and remission. A severe attack which can be a potentially fatal condition, is sometimes the first manifestation and is observed in about 15% of the patients with this disease. The introduction of corticosteroids sharply reduced the death rate. Corticosteroids remain as a pillar in the treatment of a severe attack of ulcerative colitis. The importance of achieving remission is undermined by the fact that the long-term colectomy rate in patients with a severe attack who achieve clinical and endoscopic remission after steroids is similar to those with a moderately severe or mild attack. The dependence on corticosteroid is a continuous problem. Steroid dependent patients may develop osteoporosis as they get older, in addition to dealing with weight gain, moon face, increase in appetite, as well as, acne and joint pain. Furthermore, the likelihood of a colectomy within the first five years from diagnosis ranges from 9% in patients with distal colitis to 35% in patients with complete colitis, normally because of unsuccessful medical therapy. The risk of recurring inflammatory bowel disease in the form of pouchitis ranges from 15.5% 1 year after the procedure to 45.5% 10 years after the procedure. Accordingly, new treatments for ulcerative colitis are needed (CCFA.org, 2015).

In recent years biological therapy has been the new method of treatment for those who are steroid dependent and or failing to respond to other treatments. Tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) is a key pro-inflammatory cytokine in patients with Crohn's disease, but is also found in amplified amounts in the intestinal tissue, stools, blood and urine of patients with ulcerative colitis. Infliximab, also known as Remicade, is an IgG1 monoclonal antibody that binds with intense strength to TNF- $\alpha$ , neutralizing its biologic activity (Simmons & Jewell, 2002). The question now becomes; will biological therapy be the answer to help induce clinical remission in those suffering from UC?

## Material and methods

In order to answer the question proposed above, many research articles with relation to this topic have been read. Touro College's library database, the national website of Pubmed and Google Scholar were all used to obtain information for this paper. All of the articles and information that was accumulated through this research have been used in an attempt to conclusively determine if biological therapy will induce a remission in patients living with active state of ulcerative colitis.

## Different types of ulcerative colitis

The symptoms present for a person with ulcerative colitis will vary based on the amount and severity of inflammation present and the site of the disease in the large intestines. Ulcerative proctitis, the mildest form of ulcerative colitis, is limited to the rectum (about 6 inches or less). Proctosigmoiditis is colitis that affects the rectum and sigmoid colon-which is located superior to the rectum. Proctosigmoiditis can be determined sometimes by the symptom of tenesmus (straining to have a bowel movement.) In addition, moderate pain on the lower left side of the abdomen may occur while the disease is present. The third version of colitis is known as left sided colitis. This presents as continuous inflammation that begins at the rectum and goes up as far as the splenic flexure (a bend in the colon near the spleen in the upper left abdomen). Symptoms for left side colitis

include severe pain on the left side of the abdomen, in addition to loss of appetite. Lastly, the most severe form of ulcerative colitis is pancolitis. Pancolitis affects the entire colon causing terrible abdominal pain, weight loss and complete loss of appetite (CCFA.org, 2015).

### Climbing the medical ladder

Today, there are very effective treatments on the market to help control the disease with the goal of putting it into remission. The main aim of the different treatments is designed to decrease inflammation. This not only allows the colon to heal, but also relieves the symptoms of diarrhea, rectal bleeding and abdominal pain accompanied by the flare. Unfortunately, there is no “one size fits all” treatment plan for everyone suffering with this disease. One person may do just fine with the most minor suppository and live fine the rest of his life, while another may require more invasive treatment or need a complete colectomy. Therefore, the approach must be customized for each individual person because each person’s disease is different. With the correct medical treatment, a patient can achieve remission with the hope of the state of remission lasting for months or even years. Nonetheless, ulcerative colitis may flare up at times from the reappearance of inflammation or from a specific trigger. Reoccurrence of a flare usually indicates the need to change the medication regimen for the patient. The choice of drugs to treat a patient is dependent upon the severity of the disease. The most commonly prescribed drugs fall into three basic categories:

**Aminosalicylates:** This class of drugs encompass 5-aminosalicylic acid commonly known as the 5-ASA. This class of drug is available in an oral form in addition to a suppository or an enema. The goal of the 5-ASA is to line the GI tract with the hope to decrease inflammation. In addition, Aminosalicylates are beneficial as a maintenance treatment in order to prevent a relapse of the disease (Colombel et al., 2010).

**Corticosteroids:** The job of steroids is to prevent the body from launching an inflammatory cascade. Additionally, corticosteroids work to keep the immune system properly balanced. Steroids are effective for short-term control of flare-ups. This class of drugs is known to help “put out the fire”. Yet, corticosteroids are not recommended for long term use or as a maintenance drug for UC because of their tremendous amount of side effects. The goal of steroids is to buy the patient time, not fix the problem. A person who is unable to come off steroids without experiencing a relapse of the disease will then be prescribed a higher class of drugs to help manage the disease (Colombel et al., 2010).

**Immunomodulators:** Immunosuppressants work to control the body’s immune system response in order to prevent

ongoing inflammation. Immunomodulators are mostly used in people who have failed to respond to the 5-ASA and steroids have not been effective or have been minimally effective in controlling the disease. Furthermore, immunosuppressants are geared to allow people who are steroid dependent to eliminate the use of them (Sood et al., 2002).

Though these three possibilities of treatments have been shown to be effective, there are always patients who fail to respond to the three treatment options. These patients were left with no choice but to have surgery to rid them of the disease leaving them without a colon and remaining with a pouch for the rest of their lives. It had become evident that there was a need to help prevent patients from undergoing a complete colectomy.

### TNF-a and inflammation

All humans have a large gastrointestinal mucosal surface that is continually being exposed to billions of potentially harmful antigens from our food, bacteria and the environment. However, the mucosal surface of the GI tract possesses an immune system that strongly controls the balance between immunogenic responsiveness and non-responsiveness-tolerance. Response to luminal antigens provokes “controlled” inflammation that is rapidly down regulated after eradication of the pathogen. However, this is not the case for someone with ulcerative colitis. In this disease this state of equilibrium is disturbed resulting in a state of chronic inflammation. During the start of the inflammation, antigen presenting cells, such as macrophages, cause the activation of T lymphocytes. T lymphocyte cells divide into two T helper T1 and T2 cells. T1 cells secrete interferon gamma (IFN $\gamma$ ) which then enables macrophages to produce TNF $\alpha$  (Simmons & Jewell, 2002).

TNF $\alpha$  is an inflammatory mechanism that has been shown to play a vital role in the pathogenesis of IBD. The transcribing of the TNF $\alpha$  gene in stimulated macrophages, platelets, monocytes, adipocytes and T cells results in the secretion of TNF $\alpha$ . Circulating TNF $\alpha$  binds to 2 TNF $\alpha$  receptors facilitating numerous biological effects including activation of other macrophages, further expansion of the T cell response, and initiation of granuloma formation. TNF $\alpha$  also lengthens inflammation by triggering NF- $\kappa$ B dependent pathways, which contribute to ulceration and degradation of the mucosa through the release of MMP (matrix metalloproteins). TNF $\alpha$  triggers other inflammatory mediators, such as, IL6 therefore strengthening the early sequence of the inflammatory cascade. Increased TNF $\alpha$  production and NF $\kappa$ B have been shown to be abundant in lamina propria mononuclear cells derived from UC patients with ulcerative colitis through stool, urine and blood tests (Hassan et al., 2007).

Recent advancement in bio-technology have thankfully led to

the development of a large array of new therapeutic agents intended to target the exact site in the multifaceted cascade of cytokine and chemokine effector molecules involved in UC pathogenesis. In particular, the introduction of the chimeric monoclonal antibody to TNF $\alpha$  has deeply affected the clinical treatment of moderate to severe ulcerative colitis, opening the door to a new era in the treatment of this disease.

### **Infliximab**

Infliximab is the first and most widely studied biological agent for ulcerative colitis. As of 2006, Infliximab has been approved by the FDA for the treatment of ulcerative colitis. "This biologic is a chimeric monoclonal antibody to TNF $\alpha$  composed of a "human" IgG1 constant region (75%) and murine-derived antigen binding variable region (25%)" (Sands & Kaplan, 2007). Infliximab is able to bind powerfully to both soluble and membrane-bound TNF $\alpha$  receptors. The actual mechanism of Infliximab is not completely understood, direct neutralization of TNF $\alpha$  does not entirely explain its effect. Infliximab has been found to apply a proapoptotic effect on monocytes and T cells in the lamina propria of the gut. The proapoptotic effect in infliximab may be exerted, preventing the production of granulocyte macrophage colony-stimulating factor, which is a growth factor that stimulates granulocyte growth and differentiation and activates neutrophils with greater adhesion (Sands & Kaplan, 2007).

Patients were eager to try this treatment option hoping to get their disease under control. A study was conducted in March 2002 testing the safety and efficacy of Infliximab. This study consisted of two large double-blind, placebo controlled trials known as the Active Ulcerative Colitis Trials 1 and 2 (ACT 1 and ACT 2). Both trails were done on 364 patients with moderate to severe ulcerative colitis. All participants in ACT 1 who were considered eligible were screened for a confirmed diagnosis of ulcerative colitis via biopsy and endoscopies. Eligible patients had active colitis with a Mayo score of 6 to 12 points (scores range from 0-12 with higher scores indicating more severe disease activity). Additionally, eligible patients also had active disease on the sigmoidoscopy exam results (despite simultaneous treatment with steroids alone or in combination with immunosuppressant's, aza or 6mp). ACT 2 allowed study participants who had only failed 5 ASA's to participate. Patients formerly exposed to infliximab or any other anti-TNF agents were omitted. Patients who were eligible were randomly assigned to be administered intravenous infusions or infliximab at a dose of 5mg or 10mg per kilogram of weight or a placebo at weeks 0, 2, and 6 and then every 8 weeks. Patients were monitored through week 54 in ACT 1 and week 30 in ACT 2 (Rutgeerts et al., 2005).

The main end point was a clinical response at week 8. Secondary end points included a clinical response or remission with stoppage of corticosteroids at week 30 in both studies and at week 54 in ACT 1.

Of the 364 patients in ACT 1, 121 were given a placebo, 121 received 5 mg of infliximab, and 122 received 10 mg of infliximab. Treatment was stopped early by 74 patients in the placebo group, 45 patients in the group receiving 5 mg of infliximab, and 49 patients in the group getting 10 mg. Of the 364 patients in ACT 2, 123 were given a placebo, 121 received 5 mg of infliximab, and 120 were administered 10 mg. At week 8, in ACT 1, clinical remission was achieved in 69.4% of patients receiving 5 mg of infliximab and 61.5 % of patients in the group receiving 10 mg of infliximab. However, in the placebo group only 37.2 percent of patients achieved clinical response. In ACT 2, at week 8, 64.5 % of the patients in the group receiving 5 mg of infliximab and 69.2 % of patients receiving 10 mg of infliximab exhibited a clinical response, in comparison to the 29.3 % of patients in the placebo group. Additionally, at the end of weeks 54 and 30 significantly higher percentage of patients receiving Infliximab as opposed to the placebo went into complete remission and were able to discontinue the use of steroids (Rutgeerts et al., 2005).

### **Antibodies against Infliximab**

A common concern when starting biologics is the possibility of a buildup of antibodies towards the drug. One study tested serum samples from participants to check for a buildup of antibodies to the drug. At week 54, 229 patients in ACT 1 had serum samples available for testing of antibody build up to Infliximab. Only 14 patients had a positive test after the first infusion, 36 presented negative tests and 179 patients had inconclusive tests. In ACT 2 from the 188 patients who had serum samples available for testing, 12 presented a positive test for antibodies, 34 negative, and 142 inconclusive. Furthermore, in ACT 2 a clinical response still occurred in 11 patients who tested positive for antibodies.

The study further tested the safety of this drug. In both studies, the proportions of patients with adverse events were similar in the placebo group and the two Infliximab groups. In ACT 1, serious adverse events occurred in 25.6% of patients in the placebo group, 21.5% of patients receiving 5 mg of Infliximab, and 23.8% percent of patients receiving 10 mg of infliximab. In ACT 2, the percentages of serious adverse events were 19.5% in the placebo group, 10.7% in the 5mg group, and 9.2% in the 10mg group. Based on these numbers, it was concluded that adverse events were more common in the placebo group showing that the medication was effective. Serious adverse events were most commonly related to the gastrointestinal system.

Among adverse events in ACT 1, basal-cell carcinoma developed in one patient treated with 10 mg of infliximab. In ACT 2, basal-cell carcinoma developed in one patient who received placebo, and rectal adenocarcinoma developed in one patient treated with 5 mg of infliximab. Three neurologic events occurred only in patients treated with infliximab. One patient in ACT 2 (receiving 5mg of infliximab) presented a lupus-like reaction (Rutgeers et al., 2005).

The incidence of infections was similar among the groups in both studies. In ACT 1, infections occurred in five patients in the placebo group, three patients in the group receiving 5 mg of infliximab, and eight patients in the group receiving 10 mg of infliximab. In ACT 2, severe infections occurred in one patient in the placebo group, two patients in the group receiving 5 mg of infliximab, and three patients in the group receiving 10 mg of infliximab. In ACT 1, tuberculosis developed in one patient treated with 10 mg of infliximab (Rutgeers et al., 2005).

This study proves that Infliximab is twice as likely to induce clinical remission in patients with moderate to severe ulcerative colitis in comparison to a placebo. Because the results of the study proved to be positive for adult patients put on Infliximab doctors wanted to use this drug to treat young children failing to respond to other medical therapy as well. Consequently, a study was done to test the safety and efficacy of the use of Remicade in pediatric patients with a moderate to severe form of the disease. A total of 60 pediatric patients ranging from ages 6-17 years old who had active ulcerative colitis and had not responded to or tolerated other treatment options like immunosuppressants, steroids or 5ASA, were given 5 mg/ of infliximab at weeks 0, 2, and 6. The primary end point was response at week 8. At week 8, those whose responded well to the drug were randomly assigned to groups given Infliximab every 8 or 12 weeks and were monitored through week 54 (Hyams et al., 2012).

### Results

The study done proved Remicade to be highly effective in treating children with moderate to severe form of the disease. At week 8, Infliximab induced a response in 73.3% of patients. Additionally, 68.3% of patients achieved mucosal healing at week 8. Those who achieved remission at week 8, were randomly assigned to receive Infliximab, 5mg every 8 weeks through week 46 or every 12 weeks through week 42. At week 54, twice as many patients in the group that received Remicade every 8 weeks achieved remission compared with the group that received Remicade every 12 weeks. Serious unfortunate events and infusion reactions occurred in comparable quantities in the 8 and 12 week groups. No deaths, cancers, serious infections, or tuberculosis were reported in either group given Remicade. Infliximab was safe and effective, inducing a response at week

8 in 73.3% of pediatric patients with moderate to severely active ulcerative colitis who did not respond to other medical intervention. Those given the every 8 week infusion ended up with a higher clinical remission rate than those who were given infusion every 12 weeks (Hyams., 2012) .

Today, Remicade is approved for children and adults. It is known to be one of the most effective methods of treatment for those suffering with an active state of the disease. Remicade is known to save people from undergoing a complete colectomy in addition to giving them a new lease on life. The studies above help strengthen this point showing how effective this biologic can be.

### Adalimumab

Another biologic on the rise is known as Humira has been approved by the FDA in 2012. Humira is a treatment method for moderate to severe active ulcerative colitis in adult patients who have failed to respond to conventional therapy. Humira is a fully humanized monoclonal antibody against TNF $\alpha$ . Unlike Remicade, Humira is administered subcutaneously. Because Adalimumab is fairly new on the market it is still being tested today. The main studies that assessed the effectiveness of Adalimumab in UC are the induction and maintenance trials, known as ULTRA 1 and ULTRA 2 (Ammuzi et al., 2013).

ULTRA 1 was an 8 week randomized, double-blind, placebo controlled trial studying the use of Humira as an induction therapy in patients with moderate- severe UC despite the use of conventional therapy. In the ULTRA 1 trial 576 patients were randomly selected to receive either placebo or one of two different regimens of Adalimumab. The first regimen was 160/80mg. patients were given 160mg at week 0 followed by 80mg at week 2 and 40mg at weeks 4 and 6. The second regimen was 80/40mg. 80mg were given at week 0, 40mg were given at week 2, 4, 6. At the same time patients were still receiving stable treatment with oral corticoid steroids or immunosuppressants (Sandborn et al., 2013).

The primary endpoint of the trial was clinical remission at week 8. The patients who participated had moderately to severely active UC, with a Mayo score of 6–12 and simultaneous treatment with at least oral corticosteroids, mercaptopurine/AZA or did not respond or could not endure prior corticosteroids or immunomodulators.

The primary endpoint of clinical remission was achieved with the higher dose of Adalimumab 160/80mg showing results of 18.5% percent response and 9.2% placebo. The amount of patients who achieved clinical remission at week 8 in the placebo group compared to the group receiving Humira at a regimen of 80/40mg and 160/80mg was a difference of point .6%. These

results show that Humira is much more effective when given to patients at a higher regimen. Additionally, Adalimumab treatment was tolerated well at both doses. The safety profile was comparable to that of placebo. Ulcerative colitis was the most common adverse event which led to discontinuation of 4% of the placebo group, 3.8% of the 80/40mg group and 3.6% of the 160/80mg group (Sandborn et al., 2013).

ULTRA 2 was a 52 week study evaluating the efficacy of Humira as a maintenance therapy in UC patients. The ULTRA 2 trial included 494 patients displaying moderate-severe ulcerative colitis and failed to respond to conventional treatment. ULTRA 2 evaluated patients through week 52 but did not have an open-label phase after induction like in ULTRA 1. ULTRA 2 included two treatment possibilities: 248 patients received Adalimumab 160/80mg (160 mg at week 0, 80 mg at week 2, and 40 mg every other week starting at week 4) and a placebo group consisting of 246 patients. Participants were randomly assigned to treatment groups. The two primary endpoints were remission at week 8 and 52 (Sandborn et al., 2013).

Overall, clinical remission at week 8 was achieved in 16.5% of patients on Humira compared to 9.3% on placebo. The results for week 52 for the Adalimumab and placebo groups were 17.3% and 8.5%. Secondary endpoints encompassed clinical response and mucosal healing. The differences between adalimumab and placebo were substantial at weeks 8 and 52 in favor of adalimumab (Ammuzi et al., 2013).

A subclass analysis of ULTRA 2 evaluated the one-year maintenance outcomes in patients who responded to therapy with Adalimumab. Patients who attained clinical response at week 8 were evaluated at week 52 to determine if they achieved several outcomes such as clinical remission, clinical response and mucosal healing. At week 8, approximately half of the Humira treated patients achieved clinical response. Of those, 30.9% from the placebo, 49.6% for the 160/80mg and 43.1% for the 80/40mg achieved clinical remission, clinical response and mucosal healing at week 52. Furthermore, of the week 8 responders who were dependent on corticosteroids, 21.1% achieved steroid-free remission and 37.8% were able to discontinue steroids at week 52 (Sandborn et al., 2013).

Aside from the ULTRA trials, there is further clinical experience on the use of Adalimumab for UC. Data from a study on a small group of 30 patients from Spain validated that Adalimumab induction and maintenance therapy was effective in patients who previously failed other therapies including infliximab. At weeks 4 and 12, clinical response was achieved in 16 (53%) and 18 (60%) of patients, and clinical remission was achieved in 3 (10%) and 8 (27%) of the patients. Adalimumab was continued in 50%

of the patients after the 48week follow-up. Total colectomy was necessary in six (20%) of patients. Nevertheless, patients who reached clinical response at week 12 evaded colectomy over the long term (Denese et al., 2013).

### **Adalimumab vs. Infliximab**

Real-life data on the use of anti-TNF agents in UC was obtained from a Canadian group. This was a forthcoming study with a long-term follow-up of 53 patients treated with either Remicade or Humira. Effectiveness was evaluated using physician's worldwide assessment focusing on stabilization of bowel frequency, non-appearance of blood with defecation and tapering of corticosteroids until it can be discontinued. Responses to induction therapy were 96.4% for infliximab and 80% for Adalimumab. Responses to maintenance therapy were similar: infliximab 77.8% and Adalimumab 70.0% (Denses et al., 2013).

Based on research present above, Remicade tends to produce better results when given to patients with active disease. There haven't been any studies found showing that Remicade does not induce remission to those experiencing moderate to severe ulcerative colitis.

### **Administering biologics**

However, due to the way the two biologics are administered may be enough of a reason for a patient to prefer one over the other. Remicade is administered as a 2-hour IV infusion so that it goes right into the bloodstream and starts to work. Doctors will normally give a patient 5mg per infusion. Some adult patients who at first respond to treatment or completely fail to respond to 5mg/kg may do well if their dose is increased to 10mg/kg. Remicade is first given at weeks 0, 2, and 6. Patients will then stay on maintenance therapy, which is every 8 weeks, which could be as few as 6 times per year (REMICADE.com, 2014).

Humira, on the other hand, is given via self-injection under the skin, typically every other week (after the initial starting doses). Humira needs to be kept in a refrigerator in its original bottle and protected from light until it's ready to be used. The recommended dose regimen for adult patients taking Humira is 160 mg initially on Day 1 (given as four 40 mg injections in one day or as two 40 mg injections per day for two sequential days), followed by 80 mg two weeks later. When this is completed patients continue two weeks later with a dose of 40 mg every other week (HUMIRA.com, 2013).

### **Possible side effects**

As always, all drugs come along with side effects. The usual side effects of biologics are nausea, headaches and fatigue. However, with time these side effects usually subside. More serious side effects have been experienced by patients especially those 65 years and older. Patients have had serious infections caused by viruses,

fungi or bacteria that have spread all through the body, including tuberculosis (TB) and histoplasmosis. Some of these infections have been deadly. Rare cancers have been reported in children and teenage patients taking TNF-blocker medicines. T-cell lymphoma, an uncommon form of deadly lymphoma, has transpired typically in teenage or young adult males with ulcerative colitis who were taking Remicade or Humira. (REMICADE.com, 2014; HUMIRA.com, 2013). To help prevent serious side effects, patients are required to go for blood work initially every other week and then monthly in order to monitor their blood count. Though the side effects listed above are scary, a patient needs to weigh the benefits and the risks together. Prolonged, untreated inflammation is the number one cause of colon cancer a number one leading death in the United States today.

### Immunosuppressants + Biologics

Seeing the results biologics produce on their own, doctors questioned if combining immunosuppressants with biologics to treat patients would have an even greater outcome than just using biologics alone. Their thought process was as follows; the job of immunosuppressants is to stop the production of white blood cells by interfering with transcribing DNA and preventing the division and multiplying of many more white blood cells. Biologics on the other hand, are geared to stop an inflammatory response by binding with TNF $\alpha$ . Therefore, it was thought by combining these two drugs the likelihood of the patient achieving a remission is two times more likely than just treating them with one drug.

Initial data is available from a UC study that had a similar design to SONIC (which was a study designed to test the same combination in Crohn's patients) (Colombel et al., 2012). The study included 239 patients with moderate-severe UC who were unexperienced to biologics, were failing corticosteroids and were either unexperienced to AZA or had stopped AZA three months before entering the study. Patients were randomly assigned to receive AZA 2.5 mg/kg, infliximab 5 mg/kg or infliximab 5 mg/kg plus AZA 2.5 mg/kg for 16 weeks. The primary endpoint was steroid-free remission at week 16 and secondary endpoints included response and mucosal healing both at week 16. Preliminary results showed that the primary endpoint was achieved in the infliximab plus AZA group compared with the AZA alone. Additionally, infliximab plus AZA was greater to AZA or infliximab monotherapy in inducing steroid-free remission in patients. Patients treated with an infliximab plan of action were more likely to achieve response and mucosal healing than those treated with AZA monotherapy. What does this study mean for anti-TNF therapy moving forward? It is tough to make any precise conclusions and more data is needed. Nonetheless, the limited data (only from infliximab experience) point that anti-TNF therapy in combination with immunosuppressants may be more effective in early UC compared with monotherapy at

16 weeks. Though remission is achieved in a greater percentage when the two classes of drugs are combined, it is not recommended to combine the two because of increased risk of side effects (Denese et al., 2013).

### Conclusion

Recent advancement in bio-technology have thankfully led to the development of a large array of new therapeutic agents intended to target the exact site in the multifaceted cascade of cytokine and chemokine effector molecules involved in UC pathogenesis. In particular, the introduction of the chimeric monoclonal antibody to TNF $\alpha$  has deeply affected the clinical treatment of moderate to severe ulcerative colitis, opening the door to a new era in the treatment of this disease. Studies discussed in this paper show the effectiveness of both Remicade and Humira given to patients with an active state of this disease. Additionally, doctors tested the possibility of combining a biologic with an immunosuppressant. Though remission nearly doubled, side effects were likely to double as well. Therefore, doctors are not pushing the combination of the two drugs unless there is no other option.

With the recent introduction to biologics, patients suffering with ulcerative colitis in an unmanageable state are able to try a new treatment option with the hope of avoiding surgery. Remicade and Humira have both been proven to put patients with an active state of the disease into full remission.

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# What are the Possible Causes for Autism Spectrum Disorder?

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## Abstract

Ever since the mid 1980's when Autism Spectrum Disorder (ASD) started to become increasingly prevalent, researchers have been trying to find a possible cause for it. Autism Spectrum Disorder is a developmental disorder that manifests itself in children who are between 18-30 months old. People with autism have reduced social skills, and they have a difficult time communicating verbally and non-verbally. Autism is diagnosed through a questionnaire and other instruments that allow psychologists, psychiatrists, speech therapists, neurologists and many other doctors to determine if a child has a form of ASD. There are many theories about what causes autism. It has been widely believed that vaccines, specifically the MMR vaccine causes autism, but that theory has been disproven. Another possibility is the role genetics plays in ASD. Many studies have been done to determine which genes might cause autism. Because of the heterogeneity of the symptoms and behaviors of people with autism and general genetic complexity it has been hard to find a specific genetic cause of autism. While a confirmed gene has not been determined to cause autism many studies have been done, some using whole-exome sequencing (WES) to see if mutations in parent's genes caused their children to have autism. It is also possible that autism can be caused by hereditary or parental factors. There have also been theories that environmental factors play a role in autism such as exposure to tobacco, pollutants, and metals, and including maternal conditions that could affect the fetus. While many of the studies were inconclusive, and a confirmed cause of autism has not been found, the studies do show that genetics does play a major role in ASD, though more studies will have to be done to determine how and which genes cause it.

## Introduction

Autism Spectrum Disorder (ASD) is a neurological impairment where the child has a problem interacting with others, has trouble communicating verbally and non-verbally, and exhibits odd behaviors such as repeating certain actions over and over again and acting out if something does not go according to routine (Block et. al., 2006). A child with ASD will have trouble making eye contact with those interacting with him and will be unable to understand what others are thinking or feeling because they have trouble picking up on social cues ("What is Autism", 2009) ASD is predicted to occur in three to six children for every 1000 who are born. It is 5 times more likely for a male to get autism than a female, however if a female does have autism her symptoms are more severe. Autism Spectrum Disorder is categorized as a pervasive developmental disorder because there are many different symptoms and behaviors for people with autism. Someone who has severe symptoms of autism can be placed on one end of the spectrum, while someone with a milder case can be on the opposite end of the spectrum. Different forms of ASD include Rett's disorder, childhood disintegrative disorder, and Asperger's syndrome. (Block et. al., 2006).

## Methods

The information that was collected for this research paper was obtained through Proquest, Ebsco, Pubmed, and the NIH website. These resources were provided from the Touro College Library. Key words included autism, ASD, vaccinations, genetics, and environmental factors.

## Discussion: Diagnosing Autism

There is no specific test done to determine if a child has ASD because the symptoms and behaviors are so broad and can vary

significantly between each child. Although children can start showing symptoms between 15-18 months of age, the average age of diagnosing is four to five years old. Diagnosis for ASD should be done by gathering developmental history of the child, and by direct observation of the behaviors of the child. There should be a team of different professionals who can assess symptoms and behaviors of the child. The team should include a pediatrician, a child psychiatrist, and a speech and language therapist, and a child psychologist. Behavior of the child should be observed in different settings such as in his home and in his school. The team should be looking for different behaviors that are prevalent in children with ASD. Prognosis of each case is done on an individual basis because the behaviors of the child can vary greatly, and the behaviors can even change over time. Some people with autism can lead productive lives, such as those with high functioning Asperger's syndrome, while others will need assistance all through their lives (Yates et. al., 2013).

## MMR Vaccine and Autism

There has been much talk over the years about a possible link between the Mumps, Measles and Rubella (MMR) vaccine and autism. The supposed link caused major panic because vaccines are needed to prevent, in this case, mumps, measles and rubella from spreading. Vaccines were a vital discovery which prevented many infectious diseases from recurring over and over again around the world.

The link between the vaccine and autism was introduced by Andrew Wakefield in 1998 (cited in Richards, 2011), when he conducted an experiment using twelve children to prove the link. It came out later, while his experiment was under

investigation, that he altered many of the details of the children's previous symptoms and behaviors. Therefore his study had no scientific basis to prove a link. Many studies have been done since then to disprove the supposed link between the MMR vaccine and autism (Richards, 2011).

One reason why people may think there is a link between the MMR vaccine and ASD is because children start showing signs of autism right around the time that they get the vaccine. The explanation for this is that the vaccine is given to children only after they are a year old, which is when children start showing symptoms and unusual behavior. Just because these events happen around the same time does not mean that there is any basis for a connection. Many studies were done to try to prove a link between the vaccine and autism but none of them have been successful ('Autism and the MMR Vaccine', 2001).

### **Investigations done to disprove the link**

In 2000, the Institute of Medicine at the National Academy of Science, under the request of the CDC and NIH conducted a review of all the evidence linking the MMR vaccine to ASD. They took under consideration all the studies and concluded that the evidence did not support an association between the vaccine and autism. Furthermore in 2000, the American Academy of Pediatrics held a conference to review the information and found no evidence in support of a linkage ('Autism and the MMR Vaccine', 2001).

In 1999, Taylor and colleagues published a study to determine a connection between the MMR vaccine and autism ('Autism and the MMR Vaccine', 2001). He took all the known cases of ASD in children living in certain districts in London who were born in 1979 or later and matched the ASD patients with an independent registry of vaccinations. His results were: 1. the number of ASD cases had increased steadily since 1979 but there was no steep incline in cases after doctors started using the MMR vaccine. 2. Children showed symptoms and were diagnosed with ASD all at around the same age, regardless if they got the vaccine before or after 18 months of age. This is important because if the children were vaccinated they would have shown signs of ASD earlier than those children who were not vaccinated. 3. Lastly, by age two the number of children who got the vaccine and showed signs for ASD was almost the same amount as children who got the vaccine but did not show any symptoms. If there was a connection between the MMR vaccine and autism more children should have shown symptoms for ASD (NICHD, 2001). Although the MMR vaccine was a popular theory concerning the causes of autism, many later studies failed to show a connection. This still remains controversial and continues to be studied.

### **Genetic factors that may cause ASD possible genetic mutations that cause autism**

Another possible cause of autism is genetic mutations in the genome of the affected person. Mutations in genes that are responsible for encoding proteins which are involved in molecular machinery regulating synaptic protein synthesis is strongly connected to autism. Autism possibly can be caused due to mutations known as Fragile X Syndrome and tuberous sclerosis (TS). Both of these syndromes are caused by abnormal mRNA translation, resulting in excess protein synthesis.

Fragile X Syndrome (FXS) is caused by an unstable expansion of the CGG repeat in the FMR1 gene. The prevalence of autism in the FXS mutation is 30%. This gene mutation causes abnormal methylation production, FMR1 transcription silencing, and decreased FMRP protein levels in the brain. ASD in FXS is mainly characterized by deficits in peer interaction. Tuberous sclerosis is an autosomal dominant disease that is caused by a mutation on either the TSC1 or TSC2 gene. Clinical manifestations of tuberous sclerosis included epilepsy, learning difficulties, and behavioral problems. There are a variety of mutations that cause tuberous sclerosis including nonsense, missense, insertion, and deletion mutations. Autism is significantly more frequent among TS patients than in the general population. Because there is a large phenotypic variability in FXS and TS with patients who have autism, research is being done to make a clear correlation (Perisco et. al., 2013).

### **Using Whole-Exome Sequencing (WES) to determine possible genetic links to ASD**

Whole-Exome Sequencing (WES) is a technique which captures and sequences the exons in the genome to reveal all de novo and low frequenting alleles that contribute to genetic risk for disease. This method is cheaper than Whole-Genome Sequencing, because instead of searching through the entire genome, it only goes through the exons in the genome, which only takes up 2-3% of the genome. This technique is better than the previously used technique of Genome Wide Association Studies (GWAS) because GWAS only reveals risk factors that are common in the population (Icahn School of Medicine, 2012). WES is particularly good for finding the cause of ASD because ASD can be caused from different genes in many different locations with a weak genotype and phenotype correlation (Perisco et. al., 2013).

A study was done applying WES on families where the spouses were related and on families where cousins shared the same disease. This way it is easier to identify specific mutations that cause ASD. The mutations that were identified on the genes were hypomorphic mutations, meaning that the altered gene product has a reduced level of activity, which can explain the wide spectrum of ASD. This analysis was done on three families. In this paper the first family will be discussed.

The first family had three children with ASD and two unaffected children. The parents were first cousins. WES was performed and it showed a single linkage peak in a large homozygous interval, and suggested a 900:1 likelihood that the responsible mutation was found in this homozygous interval. Then WES was performed on one of the affected children. The linked interval showed only a single rare change that was absent in the known databases and population matched controls.

This mutation was on a gene that is responsible for the production of the enzyme AMT, aminomethyltransferase. This enzyme is needed for the breakdown of glycine. Sanger Validation confirmed that the mutation was heterozygous in both parents, homozygous in the affected children, and absent or heterozygous in the unaffected children.

Mutations in AMT cause nonketotic hyperglycemia (NKH), which is a neonatal syndrome that causes lethargy, hypotonia, several seizures and death within the first year. Rarer, atypical forms of NKH have been described in association with hypomorphic missense AMT mutations. This manifests at a later age and causes delay in expressive language, behavioral problems and seizures. While individually non diagnostic, when all three children were tested they exhibited a range of neurological symptoms that were strongly suggestive of NKH (Yu et. al., 2013).

### **The amygdala theory of autism**

There is a network of neural regions that comprise the “social brain”: the orbit-frontal cortex, the superior frontal sulcus, and the amygdala. This region of the brain is responsible for social intelligence, a quality which ASD patients are known to be lacking. The Neuroscience and Behavioral Reviews has come up with The Amygdala Theory of Autism, where they hypothesize that patients with ASD have an amygdala deficit which causes the deficit in social skills.

An experiment was done on rhesus monkeys which showed that ibotenic acid lesions of the amygdala affect the social behavior of the monkeys and they also became socially isolated. When the amygdala-lesioned monkeys were sent into the wild, they were unresponsive to group members, failed to display appropriate social signals and they withdrew from other animals. When another study was done, where lesions were made to the monkeys' anterior temporal lobe, which included the amygdala, the monkeys showed specific symptoms, for example they had a tendency to overreact to all objects, they were hyperemotional, they had a loss of fear, and they started to investigate objects with their mouth instead of their hands.

Then a study conducted with humans was done. They used single photon emission computed tomography that showed that

patients with ASD show significant reductions in the temporal lobe blood flow. The study was done in fuller depth where an fMRI (functional MRI) was done on adults with ASD that showed significantly less amygdala activation during a mentalizing task called “Judging the Mind in the Eye” task. In this experiment, six people with autism were matched for mean age, handedness, IQ, socioeconomic status and educational level with twelve people in the normal group.

In the fMRI scanner a blocked periodic ABA design was used. There were two tasks. The first task, Task A, was supposed to induce periodic MR signal changes, with signal maximum in brain regions relatively specialized from gender recognition from facial stimuli. In this task, the subjects were visually presented with pictures of eyes and were asked to indicate by right button pressing if the picture was a male or female.

The second task, Task B, was checking the periodic MR signal change with signal maximum in brain regions relatively specialized in mental state recognition from facial stimuli. In this task, a response involved choosing between two words that described the mental state of the photographed person.

The key difference between the two tasks was seeing the subject's judgement between the two pictures. Social intelligence is about picking up social cues, such as the expression of people's faces to understand what the person is thinking.

The subjects who had ASD had a hard time doing task B. The fMRI data was analyzed in two stages. First, generic brain activation maps were constructed separately for the control and autistic groups. By doing so, they were able to determine which voxels were activated in each group by each of the two tasks. Secondly, ANOVA was used to determine the voxels that demonstrated a significant difference between groups in mean power of response to each task.

The autistic group activated the frontal components less extensively than the control group, and they did not activate the amygdala at all. The control group demonstrated significantly greater power of response in their bilateral superior temporal gyrus. The autistic group seems not to use the amygdala for the task, and compensated by using a greater processing load on temporal lobe structures, which specializes in verbally labeling complex visual stimuli. This may be because of compensation for an amygdala abnormality (Baron-Cohen et. al., 2000).

### **Maternal diabetes and autism**

A study was done to investigate if Gestational Diabetes Mellitus (GDM) in the mother may result in the child having ASD. Specifically, researchers were determining if the timing of when

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the mother developed GDM had any significance whether the child would develop ASD.

This study included children who were born at 28-44 weeks gestation. Women who had type 1 diabetes and congenital anomalies were excluded from the study. The primary variable was maternal type 2 diabetes or maternal GDM during pregnancy. Exposures were broken up into three categories- 1. No exposure to maternal diabetes, 2. Exposure to maternal type 2 diabetes, and 3. Exposure to maternal GDM. Other variables that were included were maternal age at delivery, education, self-reported maternal race/ethnicity, and the gender of the child. For the GDM exposed group, the different methods that were used to diagnose were also recorded. The gestational weeks were also split up into three categories- 1. Diagnosed 26 weeks or earlier, 2. Diagnosed after 26 weeks but before 30 weeks, 3. Diagnosed at 30 weeks or later.

The primary data analysis was collected from 322,323 children. The children were checked up on approximately five and a half years after birth. During this time, 3,388 children were diagnosed with ASD. A large amount of mothers who got GDM during the first 26 weeks of pregnancy gave birth to a child with ASD. It is also interesting to note that among the children who had ASD, 121 of them had older siblings who also had ASD.

To summarize, mothers with pre-existing type 2 diabetes were not significantly associated with risk of ASD, but mothers who got GDM earlier than 26 weeks of pregnancy were significantly associated with risk of ASD (Xiang et. al., 2015).

### De Novo Mutations

De novo mutation is an alteration of a gene that was present for the first time in one family member as a result of a mutation in a germ cell (egg or sperm) of one of the parents or in the fertilized egg itself (Genetics Home Reference, 2015). De novo mutations are important in finding the cause of ASD.

Studies show that the majority of de novo mutations do not cause the disease, they only increase the risk of getting the disease. There may be several genes in which a high risk of de novo mutations can occur from. Through these studies many de novo mutations were found that predicted that these mutations would disrupt the gene function in a child with ASD, however these mutation are not necessarily what causes the disease. Two studies also found that de novo mutations are caused from the paternal side and are age dependent. There is a detectable increase in autism risk with children who were born to older fathers (Perisco et. al., 2013).

### Advancing parental age and autism

There are a number of studies which are trying to determine if the age at which the parents conceived the child increases the risk of the child being born with ASD. Whole-Exome Sequencing links older fathers to de novo mutations and increased risk of having a child with ASD. The linkage of older mothers having a child with ASD follows different pathways.

Prevalence of ASD has increased from 5 cases per 10,000 people in the 1980's to the latest CDC's estimate of 1 case in every 68 people. This may be due to a variety of reasons, including changes in diagnostic practice, heightened awareness, or even an actual increase in the disorder. One factor that might cause ASD is parental age, since there is a tendency in recent years for people to become parents later in life. In Spain and England the proportion of mothers who had children after 35 years old in 1980 was 14% and 25% respectively. In 2007, the proportion of mothers who had children after 35 years old increased to 25% and 40% respectively.

There are more than forty studies being done trying to link age of parents when they had kids to ASD. While individual studies are not consistent with their findings, this can be because of a number of factors. The discrepancies can be caused by inconsistent sample sizes and characteristics, or missing data. The studies did conclude an increased risk of ASD from both older parents, an older father but not older mother, and older mother but not older father, and neither an old mother or old father. Inconsistencies could have also been affected by the fact that these studies did not take into account socioeconomic status of the families or co-parental age.

The general consensus is that advancing paternal age (APA) and advancing maternal age (AMA) are independent factors that can increase the risk of having children with ASD.

In 2012 meta-analysis focused on advancing maternal age and a link to autism, using data from over 25,000 ASD patients, and eight million controls aggregated from 10 studies. The results were that mothers who were 35 years or older when they had the child had a 1.5 fold increased likelihood of having a child with ASD compared to mothers who had children at 25-29 years of age. In 2010, a study was done to test the theory of advancing paternal age and its link to autism. Meta-analysis was done on eleven studies and found that fathers who were 40-49 years old when they had children, had a 1.8 fold increased likelihood of having a child with ASD, compared to fathers who were 29 years old or younger.

### APA effects on ASD

At this point the question is how APA and AMA are associated

with a child being born with ASD. One possibility of why age affects ASD is age-related mutagenesis in the male germ cell. While a female germ cell undergoes 22 mitotic cell divisions in utero, male germ cells undergo 30 mitotic cell divisions in embryogenesis, and then divide every 16 days from puberty and on. Because the male germ cell undergoes many more cell divisions during his reproductive age, there is more of a possibility of gene copying errors, which can lead to de novo mutations in the male's child, which can cause ASD.

Multiple studies that are using Whole-Exome or Genome Sequencing found that higher numbers of de novo loss-of-function single nucleotide variants are seen in fathers of increasing age. While it is fine for a new born to have between 30-100 de novo point mutations, there is an increase of about one to two mutations for each increasing year of a father's age. If the mother is also older, it increases the likelihood of more de novo mutations, because AMA and APA can be linked together.

Two studies were conducted to disentangle APA effects from AMA effects on the child. The studies used multiple regression which was adjusted so that both parental ages were entered as predictors. It was found that APA remained a significant predictor in de novo mutations while AMA effects were negligible. Two thousand, five hundred families who had one child with ASD were analyzed and found that a majority of the de novo mutations originated from the father. It should be pointed out that although de novo mutations can affect any biological system, there is reason to believe that de novo mutations differentially impact brain development because the brain may have fewer redundancy mechanisms that are needed to inhibit mutations (Lee et. al., 2015).

They hypothesized that if mutations in germ cells result in a slightly faster rate of cell division, then cells with these mutations will expand within the testes and logically they will contribute to the larger proportion of sperm. This process is known as "selfish spermatogonial selection". This mechanism can favor the propagation of germ cells which are carrying mutations that prefer to impact certain cellular signal pathways. (Lee et. al., 2015).

### **AMA effects on ASD**

One possible correlation between AMA and ASD would be assisted reproductive technologies which are used by couples more commonly with increasing age. A Swedish study was done which involved 2.5 million infants. The results were that in vitro fertilization procedures had no association with risk of ASD, while a specific procedure called "Intracytoplasmic Sperm Injection (ICSI)" has a slightly increased risk of having

children with ASD. ICSI is done when there is paternal infertility. The reason why ICSI can increase risk of ASD is because the injection process can damage the egg which can increase the risk of adverse outcomes.

Another possible correlation can be the fact that a person accumulates more and more environmental toxins with increasing age. Environmental factors such as pesticides, heavy metal, and organic pollutants have been linked with increased risk of ASD. Human brain samples were examined and found that exposure to polychlorinated biphenyl 95 strongly predicted maternal 15q11-q13 duplication, which is one of the most common CNV findings in ASD cases (Lee et. al., 2015).

### **Other factors that effect advancing parental age and autism**

A study was done using 1,251 ASD cases occurring in over 250,000 births throughout the United States. This study found a connection between parental age and birth order with increasing risk of ASD. Younger parents (mother younger than 35, father younger than 40), on their first birth, had a 1.7 fold increased odds of having a child with ASD compared to other young parents on their third birth and later. Regardless of the age of the parents, the risk of ASD is increased in first born children. Older parents (mother older than 35, father older than 40) on their third child or later had a 1.8 fold increase on the likelihood of having a child with ASD. Older parents on their first child had a 3.1 fold increase on the odds of having a child with ASD. To conclude, the effect of parental age on ASD is amplified if it is their first birth (Lee et. al., 2015).

### **Environmental chemical exposures and autism spectrum disorder**

Autism can be caused by a mixture of genetic and environmental influences, and studies are being done to understand the interplay of both contributions. These contributions can be different from one person to the next. A study in Sweden was done which included 14,000 children with autism. The study showed that heritability contributed to 50% of a possible cause of autism while environmental factors contributed an equally strong role. Genetic and environmental factors may work together to disrupt the normal process of nervous system development, interfering with neuron formation and migration, synapse formation, or neurological connectivity which can cause ASD.

In this study, the chemical exposures that were included are ones that can be reduced in exposure, which opens up possibility for prevention of autism. Of course it can only be reduced because not all exposure can be controlled, such as air pollution and any toxic chemical that are unknown to the patients.

### **Maternal smoking in pregnancy and ASD diagnosis**

Seven studies were included which were done in the United States, Canada, Sweden, Norway, Finland, and England/Wales. These studies showed no association between maternal smoking during pregnancy and ASD diagnosis. Some of the studies focused on the spectrum of autism. There was an elevation in two of the studies between maternal smoking during pregnancy and higher functioning autism, such as Asperger's syndrome, compared to lower functioning autism. Overall, there is no conclusive evidence that maternal smoking or tobacco exposure to a fetus has any effect on a child being born with autism (Kalkbrenner et al., 2014).

### **Regulated and traffic related air pollutants exposure and ASD**

Air pollutants include hundreds of chemicals which can be categorized into three categories: 1. Point pollutants arising from spatially separated large buildings, for example factories and power plants. 2. Area pollutants associated with population density, for example gas stations and dry cleaners. 3. Mobile pollutants associated with vehicles. In this paper we will be discussing the third category. When these pollutants enter the atmosphere they undergo reactions that create new pollutants. Specific chemicals that arise from traffic that will be discussed here are particulate matter (PM), NO<sub>2</sub>; nitrogen dioxide, and O<sub>3</sub>; ozone.

Air pollutants enter the body through inhalation and direct transportation from the nose to the brain via the olfactory bulb. Some chemical pollutants are known to induce oxidative stress and cause a systemic inflammatory reaction, which can alter normal neurodevelopment. Fetuses itself can come in contact with chemicals from air pollutants or they can be exposed to them from the elevated levels of inflammatory cytokines in the maternal circulation.

Seven studies were done in the United States with adequate sample sizes ranging from 284 to 7,594 children with autism. The studies also had controls under consideration such as maternal age and the parent's level of education. Season of birth is also important to take under consideration because occurrence of autism varies by season of conception for unknown reasons. It is also important because certain air pollutants have a stronger concentration in some seasons over other season, such as influenza infection and vitamin D levels.

Results for this study concluded that exposure to pollutants such as PM<sub>2.5</sub>, PM<sub>10</sub>, and NO<sub>2</sub>, were associated with the risk of getting autism. For almost every pollutant in every study, associations were stronger for exposures in the third trimester

of pregnancy and the first year of life compared to earlier on in the pregnancy. All of these studies estimated exposure to outside levels of these air pollutants using historical data that could be related geographically to the home residence of the pregnant woman or infant, because retroactive direct person-based air sampling was not possible (Kalkbrenner et al 2014).

### **Metal (Ethylmercury) from vaccinations and ASD**

Connecting this discussion back to vaccinations, thimerosal was thought to be a toxic compound that increased the risk of autism. Thimerosal is a vaccine preservative that contains ethylmercury which is considered a less toxic form of mercury compared to methylmercury.

Although the Institute of Medicine already stated that there is no concrete evidence that the MMR vaccine causes autism, people are still skeptical. No one suspected thimerosal to be toxic, seeing as it was found in such small doses in vaccines. In the 1970's it was known that large doses of mercury were harmful. It was only in the late 1990's that scientists suggested that even relatively low exposure to organic mercury could be dangerous to fetuses and young infants (Baker, 2008). The U.S. Food and Drug Administration suggested removing thimerosal from vaccines in 2001, because of the biological plausibility that thimerosal may pose as a neurotoxic harm. However, trace amounts of thimerosal are present in influenza vaccines which infants and pregnant women may be exposed to. Six studies were included, testing for a link between thimerosal-containing vaccines and autism. The results of the studies suggested that there is no connection between thimerosal and increased risk of autism (Kalkbrenner et al., 2014).

### **Conclusion**

The causes of autism are still vague. Because it is characterized as a spectrum disorder there can be many different variables that can play a role in causing different levels of it. The theory that vaccinations cause autism has become a popular idea, therefore much research has been done to make a correct conclusion. Until the causes of autism are confirmed research is still being done to see what role vaccinations have on autism, however the research that has been done until now has concluded that vaccinations do not have a link to ASD. Another highly researched cause of autism is a genetic cause, be it a genetic mutation or a number of other different genetic variables. In this paper the idea that different genetic mutations that can cause autism has been discussed, and while some studies have brought optimistic results, further studies have to be conducted. Other genetic factors that have been discussed are the effect of maternal diabetes on the fetus, and parental age

when the child was conceived. While further research is needed to confirm these factors, the studies that have been done have given additional insight into what causes autism. Another possible cause for ASD that was discussed was a mixture of genetic and environmental factors that the child was exposed to either as a fetus or as an infant. While some environmental factors that have been tested had no correlation to ASD, other environmental factors did show a correlation, although more research is required.

Many studies are constantly being done, and researchers are discovering more factors that might be a cause for ASD. The closer they come to finding a cause, the closer they get to trying to find a way to prevent autism from becoming more prevalent, and maybe even closer to finding a possible cure for Autism Spectrum Disorder.

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# The Neurological Underpinnings of Hypnosis and its Clinical Applications

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## Abstract

The brain is so complex that it is almost impossible to select one variable as the reason for a specific observation. This paper will discuss the neurological basis of hypnosis, and how hypnosis has made unique contributions to the refinement and development of cognitive neuroscience. In addition, hypnosis has been proven to cure many psychological and neurologically based diseases. Due to in-depth study of the neurological underpinnings of hypnosis, much advancement has been made in elucidating the relationship between the complex neural circuitry of the brain, its direct correlation to consciousness, and both the efferent and afferent neurological systems. New neuroimaging techniques, such as fMRI and other brain scanning methods such as Electroencephalography (EEG) and positron emission tomography (PET), have made it possible to localize task related regionally specific brain activity and cognitive mental state, which allows researchers to scientifically examine and construe the many obscure theories surrounding the phenomenon of hypnosis.

## Introduction

Modern views on the experience of hypnosis are largely dominated by the belief that the “hypnotist” possesses the ability to generate a “sleep-like state” within the individual being hypnotized. It is then presumed that the hypnotist possesses a supernatural control over the person’s mind, causing him to behave in an irrational manner. In actuality, hypnosis is a highly complicated component of neuroscience related to the intrinsic workings of the human brain. Neurobiologically, the induction of a hypnotic trance can be viewed as an alternate state of consciousness due to the modulation of brain activity critically related to areas in the brain that oversee the regulation of the conscious state of being (Kihlstrom, 2013). Hypnosis is also characterized as an increase in mental relaxation and mental absorption mainly related to changes in the anterior cingulate cortex (ACC), various areas of the prefrontal cortex and frontal lobes, cortical and sub-cortical areas, the ponto-mesencephalic brainstem, and changes of regional cerebral blood flow (rCBF) in these areas (Rainville et al., 2002). Contemporary scientific theories of hypnosis emphasize changes in the engagement or disengagement of specific neurocognitive processes, and their effect on performance and psychophysiological activity such as executive control and attention. Additionally, there are individual psychological characteristics, partially relating to genetic brain structure, predicting hypnotic susceptibility. Moreover, scientific experiments have proven that hypnosis can be effective as an analgesic. Hypnosis can reduce acute pain associated with invasive medical procedures, burn care pain, labor pain, as well as reduce chemotherapy side effects. Hypnosis can also decrease chronic pain such as headaches, backaches, and fibromyalgia. The study of hypnosis and its clinical applications is an ever-evolving field that can greatly advance the understanding of the conscious versus the subconscious mind and the complex structure of the human brain.

## History of Hypnosis

In the 1770’s Anton Mesmer wrote his doctoral thesis titled: ‘De influxu planetarum in corpus humanum’ (On the Influence of the Planets on the Human Body), in which he revisited the ancient belief that the solar system emits invisible rays that affect our

bodies. Mesmer called this idea the “animal magnetism” effect. He practiced his healing through animal magnetism, capturing the “magnetic fluid” through pieces of iron and conductive metals that he fixed upon the diseased areas on the patients’ bodies. He concluded that one could attain “magnetic” effects through the laying of hands, or even simply by speaking to the patient. His teaching became known as mesmerism. This theory was accepted until the mid-1800’s. James Braid disproved this idea of mesmerism in 1840 (Gauld, 1992). Braid demonstrated, through various experiments, that hypnosis was nothing more than a fixation of attention rather than an occult shadow of mesmerism. Braid concluded that there is a biological and physical basis to what was previously known as “mesmerism”, and coined the term “hypnosis”, which comes from the Greek word “Hypnos”, which means sleep (due to the trance-like state of the subject). Subsequently, many famous psychologists such as Milton Erickson, who introduced the Neuro-Linguistic Programming via hypnosis, used hypnosis to cure clients of psychological ailments (Gauld, 1992). The trance-like state of hypnosis is now known to be a reflection of biological circuitry and a form of focused attention as proposed by Braid. The future of hypnosis will be to uncover fully all the underlying neurological components of hypnosis and discover its many clinical applications.

## Materials and Methods

In researching the neurological underpinnings of hypnosis and its clinical advantages, many articles and journals were compiled to properly explore and present this topic. References were obtained through PubMed, and Touro College’s Database, in addition to Google scholar and EBSCO multisearch. Key words, such as; hypnosis, hypnotic susceptibility, clinical benefits of hypnosis, and hypnotic analgesia were used to find pertaining articles that are cited throughout this paper.

## Contemporary rendition of hypnosis

Succeeding the Braidian definition of hypnosis, researchers argued regarding the exact definition of hypnosis and its causes. Hypnosis refers to a change in mental activity following an induction, which usually results in increased attention,

dissociation, and an increased absorption in pertaining stimuli (Spiegel, 2007). Typical hypnosis includes alterations in sensory experiences, motor control, and even amnesia. During a hypnotic induction, specific neural synaptic circuits are activated to express one's character, and personality in relation with his/her character traits specifically portrayed during hypnosis. Herbert Spiegel (2007), an American psychiatrist who popularized hypnosis as a treatment for pain and other disorders, identified three characteristics of hypnotized individuals:

1. Dissociation is the conscious versus unconscious separation of memory, perception, and motor response from one's main awareness. The capacity to dissociate is biologically determined and is reflected in the Eye Roll (ER) movements controlled by the external ocular muscles (as explained below).
2. Absorption is the decrease in peripheral awareness to facilitate greater focal attention. The intensity and duration of this absorption is influenced by bio-psychological components of intelligence and motivation. Absorption is diminished by attention deficit disorders, impaired concentration, and some medications.
3. Suggestibility is characterized by how prone an individual is to accept new information as fact with a relative suspension of critical judgment.

Rainville et al. (2002) described hypnosis as a state of mental relaxation and mental absorption, which are both associated with the instructions used to induce a hypnotic state. Hypnotic relaxation results from the direct instruction to relax prior to a hypnotic induction, which leads to positive bodily feeling, drowsiness and mental ease. Mental absorption, otherwise known as fixed attention, as "total attention that fully engages one's representational resources and results in imperviousness to distracting events" (Rainville et al., 2002).

Individuals who were hypnotized reported having been in an altered state of consciousness, describing this state as an increase in mental relaxation, automatic response, slight disorientation of time, increased imagery, focused attention, dissociation of irrelevant stimuli, and a disorientation toward their sense of self (Oakley and Halligan, 2009).

### **Hypnosis susceptibility**

Hypnotic susceptibility is unique to each individual. Some people are easily hypnotizable while others are virtually unaffected by hypnotic induction. Hypnotic suggestibility scales are the primary way to measure hypnotic susceptibility. Two such scales include the Stanford Hypnotic Susceptibility Scale (SHSS) and the Harvard Group Scale of Hypnotic Susceptibility (HGSHS).

These tests are constructed for standardized group administration and are scored by self-report. They consist of a recorded verbatim hypnotic induction, which is scored according to how similar the subjects responses are in relation to previously measured highly susceptible individuals. There are many other ways to measure hypnotic susceptibility, but these two scales are most commonly used in scientific experiments.

Hypnosis is thought to be a state of fixed attention and absorption. It can therefore be postulated that the individuals who have the highest score in hypnotic susceptibility are more able to focus intently on one specific stimulus, disregarding other competing "noise" (Galbraith et al., 1970). There is much controversy as to whether or not hypnotic susceptibility depends on the individual's ability to selectively attend to the hypnotist's instructions, or whether it has to do with the ability to shut off distracting stimuli, creating a mental state where the subject is more able to capture the hypnotist's commands. A study was done to measure hypnotic susceptibility via an electroencephalogram (EEG). Subjects were asked to focus intently on a dim light. The EEG showed that those who scored highest on hypnotic susceptibility were more able to fix their attention on the dim light, which directly led to their ability to ignore all other stimuli (Galbraith et al., 1970). This discovery discounts the findings that hypnosis is an inhibitory response and lends credence to the fact that hypnosis is a result of fixed attention.

To further research this phenomenon, a case study was done to determine the differences in cortical activity in "high" and "low" individuals (in regard to hypnotic susceptibility). The EEG showed greater theta activity (4-8 Hz) in highly susceptible individuals in the anterior frontal cortex, as well as in the occipital cortices. Theta waves in the frontal lobes and occipital cortices are associated with vivid visualizations, and great imagination. This shows a pattern of EEG dimensionality more consistent with imagery processes, which are controlled by various parts of the frontal, occipital and parietal regions of the brain. Low susceptible individuals exhibited a pattern more consistent with cognitive activity such as mental math (Blai et al., 1998). This study was done in conjunction with another study involving neuropsychological tests. These tests were administered to both "high" and "low" individuals. The tests were selected to examine potential differences in tasks using the prefrontal cortex, as well as verbal and visual-spatial modalities. The WCST (Wisconsin Card Sort Test) tests the ability to detect relevant information by dissociating the irrelevant. Overall, a faster performance was observed in the highly susceptible individuals, which indicated that highly suggestive participants are more flexible in their ability to shift cognitive sets, which is consistent with the results of the EEG (shifting cognitive sets more easily insinuates a greater imaginative ability) (Blai et al., 1998).

In 1992, Herbert Spiegel presented three different personality styles based on the way an individual related to the self and to the world. Those who score high on hypnotic ability tend to be more trusting, have a higher degree of malleability, and an extreme propensity to dissociate. This leads to total absorption with a complete abandonment of peripheral awareness. Those who are not susceptible tend to place logic at highest priority and have a limited experience of dissociation, having constant peripheral awareness. Those in midrange exhibit trends toward oscillating between relative periods of action and inaction. They tend to fluctuate between feeling and thinking and have a moderate ability to express dissociation.

In a study conducted by Herbert Spiegel in 2006, the measure of what was referred to as the “eye roll” determined hypnotic susceptibility. This proved that there was a discernable biological component in the ability to experience a hypnotic state. The eye roll is the distance between the lower eyelid and the bottom of the cornea. Spiegel hypothesized that hypnotic susceptibility was based on the amount of sclera seen in the eye while hypnotized. Consequently, experiments showed that his hypothesis was correct. When he asked his patients to look up during the induction phase of hypnosis, he found that if the eye roll was so high that nothing but the sclera was showing, that individual has a higher neurological capacity for dissociation and focused attention, thereby having the potential to be highly hypnotizable. This is attributed to the basic biological circuitry of the brain unique to each individual. This complex circuitry involves spinal cord pathways, the trigeminal nerve that includes the ocular motor muscles (which explains the ER phenomena), as well as the vagus complex and many other nuclei and neural circuits. Conversely, if little sclera is seen between the lower lid and the cornea, that individual has a lower biological dissociative ability and is therefore only capable of low hypnotic capacity. This study was further proven in conjunction with the Hypnotic induction profile, which provides an assessment for mental concentration, the ability to internalize new ideas, disassociation, and the capacity to experience sensory alteration. This proves that the ER can be regarded as a surface indicator of underlying synaptic circuitry.

To enable those who have low hypnotic susceptibility to benefit from hypnotherapy, studies have been done to determine whether hypnotic susceptibility can be increased. A study done by Kinny and Sachs (1974) demonstrated that hypnotic susceptibility could indeed be increased in some individuals. Additionally, this experiment determined whether the permutation in hypnotic susceptibility is attributed to actual cognitive and perceptual changes or to a response alteration due to expected behaviors. The experiment included training that was found to improve hypnotic susceptibility in past experiments.

Participants were taught how to imagine certain sensations so acutely until the perception of the sensory explanation was perceived as being genuine. The goal of this learning process was to teach participants how to feel the sensation that was imagined, in addition to blocking out other competing variables. Therefore, it can be inferred that the more imaginative a person is, the more susceptible they are to hypnosis. The SHSS was given after each training session to measure the progress of the participants. The result of this experiment proved to be exceedingly intriguing. Overall there was an increase in hypnotic susceptibility among most of the subjects. Researchers postulate that the reason for this change can be attributed to three variables: learning, attitude and motivation. This can essentially be positively correlated to the learning process of all other skills. Subjects practiced attending to specific sensations and blocking out others. Moreover, subjects were allowed to advance at their own pace to ensure optimal results. There were also changes in the attitude previously attributed to hypnosis. Many subjects were originally skeptical regarding the legitimacy of hypnosis. Once they accepted the fact, for example, that their hand could be lowered involuntarily, they were more willing to capitulate to the hypnotic induction. Subjects then reported that they were better able to concentrate, and believed that they had greater autonomy over their actions during hypnosis. The subjects who originally portrayed controlling, rigid and/or fearful personalities failed to show large improvements in their ability to be hypnotized. They were afraid of losing control and were concerned that their mind would betray them during the hypnotic stage (Kinny and Sachs, 1974). Furthermore, hypnotic susceptibility has been shown to be a stable trait due to studies that tested the hypnotic susceptibility of the same individuals at different ages. Therefore, it can be deduced that hypnotic susceptibility can be attributed to personality traits that are inherent in each individual, which also control their ability to imagine, focus attention, and absorb internal stimuli.

### **Neurological underpinnings of hypnosis**

There is much controversy regarding the neurological basis of hypnosis. This is attributed to the fact that much remains unknown regarding the various structures and networks present in the brain. Although many studies show conflicting results, there are some conclusions that can be deduced from the many studies that examined this topic. The following comprehensive study was done by Rainville et al. in 1999 and was later repeated in 2002, attaining similar results. Therefore, it can be assumed that the information presented in these studies can be considered rather factual, as opposed to purely theoretical. The effects of hypnosis on regional cerebral blood flow (rCBF) were measured using positron emission tomography (PET), which gauges the rCBF in the brain. “Pure” hypnosis (hypnosis without suggestion) was accompanied by a considerable increase in rCBF in the following

regions: the occipital region, the right anterior cingulate cortex, and bilaterally in the inferior frontal gyri. Decreases in rCBF were found in the right inferior parietal lobe, the posterior cingulate gyrus, and the left precuneus. Hypnosis with suggestion showed additional increases in rCBF in the frontal cortices, chiefly in the left side of the brain (Rainville et al., 1999). This is attributed to the fact that the proposal for an altered perception reflects the verbal arbitration of suggestion and top-down processing involved in the reinterpretation of the perceptual experience (Kihlstrom, 2013). An increase in delta rhythms shown in the EEG performed along with the PET, supports the theory that hypnosis reflects an altered state of consciousness which is associated with decreased arousal. Moreover, findings show a great increase in occipital rCBF, which supports the theory that hypnosis facilitates visual imagery.

### rCBF differences in “pure” hypnosis

Hypnotic induced relaxation showed a wide-spread increase in rCBF bilaterally in the occipital lobes (Figure 1). Interestingly, comparable effects have been reported during visual imagery. In this study, subjects were not encouraged to engage in imaginative thinking, but spontaneous visual imagery was reported in many subjects. This phenomenon could be attributed to the establishment of deep relaxation, which has been proven to facilitate visual imagery processes (Brann et al., 2012). Other areas associated with an increase in rCBF included: the inferior frontal gyri, which are associated with prepotent responses, and the right anterior cingulate cortex (ACC), as seen in Figure 1. The ACC is an area in the brain that is connected to functions related to conscious experiences and the emotional interpretation of pain. Greater rCBF in the ACC was present in more emotionally aware females (Lane et al., 1998). Many studies have found that the ACC is involved in functions such as anticipation tasks, attention, and motivation (Bush et al., 1999). Moreover, focus of attention is associated with the anterior cingulate gyrus, which is consistent with the definition of hypnosis as being a state of focal attention and increased concentration.

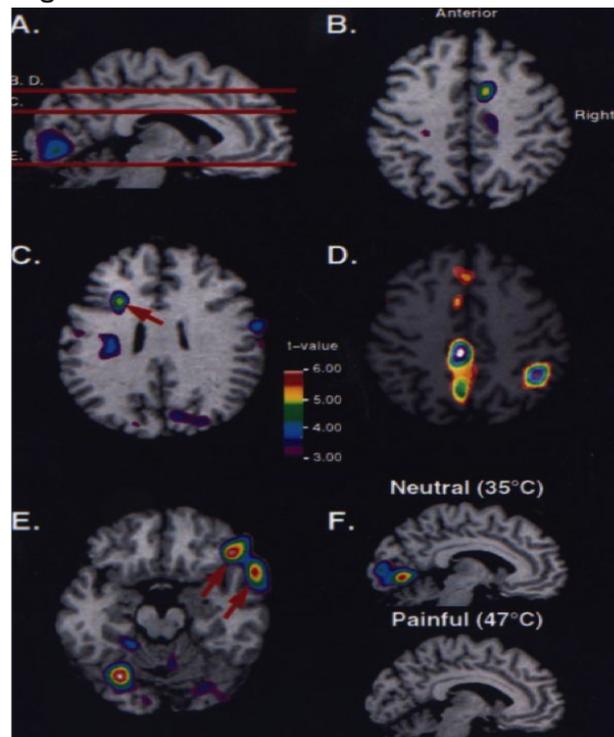
Decreases in rCBF were associated with the inferior parietal lobule, which involves language and mathematical operations. It is remarkable to note that individuals with low hypnotic susceptibility tend to exhibit a greater preference to cognitive activities such as mental math (as aforementioned). Specific parts of the posterior parietal cortex also showed a significant decrease in rCBF (Figure 1). The posterior parietal cortex attends to processes involving spatial attention, orientation to external stimuli, and self-representation. A decrease in rCBF in this area reflects the decreased orientation to extrapersonal and somatic stimuli observed in individuals under hypnotic influence. Additional decreases in rCBF were found in the medial precuneus, which is involved in self-processing operations, and is part

of the network of the neural correlates of self-consciousness and self-related mental representation (Cavanna and Trimble, 2006). A reduction in rCBF was observed in the left posterior cingulate gyrus, which has been proven to become deactivated during effortless mind wandering, while controlled awareness corresponded to activation in the posterior cingulate (Garrison et al., 2013), the left medial superior frontal gyrus, which is involved in self-awareness in conjunction with sensory system, and left posterior middle temporal gyrus, whose function remains unknown (Rainville et al., 1999).

### rCBF differences in suggestion related hypnosis

Increases in rCBF were seen predominantly within the medial superior and left dorsolateral regions of the frontal lobes, in

**Figure 1**

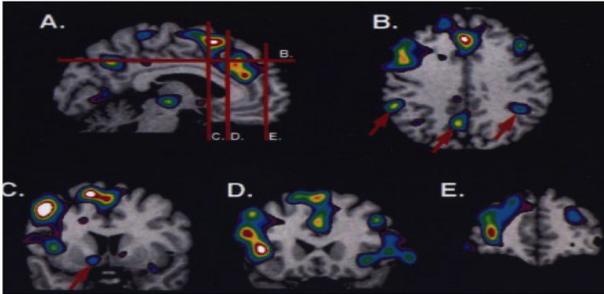


Statistical (*t*) maps of hypnosis-related increases in rCBF, across stimulation conditions, in occipital (A), right anterior cingulate (B), left frontal (arrow in C), right frontal and right temporal cortices (arrows in E). Decreases in blood flow were found in right lateral and medial posterior parietal cortices (D). When analyzed separately in each stimulation condition, the hypnosis-related increase in occipital rCBF was observed mainly in the neutral stimulation condition, as shown in F. (Rainville et al., 1999).

addition to the right dorsolateral frontal lobule (Figure 2). This can reflect the verbal mediation of hypnotic suggestions, working memory processing, and top-down mechanisms involved in the reinterpretation of the sensory experience sometimes used

in the induction of hypnosis. Similar effects have been found in subjects who were asked to listen and list words that include verbal lexical-semantic processing (Oakley and Halligan, 2009).

**Figure 2**



Statistical ( $t$ ) maps of suggestion-related changes in rCBF show increases in medial (A) and dorsolateral frontal cortices (C, D, and E), and in medial and lateral posterior parietal cortices (arrows in B). Arrow in D shows significant subcortical increase in left nucleus accumbens. (Rainville et al., 1999).

Therefore, it was postulated that the left anterior lobes are largely involved in the internally generated reinterpretation of stimuli, which can lead to an alteration in perception known to affect hypnotized individuals (Rainville et al., 2002). Significant increases were also seen in the left medial parietal and bilateral posterior parietal cortices (Figure 2). This can be attributed to the specific content of the suggestion, which may cause specific somatic interpretation of perception. Decreases were found in the right uncus, bilateral in posterior orbitofrontal regions, and the left lateral cerebellum (Rainville et al., 1999).

### Hypnotically induced changes in neural oscillations as measured by EEG

When searching for neurological changes in the brain due to hypnosis, Electroencephalography (EEG) can directly measure the electrical activation of the various parts of the brain. An EEG records the frequency (measured in Hz) and amplitude (measured in microvolts) of waves produced by electrical brain activity. Four simple periodic rhythms are recorded in the EEG: alpha, beta, delta, and theta. These rhythms are associated with the frequency of the waves. Alpha waves are typically 8-13 Hz. Alpha rhythms are usually prominent in adults who are awake, but in a very relaxed state (e.g. eyes closed). These alpha waves diminish when subjects tune into external stimuli and are usually observed to be of the greatest amplitude in the parietal and occipital regions of the cerebral cortex. In contrast, Beta rhythms (13-30 Hz) occur in individuals who are attentive to external stimuli, or who exhibit specific mental stimulation. In essence, Beta waves represent the arousal of the cerebral cortex to higher degrees of alertness and attention. Delta waves (1-5 Hz) are generated in deep meditation, and suspend external awareness. It has been proven that healing and regeneration occur in this state as well. Theta waves (4-8 Hz) are low-frequency

rhythms that are dominant in deep meditative sleep. Senses in this state ignore external stimuli and focus on the subconscious. Vivid imagination beyond normal conscious awareness is present in this stage as well (Lee et al., 2007). Not surprisingly, these rhythms directly correspond to the EEG brain waves associated with different stages in hypnosis.

A case study was done to determine whether an EEG during “pure” hypnosis (hypnosis without suggestion) would differ from a normal non-hypnotic EEG. Pure hypnosis can be categorized as a state of heightened attention and increased alertness, reflected in neuronal activation (Rainville et al., 1999). These neural changes account for the susceptibility to suggestion after a hypnotic induction. This study concluded that hypnosis affected all of the EEG electrodes. Occipital and frontal EEG channels were most affected by hypnosis. There was up to an 89% increase in Spectral Pattern (SP) from baseline to hypnotic state in the frontal lobes. Right parietal and mid-frontal EEG channels increased 11%. Comparative analysis demonstrated that hypnotic conditions caused a large increase in delta, beta, and theta rhythmic segments in various areas of the brain when compared to the non-hypnotic state EEG. Although all EEG channels were affected by hypnosis, the prefrontal cortex (Fp1 and Fp2 electrodes) and the right occipital electrode (O2) showed the greatest percentage increase (Fingelkurtz et al., 2007). This data is consistent with the knowledge that many neural changes in these areas occur during hypnosis. Hypnosis increased beta activity and decreased delta activity in the frontal lobes. This is an interesting phenomenon, for delta rhythms should increase during hypnosis while beta rhythms should technically decrease when measured in accordance to the mental states that they represent. However, it can be inferred that the EEG measured hypnotic state after induction, which can account for the increase in beta waves due to heightened attention, while an increase in delta waves may have been observed during the induction, but decreased thereafter. A majority of beta rhythmic conditions appeared in the EEG only after the induction of hypnosis. This unique composition in brain oscillation in the prefrontal cortex during hypnosis reinforces the premise that this area is of major import in the hypnotic state (Fingelkurts et al., 2007). In addition, these findings disprove previous views that hypnosis constitutes a sleep-like state. This study further demonstrates that in actuality, hypnosis is a state of increased alertness and heightened attention to internal stimuli, as proven by the increase in beta waves. Moreover, this confirms the theory that the frontal lobes are extensively involved in attention networks.

To further elucidate the notion that suggestion for specific perception under hypnotic induction facilitates the same response in cortical activity as reality, a study was done by Kosslyn et al. (2000) in which color perception in the brain was recorded. A grey-scale image was shown to hypnotically induce participants

with a suggestion to perceive a colored image. There was modulation of activity in the fusiform gyrus that is responsible for color processing in the brain, thereby proving that when hypnotically induced, the brain interprets perception as authenticity.

### Hypnotic Analgesia

The mechanism through which hypnosis reduces pain is still quite obscure; however, there is a plethora of scientific evidence proving the effectiveness of pain amelioration via hypnosis. Pain is a spinal nociceptive reflex. Once the nociceptive signal reaches the brain, a sensory and affective discriminative neural network acts to facilitate the conscious perception of pain (Perl, 2011). Structures in the brain that comprise these networks include the primary and secondary somatosensory cortices (S1 and S2), thalamus, insula, and the ACC (Rainville, 1998). Hypnotic analgesia is thought to be attention based in that incoming stimuli are inhibited while awareness is simultaneously deployed elsewhere (Eimer, 2000). The inhibition of afferent nociceptors can be attributed to the decrease in thalamic activity when hypnotically induced (Faymonville et al., 2003). Miron, Ducanan, and Bushnell (1989) conducted a study in which subjects were instructed to attend to a painful stimulus or divide their attention between the painful stimulus and a visual stimulus. Pain reduction was reported in subjects who were asked to divide their attention between two simultaneous stimuli. These results support the hypothesis that when faced with competing processes, attention is directed to other processes, thereby inhibiting the conscious perception of pain. This process depends on a supervisory attention control system that operates to relocate thalamocortical activities. Incoming painful stimuli are suppressed at cortical levels and do not enter conscious awareness, thereby reducing the degree of perceived pain by invoking physiological inhibitory processes of the brain (Faymonville et al., 2003).

Although most studies attribute the reduction in pain to cortical activity, a study was conducted to monitor nociception at the spinal cord level and how it is affected by hypnosis. A study done in 1998 demonstrated that a suggestion for analgesia directly correlates with the spinal nociceptive (R-III) reflex. Subjects showed strong inhibition of the R-III reflex at the spinal cord level in response to hypnotic induction (Danziger et al., 1998). These results are rather intriguing because they introduce a new aspect of hypnosis, independent of the cognitive model. There is no recorded scientific basis for these findings, but they do insinuate that there may be mechanisms in the peripheral nervous system that are directly influenced by hypnosis.

There has been much research as to whether hypnotic analgesia affects the sensory or affective processing of pain. Researchers speculated that hypnosis has a greater affect on the affective system because that system has a greater cognitive evaluation,

while the sensory system is modulated by nociceptive inputs from the peripheral nervous system. Studies have shown that both are affected by hypnosis. Hypnotic analgesia produces both a modulation of pain effect by producing changes in the anterior cingulate cortex, and inhibition of afferent nociceptive signals arriving at the somatosensory cortex. Hypnotic suggestion for altering pain unpleasantness affected rCBF flow to the ACC, but not to the S1 Cortex, proving the role of the ACC in pain affect. Suggestion for modulating pain intensity affected rCBF mainly in the S1 and S2 cortices and had little affect on the ACC. These results are consistent with the role of the somatosensory cortex in the sensory dimensions of pain. Interestingly, the context of the suggestion that facilitated the analgesia determined to what degree the affective and sensory systems were affected (Rainville, 1998).

Researchers at the University of Iowa conducted a case study in 2004 to determine the difference in pain perception in hypnotically induced individuals. fMRI (functional magnetic resonance imaging) was used to measure brain activity. A painful thermal stimulus was applied to the participants' left hand. The subjects were then hypnotized, and their brain activity was recorded by the fMRI. The hypnotic state was then broken and the procedure was repeated. Hypnosis was successful in reducing perceived pain in all of the individuals. Participants reported a significant pain reduction or feeling no pain. The fMRI reported decreased activity in the primary sensory cortex, which is involved in pain perception. Increased activation was seen in the basal ganglia and the anterior cingulate cortex. The increase in brain activity in these two regions could be attributed to their involvement in the inhibition pathway that blocks pain signals from reaching higher cortical areas responsible for pain perception (Schulz-Stübner et al., 2004).

The induction of hypnotic analgesia, simply known as the reduction of pain via hypnosis, can offer amelioration of pain intensity and offer an alternative to drugs that have various negative side effects. By utilizing direct suggestion such as suggesting numbness (glove anesthesia), direct suggestion for turning down pain, physical dissociation from painful areas of the body, pain relief imagery, or cognitive reframing while in an hypnotic state, pain reduction is possible (Eimer, 2000).

A study was conducted to ascertain whether pain modulation requires a hypnotic suggestion for pain reduction, or if pure hypnosis affects the ratings for pain unpleasantness. Participants were expected to submerge their left hand in painfully hot water (47°C) during pure hypnosis and then again in response to a suggestion for pain reduction while hypnotized. The relationship between pain effect and cerebral activity was recorded via PET. An increase in rCBF was seen in the insular cortex,

where a person imagines pain while looking at painful images and feels sensation of pain and its intensity. Increases were also found in the ACC, which mediates affective response to noxious stimuli, and the primary and secondary somatosensory cortical areas (S1 and S2), which are believed to involve the sensory discriminating processing of pain. Comparing hypnosis related changes in rCBF in neutral and painful stimuli conditions tested the effect of hypnotism on pain reduction. A strong lateralization increase in rCBF in the right ACC has been shown in conjunction with the experimental painful stimuli. Furthermore, although pain and hypnosis related ACC sites were anatomically close within broadmann area 24 (which is part of the cingulate gyrus), the pain related peak was medial along the cingulate gyrus and the hypnosis related peak was more lateralized in the cingulate sulcus. Occipital rCBF was less when a pain modulation suggestion was proposed in relation to occipital rCBF without suggestion. Additionally the amount of pain reduction and pain unpleasantness was directly correlated with the participant's level of hypnotic susceptibility (Rainville et al., 1999).

### Hypnosis in treating acute pain

Acute pain is defined as pain that gradually resolves as the injured tissue heals. Many studies have been done to uncover the effects of hypnosis on the reduction of acute pain. A study was performed in 1991 to determine the effects of hypnosis in treating invasive medical procedure pain. This study compared participants who received pre-surgery hypnosis prior to angioplasty surgery with participants who received standard care. The hypnotically induced patients showed a 25% increase in the amount of time they allowed the cardiologist to keep the balloon catheter inflated, and showed a substantial reduction in opioid analgesics that are vital during the procedure. The hypnotically induced group also showed a significant decrease in catecholamine blood levels relative to the control group (Weinstein and Au, 1991). Another study done in 1996 produced similar results. Sixteen patients received hypnosis with a suggestion for relaxation and pain relief imagery while fourteen patients were treated with the standard procedure. Hypnotized patients reported less pain, used less pain medication, and showed more physiological stability during the diagnostic arteriogram procedure. No statistically significant differences in heart rate or blood pressure were recorded (Lang et al., 2000).

Bone marrow transplant patients often receive supralesional doses of chemotherapy prior to the procedure. This treatment often results in severe nausea, pain from oral mucositis, and vomiting. Patients were hypnotically induced and were given suggestion for pain control and relaxation. Most patients reported a significant reduction in pain; however, no significant differences emerged regarding nausea and vomiting (Syrjala et al., 1992). Burn care patients who were treated with hypnosis

reported a reduction in burn-related pain and even facilitated wound healing (Patterson et al., 2003). Additionally, burn patients who received hypnosis used significantly less analgesic drugs than the control group (Wakeman and Kaplan, 1978). Labor pain is also a good candidate for hypnosis. There have been many clinical benefits recorded in using hypnosis to reduce labor pains. Women who were given sessions of posthypnotic suggestions for pain relief and relaxation during labor showed shorter stage I labor and reported less labor pain (Davidson, 1962). Freeman et al. (1986) conducted another study in which women received hypnosis before labor. Hypnosis involved suggestion for pain relief and for transferring anesthesia from the hand to the abdomen. No differences were reported in pain relief during labor; however, highly susceptible individuals reported that hypnosis helped reduce their anxiety during labor, thereby helping them cope effectively with the pain.

### Hypnosis in treating chronic pain

Chronic pain is defined as pain that persists beyond the healing time needed for a specific injury. Many psychological factors, such as patient coping responses, patient cognition, and environmental factors play an important role in the expression and experience of chronic pain, while acute pain is directly related to the injury itself. Therefore, different techniques in hypnosis must be used in the treatment of chronic pain as opposed to acute pain. The difficulty in treating chronic pain with hypnosis can be maintaining reduced pain awareness for an extended period of time (Patterson et al., 2003). Many studies have been performed to determine the effectiveness of hypnosis on headaches, fibromyalgia, and back pain. Other etiologies of chronic pain have not been extensively researched. Hypnosis treatment was given to 47 subjects suffering from migraine headaches. The control groups consisted of participants who were not given any treatment as well as subjects who were given prochlorperazine. Suggestion was given for visual imagery techniques, pain reduction, and for the aversion to migraine headaches. The patients who received hypnotherapy reported fewer headaches per month, a higher frequency for remission, and fewer Grade 4 headaches (Anderson et al., 1975). Hypnosis was also proven to be more effective than physical therapy in the treatment of fibromyalgia. Muscle pain, fatigue, and sleep disturbances caused by fibromyalgia were improved via hypnosis (Haanan et al., 1991). Back pain was also shown to improve when treated with hypnosis. A study done among 22 patients suffering from spinal cord injury related pain showed an 86% decrease in pain following a hypnotic induction (Jensen et al., 2001).

### Conclusion

Although there is still much obscurity surrounding the intriguing phenomena of hypnosis, much research has been done to elucidate the underlying mechanisms and benefits of this remarkable

phenomenon. Hypnosis has matured to become both a worthwhile treatment option for many medical conditions as well as a significant research tool in the quest to understand human cognition. The actual benefits of hypnosis can be seen through various experiments, both cognitively and clinically, disputing researchers who term hypnosis as being one giant placebo effect. The clinical applications of hypnosis are numerous, and more study is being done to discover viable hypnotic treatments for various illnesses. By understanding of the neurophysiological mechanisms underlying the hypnotic modulation of conscious experience, and its specific patterns of cerebral activation, one can appreciate, and potentially benefit, from the many advantages of hypnosis both in research and in practice.

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# Epigenetics as a Cure for Cancer

By Sara Rivka Margolis

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## Abstract

Epigenetics is an emerging research topic that is being tested as a potential cure for cancer. Epigenetics is a non-genetic influence that shapes the phenotype. Epigenetics effects gene expression, but does not cause any changes in the DNA. DNA methylation patterns is one such epigenetic change in the cell that has huge potential for cancer treatment. Scientists have observed that many cancerous genes express signs of either hypermethylation or hypomethylation. The key for the treatment is that epigenetic changes are reversible, which opens the door to potential drugs to cure cancer and other diseases.

## Introduction

Epigenetics literally means “above” the gene and is a new study of research in biology that explains how environmental factors impact one’s biology. Epigenetic mechanism explains how there are over 220 different cell types in an adult organism even though they all have the same DNA. All cells in each organism contains the same DNA, but their cell function differs in different cells, based on “qualitative and quantitative differences in gene expression.” (Gibney, Nolan, 2010). Bone cells, skin cells, heart cells, all have the same DNA, but their functions are completely different. This cell differentiation is a specific pattern of gene expression where certain parts of the DNA are turned on or off in each type of cell. This specific pattern of gene expression is passed down from cell to cell. Epigenetics is defined as “the study of mitotically (and potentially meiotically) heritable alterations in gene expression that are not caused by changes in DNA sequence (Gibney, Nolan, 2010). One researcher compares genes and epigenetics to a computer, where genes are the hardware and epigenetics is the software telling the hardware how to function. There is a certain balance of epigenetics that is normal and healthy for the cell, but when these balances are changed and the epigenetic mechanisms become unregulated there may be alterations in gene expression, and can ultimately lead to cell transformation and malignant outgrowths. There are three major types of epigenetic changes; DNA methylation, Covalent Posttranslational Histone Modification, and Small Inhibitory RNA-mediated signaling Pathways. Mainly, Epigenetic processes, including DNA methylation and histone modification, influence gene expression at the transcription level.

DNA methylation is the most studied epigenetic factor and that is what will be discussed in this paper. DNA methylation occurs through the covalent addition of a methyl group (CH<sub>3</sub>) to the 5 position of a cytosine generating 5-methylcytosine (Stein, Davis, 2012). Methyl groups protrude into the major groove of DNA and change the biophysical characteristics of the DNA. Cells can methylate and demethylate DNA, which effects specific gene expression. These epigenetic changes can experience multigenerational inheritance; not only does it change the phenotype of the first individual, but the methylation can pass onto further generations.

## Materials and Methods

In order to understand how epigenetics can be a potential cure for cancer, many journals and articles were retrieved from Touro College’s library database. Those articles and the references they listed formed the basis for exploring this topic.

## Correlation between DNA methylation and gene silencing

An experiment with mice and coat color phenotype and body weight measurement exposed the correlation between methyl groups and silencing of gene expression. A group of pregnant mice that were genetically identical were assembled, half the group were fed a diet rich in methyl groups (here soy), and the control half their regular diet. All these mice had the agouti gene, which when expressed gives the mouse a yellow color and causes obesity. The offspring of the mice that had the methyl rich diet were thin and brown, whereas the offspring of the mice that did not eat the methyl groups remained yellow and fat. They both have the agouti gene, but the brown mice had a methyl group attached to the agouti gene which shut it down. Bisulfite sequencing methylation analysis of CpG sites in the promoter region of the agouti gene (Avy IAP) showed a statistical increased average percentage of cells methylated of the mice given the methyl rich diet. This was a simple experiment to see how DNA methylation affects gene expression because it was easy to see when the gene was on and off based on the phenotype color switch and body measurement. Also, this epigenetic tag was passed down to the offspring’s of these mice, and continued until they ate a diet without enough methyl groups. Soy has an active ingredient that methylates DNA. This research may explain why there is a lower incidence of cancer among Asians compared to Westerners, because Asians eat diets rich in soy and may explain why Asians who emigrate to America are more likely to develop cancer than their family back home. (Dolinoy, et. al. 2006)

## DNA methylation causes gene silencing

Although a correlation was noted between gene activation and methylation, the authors wanted to prove conclusively that DNA methylation is the cause of the gene silencing. They were not positive yet which was the cause and which was the effect, they though that it might be possible for the gene inactivation to cause the DNA methylation. An experiment was carried

out that proved that DNA methylation caused gene inactivation. The experiment was conducted on the E2a region of Ad2 DNA. The region was cloned, and then half of the gene was methylated in vitro with HpaII DNA methyltransferase and then microinjected into the nuclei of *X. laevis* oocytes. The key is that the scientists ensured that the methylated DNA only remained methylated for 24 hours after microinjection. There was no synthesis of Ad2 specific RNA in the methylated DNA cells until 24 hours after injection. The control did however readily express the Ad2 specific RNA. The experiment results demonstrate that methylation plays an actual role in causing genome inactivation because at the end of the 24 hour period, there is no genome expression, yet that does not cause the methylation to continue, which proves that methylation is not a consequence of a lack of gene expression. The gene expression inactivation only occurred in the narrow window that the Gene was methylated (Doerfler, 1981).

### How it causes gene silencing

Cytosine methylation inhibits the transcription of genes. When there is an extra methyl group on the DNA, it becomes inaccessible for protein binding for gene expression. The methyl group interferes with the binding of the protein to its specific DNA to transcribe it.

### Cancer

Typically, it was believed that cancer was caused by genetic defects such as, mutations, amplifications, deletions and translocations, which affected the cancer cell and provided it with the advantage to survive and metastasize. However, today, it is clear that there is another system of equal importance that is liable in causing cancer, and that is epigenetic marks. Today cancer is considered a genetic and epigenetic disease. This epigenetic alterations which can cause cancer have been termed epimutations. This is extremely important because damage to a cell's DNA is permanent, whereas epigenetic change is reversible. (Stein, Davis, 2012).

### Proof that epigenetic changes can cause cancer

Take arsenic which is known to cause cancer of the skin, liver, lung, and bladder, but actually does not cause DNA mutations, as demonstrated in standard mutagenic assays. However in 2010, researchers found that arsenic caused cancer by epigenetic mechanisms. This was a groundbreaking epigenetic mechanism that cause cancer, and that is a major breakthrough in terms of finding a cure. One group of researchers studied CpG islands of over 14000 genes in people who were exposed to high levels of arsenic, verified by their urinary arsenic levels. It was found that these genes were epigenetically modified from the arsenic exposure, and researchers found that a tumor suppressor gene was silenced. This is called a "tumor suppressorome," which is a group of 17 confirmed or

recognized tumor suppressor genes that are silenced in human cancers, most of these through abnormal methylation patterns. (Stein, Davis, 2012)

### Two ways that DNA Methylation can have an impact on carcinogenesis

One is by hypomethylation and one is by hypermethylation. Hypomethylation is a decrease in the normal amount of methylation on DNA. Hypomethylation usually occurs at repetitive chromosomal sequences where DNA is normally methylated. Researchers found that four out of five cancer patients had significant hypomethylation in their cancer cells as compared to their neighboring cancer free cells. Hypomethylation, in decreasing the normal amount of methylation of the gene, decreases the normal amount of gene silencing, i.e. increases gene expression. This can result in an activation of genes with growth and tumor promoting functions (Lund, Lohuizen, 2004). Additionally, hypermethylation, or the increase in the amount of DNA methylation can cause a negative impact on the cells, and can lead to carcinogenesis. This hypermethylation is found at CpG islands, which are most likely found in the 5' regulatory region of the gene, which are not methylated. Here the extra methylation causes gene silencing, and tumor suppressor genes are being silenced (Feinberg, 2001). In one experiment, it was revealed that the mRNA for RL7NX3, a tumor suppressor gene involved in several cancers, is suppressed in primary cutaneous melanoma and in metastatic tumors. CpG Island, which are usually not methylated and transcriptionally active, have been found to be abnormally hypermethylated during the development of cancer, particularly in tumor suppressor genes. Genes involved in cell cycle regulation, DNA repair, (to name two) are very epigenetically susceptible (Stein, Davis, 2012).

### Hypermethylation of GSTP1 and prostate cancer

Methylation of GSTP1 is the most common epigenetic alteration described in several tumors, such as prostate cancer, endometrial cancers, and breast cancers. GSTP1 catalyzes the S conjugation of GSH, which protects the cell from cytotoxic and carcinogenic agents. Methylation of GSTP1 gene occurred in 90% of prostate cancer tissue from surgical specimens. In this experiment, the tissue samples from 144 patients who were having a prostate biopsy were examined. Forty two out of the 144 patients were diagnosed with prostate cancer. Additionally, there was hypermethylation of the GSTP1 gene in 31 out of those 42 patients, and only in 2 out of the 102 patients who did not have cancer. Since epigenetics is a cause of carcinogenesis, and, therefore, cells will exhibit hypermethylation at the onset of the cancer, there is potential for using hypermethylation as a biomarker to detect the cancer. These genes, and other genes that exhibit hypermethylation in the early onset of cancer, may eventually be useful as a biomarker to detect prostate cancer. However, methylation of

Roberto Miano, Alessandra Valentini, Stefano Germani et al.

**Table 1. Selected hypermethylated genes in human cancers.**

Gene or gene product	Function	Tumor type
Rb	Cell cycle regulation	Retinoblastoma
APC	Wnt signal transduction	Colorectal cancer and others
p14/ARF	Cell cycle regulation	Colorectal cancer
p15/CDKN2B	Cell cycle regulation	Leukemias
p16/CDKN2A	Cell cycle regulation	Melanoma cancer and others
BRCA1	DNA repair	Breast, ovarian cancer
VHL	Tumor suppressor	Renal cell cancers
hMLH1	DNA mismatch repair	Colorectal, endometrial and melanoma cancer
ER- $\alpha$	Estrogen receptor- $\alpha$	Breast cancer
GSTP1	Phase II metabolic enzyme (detoxifier)	Prostate, endometrial, breast cancer

Table 1 shows a list of that when hypermethylated become cancerous. This means that it may be possible to use hypermethylation as a biomarker to detect cancer early on.

the GSTP1 gene may indicate another cancer, and does not indicate prostate cancer, specifically. However there is potential for mapping out hypermethylation of other genes and using a multi gene profile to improve specificity for cancers. Azacytidine has been found to reverse the hypermethylation of the GSTP1 gene, and reactivate transcription (Miano et. al. 2007).

## Epigenetic tests on identical twins

The best way to study epigenetics is on monozygotic twins because they have the same genetic material, but still have difference in cell expression and their phenotype. There is a high discordance rate between identical twins for many diseases, even those thought to be genetic. Epigenetic changes through environmental variances can be the answer to the phenotypic differences of identical twins. Researchers in Madrid studied 80 identical twins in a range of 3 to 74 years old. Thirty five percent of the twin pairs had significantly different methylcytosine genomic content. Researchers observed that the difference in the methylcytosine pattern for the twins increased with the age of the twins. The youngest twin pair had their epigenetic pattern the most similar, whereas the oldest pair's epigenetic pattern was very different. Also, they found that twins who had not grown up together had the greatest difference of 5' methyl cytosine levels. These differences in methylation of their cells is what causes the gene expression and phenotype of monozygotic twins to differ. This study indicates that methylation patterns are one reason why twins with the same DNA may have different phenotypes. Additionally, the fact that the epigenetic differences were more distinct in twins who spent less of their lives together indicates that major role the environment plays into translating a general genotype into different phenotypes detectable. External factors play a great role in gene expression by changing the pattern of epigenetic modifications (Fraga, et. al. 2005).

## Epigenetics and age correlation

For every cell division there is a potential for epigenetic damage. As a person ages, their cells are forced to divide again and again to replenish any tissue damage that may have occurred. Each time this happens there is a chance for epigenetic damage through an altering of the normal methylation pattern of the cell. Scientists can take tissues from an older person and can estimate by looking at the epigenetic patterns of the DNA in a particular tissue how old the person is, because these epigenetic changes accumulate as a person ages. For a cell, aging is counted by how many times the cell has divided. The more damage a cell has, the more times it is forced to divide and replenish, and the more chances that there is going to be an abnormal pattern of methylation in this cell. For example, smoking and sun exposure can cause skin tissue injury. When a person's skin peels from a sunburn its cells repair that damage by dividing and replenishing those cells. In these cases, the sun and cigarette exposure can cause actual DNA damage, but they also just damage the tissue which leads to cellular repair of that injury through cell division, and leads to a gradual accumulation of epigenetic damage. Lungs of heavy smokers can look 20 times older than nonsmokers. This can explain why there is a higher incidence of cancer among older individuals. With age, the amount of exposure he or she has had to environmental toxins increases, which can ultimately lead to an abnormal methylation pattern (Nova, 2007).

## Epigenetic therapy

Epigenetic therapy works to repair the normal methylation pattern of the cell. This is done through DNA methylation inhibition. 5'Azacytidine and 5-aza-2'-deoxycytidine (Decitabine/DAC), the two most advanced drugs for epigenetic therapies for cancer. Both are in clinical use (Silverman, et. al. 2006). They are DNA methyltransferase inhibitors and work by trapping DNA methyl transferases thereby inhibiting methylation, and restoring the gene's normal expression. DNA methyl transferases, known as DMTAs, are the enzymes that are responsible for DNA methylation. DAC incorporates into DNA and forces the methyl transferases to form irreversible covalent bonds to DNA. These methyl transferases are then targeted for deletion in the proteasome. Then, cells divide, without DMTA and there is now DNA hypomethylation and reactivation of genes that were silenced from the extra methyl groups. However, DAC can also cause cytotoxicity when given in high doses, and therefore dosage must be monitored. A positive side to epigenetic therapy of cancer is the minimal side effects which is due to the fact that scientist can give low doses of the medication with successful results. Whereas for cancer drugs scientist have to give the highest dose possible of the medication to kill all the harmful cells, epigenetic therapy, does not necessitate high doses of the drug because it is not aiming to kill all the cells, but rather just to change the abnormal methylation patterns of the cancer cells. DAC was not tested

on young children or on pregnant women. The drug does not target normal methyl tags for two reasons. Firstly, drugs target the area of the most cellular divisions, and in this case, they are going to target the cancer cells and leave the normal cells alone. Additionally, modification of epigenetic patterns has a large effect on cancer cells but will hardly have any long term effect on the behavior of normal cells. When the drug was stopped the normal cells returned to their normal epigenetic pattern, while the cancer cells, now healthy cells with normal methylation patterns, did not revert to tumorigenesis. There is potential for all other cancers that have an epigenetic origin. Myelodysplastic syndrome, also known as MDS, and Acute Myelogenous Leukemia, also known as ALS are easier targets for these drugs because the cancer cells are in the blood and have easy access to drugs. (Issa, 2013) These drugs do not work with solid tumors yet. A reason that is likely is because it is much harder to incorporate the drugs into these cells, which are multiplying much more slowly than hematological cancers. Right now, these drugs are being tested with solid state tumors and the results have been promising. In the laboratory the scientists have been able to adjust the abnormal epigenetic pattern of the solid state tumor with these drugs, and are working to mimic these results in the body. The catch is that these drugs that prevent methylation can reactivate expression of multiple silenced genes, even genes that we want silenced, like oncogenes, which are genes that have the potential to cause cancer. Therefore, the demethylation can be harmful as well as helpful. For example, oncogene NT5E is transcriptionally silenced by methylation in breast cancer. Therefore hypomethylating that area would activate that oncogene and not be helpful. This is why in the future it is important to have some sort of epigenomic profiling- to identify potentially deleterious silenced genes before using epigenetic therapy to pharmacologically reverse resistance (Hatzimichael, Crook, 2013).

### **MDS, AML and other lymphomas**

Myelodysplastic syndrome, or MDS, and Acute Myelogenous Leukemia, are cancers of the bone marrow. MDSs are hematological disorders that usually progresses into AML. Years ago, patients diagnosed with MDS were basically given a death sentence, there was no cure, and no hope for any remission. It was soon realized that MDS was epigenetic in origin. Researchers thought to study the epigenetic pattern in patients with MDS because of the fact that the patients with the disease have an average age of 70. Diseases that target older individuals likely have an epigenetic component in it. MDS patients have aberrant methylation of their CpG loci and silencing of multiple genes. MDS was therefore an ideal candidate for epigenetic therapy, and indeed results were positive. Decitabine was given to over 100 patients for Acute Myelogenous Leukemia. In fifty percent of the patients the disease disappeared fully. Twenty five percent achieved some improvements. However, another 25% did not

respond to the drug, or the drug worked for a short amount of time but then stopped working (Nova, 2007).

### **Conclusion**

All in all, the discovery that many cancers have an epigenetic origin, has opened a new path for potential cures for cancer. This was possible because epigenetic abnormalities are reversible. It is integral to realize that everything one does affects his or her health, and the health of future generations. One's diet and what one is exposed to can affect his epigenome and subsequently his future generation's phenotype. We are not just the product of our biology, we are also its cause.

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# Why are People with Laron Syndrome Immune to Cancer?

By Raquel Margolis

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## Abstract

Laron syndrome is a congenital autosomal recessive disorder that is caused by a mutation in the growth hormone receptor. People with this syndrome have an insensitivity to growth hormone. Insulin-like growth factor I is produced by the liver in response to GH stimulus. It is responsible for systemic GH activities. If there is something wrong with the growth hormone receptor there will be decreased levels of IGF-I. Low IGF-I levels cause physical deformities notable short stature. Additionally, people with low levels of IGF-I have a natural resistance to cancer. This article discusses the ways that the decreased levels of IGF-I in Laron subjects protect the body from cancer.

## Introduction

Laron Syndrome, eponymously named after the scientist Zvi Laron who first discovered the syndrome, is a growth hormone insensitivity syndrome. People with Laron Syndrome have a genetic mutation in the growth hormone receptor. These patients have a normal level of growth hormone but a reduced level of Insulin-like Growth Factor I (IGF-I) and are characterized by dwarfism and other physical deformities. Remarkably, patients with this condition have been proven to be immune to cancer. Through research on Laron Syndrome scientist have found a link between a key growth hormone and resistance to cancer.

## Methods and Materials

Information was compiled from various articles that were obtained through Touro College's library database and Pubmed. The information was narrowed down to those directly related to the topic. The information extracted and synthesized was used to hypothesize why Laron Syndrome patients are immune to cancer.

## Background information

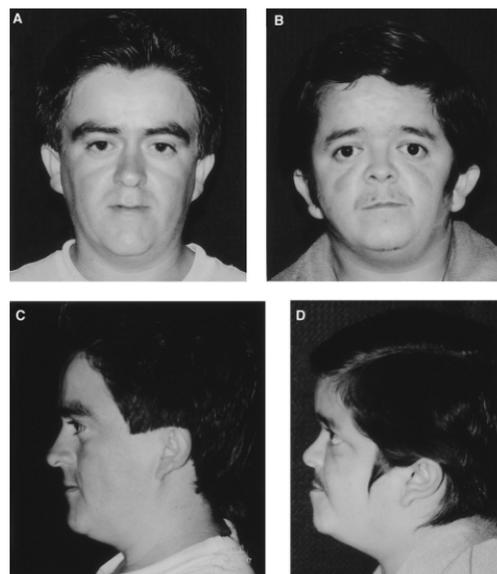
Data shows that Laron Syndrome (LS) patients have normal levels of, and a normal, GH molecule, but a defect in the Growth Hormone Receptor gene (GHR). Patients with LS have either a gene deletion or a point mutation in this gene (Wood & Savage, 1996).

Mutations in the growth hormone receptor interrupts the JAK-STAT signaling pathway which stimulates the production of IGF-I. The JAK-STAT signaling pathway brings information from chemical signals outside the cell, through the cell membrane, and into the cell where gene promoters on the DNA in the cell nucleus can cause DNA transcription and activity in the cell. The mutation in the Growth Hormone Receptor, leads to an inability of the extracellular domain to bind growth hormone and stimulate the production of IGF-I (Gastier et al., 2000).

Cited from Rosenbloom and Guevara-Aguirre that in 1966, Laron, Pertzlan and Mannheimer did research on the high levels of growth hormones in patients with clinical and laboratory signs of growth hormone deficiency (Rosenbloom and Guevara-Aguirre, 1998). There are ~251 reported people with

this syndrome. One hundred and forty eight are known to have Semitic origin. One third of the people with this syndrome live in Loja, Ecuador which is where most of the genetic studies take place. The people in Loja, Ecuador are said to have ancestors who converted to Christianity during the Spanish Inquisition (Wood & Savage, 1996).

Laron syndrome is a congenital autosomal recessive disorder. The parents of an individual with Laron syndrome must each have a copy of the mutated gene while displaying no signs of the syndrome. People with Laron Syndrome have postnatal growth failure; it begins with "subnormal birth length, retarded brain growth, acromicria, also of the facial bone, defective and crowded teeth, sparse hair, small gonads and genitalia, obesity, retarded skeletal maturation, delayed puberty, hypoglycemia. As these patients get older they have obesity, muscle underdevelopment and weakness, osteoporosis, hypercholesterolemia, hyperinsulinemia, and various degrees of glucose intolerance (Yamamoto et al, 2007)." There is wide phenotypic variability among patients with this syndrome. With that said, there is uncanny similarity between patients with LS even with variability in genotype and even if they are from different places. In the image below of two men with



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Laron Syndrome, it is evident that there is a similarity in physical features.

The man in figure A and C is 21 years old with a height of 126.6 cm. The man in B and D is 28 years old and has a height of 115 cm. Analysis of the faces indicate different craniofacial phenotype, but still both men with Laron Syndrome have an uncanny resemblance (Rosenbloom and Guevare-Aguirre, 1998).

Patients with LS have extremely low levels of insulin-like growth factors, below the 0.1st percentile for age, such as IGF-I which is the major hormone responsible for growth and it is under the control of GH. Patients with GHD also have a low level of IGF-I but those patients responded well to exogenously administered GH, whereas patients with LS did not which is a characteristic of LS. This is the primary difference between GHD and GHRD. LS patients also have extremely low levels of Insulin-like growth factor binding proteins (IGFBPs). Specifically, IGFBP-3 levels are decreased in LS patients (Wood & Savage, 1996).

### Insulin-like growth factor I (IGF-I)

IGF-I is a 70 amino acid polypeptide hormone involved in endocrine, paracrine, and autocrine functions. An original name for this hormone was somatomedin C because it was under the control and mediates the effect of the growth hormone. The name was changed to Insulin-like because it shared a similarity to proinsulin. Also, it mimics insulin activity such as stimulating glucose uptake in the cells. Additionally, IGF-I has mitogenic capabilities (Puche & Castille-Cortazar, 2012).

### Synthesis and Circulation

Growth hormone is secreted by the pituitary gland and it stimulates the production of IGF-I by acting on the liver where it is made. The pituitary gland releases GH and it works together with the liver in a negative feedback mechanism to stimulate the secretion of IGF-I; increased levels of IGF-I cause a decrease in production of GH which stimulates the production of less IGF-I (Puche & Castille-Cortazar, 2012).

Recent research by Puche and Castille-Cortazar is uncovering IGF-I as an independent and self-sufficient peptide, separate from GH. IGF-I is produced mainly by the liver in response to the endocrine GH stimulus, but it is also secreted by multiple tissues for autocrine and paracrine purposes. In tissues throughout the body it is evident that IGF-I expression is regulated and stimulated by other factors besides GH, such as estrogen in the uterus (Puche & Castille-Cortazar, 2012).

The stimulatory role of IGF-I on intrauterine growth is GH-independent. Regardless of the levels of GH, IGF-I

deficiency is the main determinant of a reduced birth size. (Puche & Castille-Cortazar, 2012).

Research done on the effects of GH to stimulate skeletal growth has proven that GH directly stimulates bone growth. Additionally, GH stimulates the production of IGF-I which will then promote bone growth. This proves, along with other findings, that IGF-I and GH work independently and synergistically to promote postnatal body growth (Puche & Castille-Cortazar, 2012).

### Central nervous system development

IGF-I binds to IGF-IR a cell surface receptor. When IGF-I binds to the receptor intracellular signaling is initiated. The AKT signaling pathway, which is a stimulator of cell growth and proliferation, and a potent inhibitor of programmed cell death, is activated.

IGF-I production coincides with periods of neuron progenitor proliferation and differentiation. The role of IGF-I in the brain is not only neuronal produced IGF-I. Systemic IGFs (mainly produced by the liver) can cross the blood-brain-barrier and are also involved in these processes. Labeled IGFs were placed in the carotid arteries of rats and were later on detected in the choroid plexus, median eminence, brain arterioles, and parenchyma. This coincides with the data that confirmed the presence of IGF-I receptors in the brain capillary endothelial cells which constitutes the BBB, and the role in internalizing IGFs from circulation to the CNS. There is evidence that growth factors, GH, basic fibroblast growth factor; nutrition; and injury influence and regulate IGF-I expression in the brain. All of the growth abnormalities that are attributed to LS patients (see above), indicate that IGF-I plays a critical role on brain development and function.

### Current therapeutic options and limitations

In 1980, Recombinant Human IGF-I (rhIGF-I), Mecasermin became available for experimental therapy for people with severe primary IGF-I deficiency. An average of 8.5 cm in height was grown the first year. Besides for an increase in height, patients with rhIGF-I treatment had an increase in testosterone levels, testicular size and stretched penile length. This shows the effect of IGF-I on sex hormones and organs in male patients (Puche & Castile-Cortazar, 2012).

Another treatment that the FDA approved was a combination of IGF-I and IGFBP-3, Mecasermin Rinfabate. This was thought to have been a smarter alternative to Recombinant human insulin growth factor I (rhigf-I) because it was supposed to extend the duration of IGF-I in the body. Interestingly enough, when the combination of IGF-I and IGFBP-3 were used to

treat patients compared to a control group who only were treated with IGF-I, those treated with IGF-I alone had better results (Puche & Castile-Cortazar, 2012).

Dosing guidelines for rhIGF-I are still being debated. The international Congress of Endocrinology has stated that both one and two injections of rhIGF-I is safe and efficient. One or two injections regimen have the same growth velocity. By carefully monitoring the IGF-I serum levels the negative side effects can be avoided. Long-term rhIGF-I has reported negative side effects. These side-effects, namely tachycardia were easily managed without treatment discontinuation (Puche & Castile-Cortazar, 2012).

A novel and efficient method for hormone replacement therapy is being developed using Sertoli cells. Sertoli cells are originally from the male testis, and they can ameliorate development and survival and function of different cell types. In the "Laron mouse" it was successfully reported that pre-pubertal sertoli cells in microcapsules can successfully promote growth. There was a significant increase in body weight and body length compared with the control "Laron mouse" treated with empty capsules. IGF-I serum levels were noticeably increased in mice treated with Sertoli cells microcapsules. The reduction in side effects, the increase in growth, and the fact that sertoli cells do not require intramuscular daily injections are all reasons that sertoli cell treatment is preferred over rhigf-I treatment (Kinam Park, 2012).

Puche and Castille-Cortazar cite a study done by Steuerman R, Shevah O, Laron Z. on the prevalence of cancer in people with Laron Syndrome compared to their family members. This study proved that IGF-I deficiency provides protection against cancer. Interestingly, this study reported that Laron Syndrome patients are protected against future cancer development, even when treated with rhIGF-I. (Puche & Castile-Cortazar, 2012).

The positive results of exogenously administered IGF-I in subjects without IGF-I deficiency, to use its anti-inflammatory, hematopoietic, antioxidant, metabolic or anabolic properties, is not clearly determined (Puche & Castile-Cortazar, 2012).

## Discussion

Studies have shown that patients with overexpression of IGF-I are more susceptible to tumors. Laron set out to determine if patients with IGF-I deficiency (LS) are less likely to develop cancer. Two hundred and twenty eight patients with IGF-I deficiency and 338 of first and second degree family members were surveyed. Cancer was not a cause of death in GHRD patients of any age. Ten to twenty four percent of the family members had a history of malignancies. Only one GHRD subject monitored had

Prevalence of malignancy in the surveyed subjects				
Diagnosis	Patients	Malignancy	Relatives	Malignancy
<b>Israeli Cohort</b>				
Laron syndrom	n=40a	0	n=99	n=24(24.2%)
M:F	18:22	2:49:00	15:08	
Age Range	3-75	19-91	42-81	
Mean age	32.3	55.4	51	
Other diagnosis	n=22	0	n=83	n=7(10%)
M:F	13:09	19:40:00	3:04	
Age Range	3-56	5-93	32-80	
Mean Age	27	51.4	64.8	
<b>Other Countries</b>				
Laron syndrom	n=129	0	n=151	n=17(11.2%)
M:F	59:70	78:73	8:09	
Age Range	6-78	27-68	45-85	
Mean Age	16	50	68	
Other diagnosis	n=31	0	n=5	n=3
M:F	19:12	3:02	2:01	
Age Range	2.5-50	40-80	68-80	
Mean Age	16.8	60	65	

(Laron & Shevah, 2006)

cancer, papillary serous epithelial tumor in the ovary. The table below shows the prevalence of the malignancies in patients and family members. Because of the results of this and further studies, IGF-I receptor blockers are being developed as drugs for cancer therapy (Shevah & Laron, 2006)

## What is the link between GH and cancer?

Data suggest that the GH/IGF-I axis shows an important role in cancer.

Growth Hormone does not have the ability to induce cancer, but it has cancer-enhancing properties. There are many different factors that influence the different effects of the IGF-I. The powerful effects of IGF-I on the stages of cancer development and behavior include "cellular proliferation and apoptosis, angiogenesis and metastasis..." Additionally, IGF-I is a powerful antiapoptotic agent. These opposing effects relate strongly to cancer. Firstly, there is increased proliferation causing epithelial cell turnover within tissues. Secondly, there is an imbalance in the control between proliferation and cell death because of the anti-apoptotic effects which leads to hyperproliferation. This is the first stage of development of many cancers. Thirdly, this imbalance between cell proliferation and cell death causes the favoring of cell survival even in damaged cells. This could

accelerate carcinogenesis. Both the second and third point are ways that cancer is accelerated not initiated (Puche & Castile-Cortazar, 2012).

Research in epidemiological studies have shown a correlation between the role of GH and carcinogenesis. People with more growth hormone are naturally taller. The Boyd-Orr study showed an SD of 1 inch height to be linked with a 42% higher risk of cancer later in life in boys. High birth weight, high stature at 14, low body mass index, and peak growth at early age were independent risk factors for breast cancer. (Jenkins et. al., 2005) Epidemiological data found a correlation between adult height and cancer. An increase in breast cancer by 22 % was noted with increased height. These studies suggest that the GH/IGF-I axis plays an important role in cancer development and behavior (Jenkins et. al., 2005). Many pharmaceutical companies are creating drugs that inhibit the IGF-I signaling to inhibit breast cancer proliferation and block the mitogenic effects of exogenous IGF-I. They are also experimenting with Inhibitors of the IGF-IR as a chemotherapeutic (Jenkins et. al., 2005).

Studies on mice were done to provide evidence to this relationship. Transgenic mice with human GH and agonist for the IGF-I receptor have increased incidence of breast tumor development. On the other hand, mice with a non-functioning GH receptor (serum GH/IGH-I levels of ten percent the normal), showed inhibition of growth of transplanted breast cancer cells. Even the serum was less mitogenic to breast cancer cells in vitro than a control serum, this changed once IGF-I was added to the serum. Recent studies have shown that mice transfected with growth hormone receptor antagonists have less incidences of carcinogenesis (Guevara-Aguirre et. al., 2011).

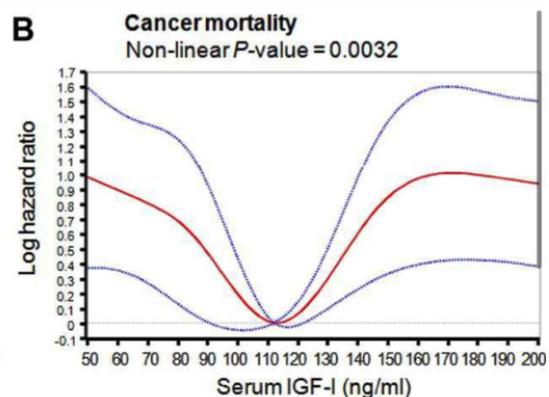
Another study done on mice was used to prove the influential effects IGF-I exerts on the metastatic power of cancers (Guevara-Aguirre et. al., 2011). This is an extremely important aspect of cancer as the metastatic spread of cancer is usually what causes mortality. A cell from the LCC6 metastatic breast cancer cell line was transfected with a truncated IGF-I receptor that silenced the expression of IGF-I receptor and inhibited IGF-I signaling. These cells were transplanted into mice and the metastatic spread was measured. Mice that were transplanted with wild type cells had multiple pulmonary metastases, but the mice transplanted with the transfected cells were completely absent of any metastases (Guevara-Aguirre et. al., 2011).

Another reason how the GH/IGF-I axis can cause cancer is by promoting an increased DNA damage. Experiments on *Saccharomyces cerevisiae* yeast indicate that mammalian growth signaling pathway genes promote an increase in DNA mutations by elevating superoxide production and increasing DNA

damage. This is particularly important because it hypothesizes that, in addition to the possibility that the GH/IGF-I axis may promote cancer by preventing apoptosis of the damaged cells, it can increase DNA damage in cells that can ultimately lead to cancer. To test this hypothesis, human mammary epithelial cells (HMECs) were placed in a medium that contained 15% serum from either Laron syndrome subjects or their relatives. The cells were then treated with H<sub>2</sub>O<sub>2</sub> followed by comet analysis to detect DNA strand breaks. The comet analysis indicated that cells incubated in serum from GHRD patients had fewer DNA breaks after treatment in comparison to cells incubated in serum from relatives. This teaches that serum from GHRD subjects can protect against oxidative DNA damage independently of cell division. It was determined that that IGF-I signaling was responsible for the sensitization of cells to oxidative damage via analysis of DNA damage in MEF cells lacking the IGF-R or over-expressing the human IGF-IR cell (R+ cells). R+ cells had more DNA damage than did R-cells. (Guevara-Aguirre et. al., 2011).

A study done by Wang and colleagues on rats explains that down-regulation of GH signaling could block carcinogenesis in rats. Human prostate cancer cell lines express GH receptors at levels greater than normal. A rat that does not have GH because of a mutation was crossed with the Probasin/Tag rat, a rat which develops prostate carcinomas at 100% incidence rate. The rats that were homozygous for the GH deficiency had prostate tumor incidence and tumor latency reduced relative to wild type rats. At 25 weeks of age, the rats with no GHR resulted in a 20% and 80% decrease of carcinoma in the dorsal and lateral lobes, respectively. At 52 weeks of age, invasive prostate adenocarcinomas were observed in all Probasin/ Tag rats positive for the Growth Hormone. Conversely, majority of the Tag rats with the mutation that makes them GH deficient did not develop invasive tumors (Wang et. al. 2008).

The potent antiapoptotic and mitogenic properties of IGF-I is linked to an increased risk in developing cancer. It is documented that IGF-I can promote carcinogenesis at the cellular



(Svensson et. al., 2012)

level, but evidence is accumulating that circulating IGF-I can also promote carcinogenesis. For example, reduction of IGF-I by dietary restriction slows tumor progression and increase apoptosis in tumor cells of animal models. And when these models were treated with recombinant IGF-I the effects were reversed. Additionally, IGF-I gene deletion in mice is linked to a 75% decrease in circulating IGF-I levels. Tumor growth and development was much higher in the control group than transgenic mice.

### Biological interactions among insulin, IGF-I and IGF1R

As stated above there are many clinical studies that suggest that high serum IGF-I levels associate with increased risk of cancer. Increasing clinical evidence suggests that low IGF-I levels are associated with cancer mortality in older men. In numerous studies, the association between circulating IGF-I levels and cancer mortality was U-shaped with increased levels of mortality at both the low and high serum IGF-I levels.

Serum IGF-I was extracted from the blood of a large cohort of elderly men from Sweden. The measurements of serum IGF-I levels were obtained. The reason for mortality was documented on the death certificate of the subjects and then used in the study. Cox proportional hazards was used to analyze the association between serum IGF-I levels and mortality by cancer. Cox regression analysis showed that both low and high serum IGF-I concentration was associated with increased cancer mortality. The patients with a history of cancer were excluded from the analysis (Svensson et al, 2012).

### IGF-1R Inhibitors as an Anti-Cancer

Because of the role that IGF signaling plays in the promotion of cell growth and inhibition of apoptosis, IGF blocking is being developed as a therapeutic potential to protect against cancer. Currently there are many clinical studies targeting and inhibiting the IGF pathway to promote anti-tumor activities. There are three main classes of IGF/IGF-1R inhibitor antibodies being studied in clinical trials; each class inhibits IGF-1R signaling but through different mechanisms. There has been some small success in treating patients with select tumor types, but many trials have been unsuccessful. Researchers are trying to identify patient selection markers to attain future success in IGF-1R inhibitor development (Chen & Sharon, 2013).

IGF blocking is also being used to overcome chemotherapy sensitivity. Resistance to chemotherapy is common nowadays. IGF is implicated in chemotherapy resistance because it promotes proliferation and inhibits apoptosis. Researchers have tested this hypothesis and have found that IGF-1R inhibitors have caused significant tumor growth inhibition. Currently,

researchers are testing the combined effect of IGF-1R inhibitors with a chemotherapeutic (Weroha & Haluska, 2008).

### Conclusion

Laron Syndrome subjects participate in groundbreaking genetic studies to explore the relationship between the deficiency in the growth hormone receptor and immunity to cancer. This syndrome is providing insight to, and revolutionizing, the way cancer is treated and prevented. Because Laron Syndrome subjects have decreased levels of IGF-I they are protected against numerous and potentially harmful effects of IGF-I including cellular proliferation, apoptosis, angiogenesis, and metastasis. The increased proliferation and decreased apoptosis causes survival of damaged cells that then spread easily throughout the body, which could lead to the development of cancer. Because Laron Syndrome subjects have decreased levels of IGF-I they are immune to cancer. The implications of IGF/IGF-1R in cancer development, maintenance, and progression, is what led to replicating the IGF-I levels of LS patients to use as an anti-cancer target. IGF-I has been successfully inhibited in animal models to prevent cancer cell growth. Now researchers are beginning to use IGF-I reducing agents and blockers as a chemotherapeutic to prevent cancer.

### Abbreviations

IGF-I	Insulin Growth Factor I
LS	Laron Syndrome
GH	Growth Hormone
GHR	Growth Hormone Receptor
GHD	Growth Hormone Deficiency
GHRD	Growth Hormone Receptor Deficiency
CNS	Central Nervous System
rhIGF-I	Recombinant Human Insulin Growth Factor I
IGFBP	Insulin Growth Factor Binding Proteins
HMEC	Human Mammary Epithelial Cells

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