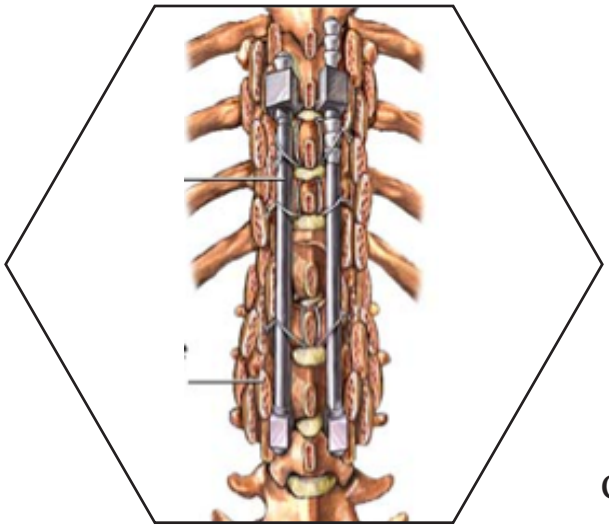

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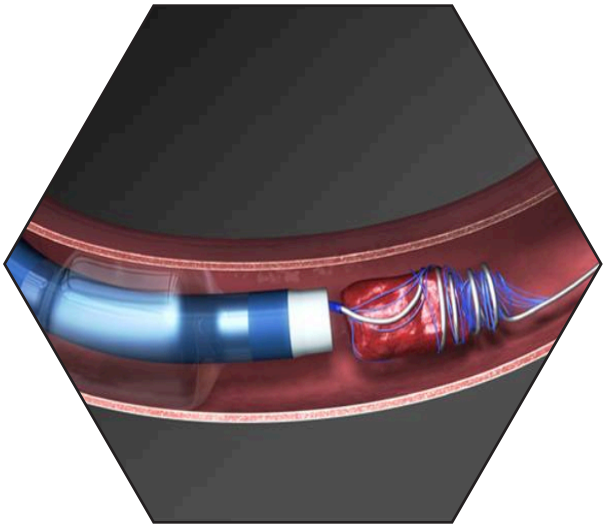

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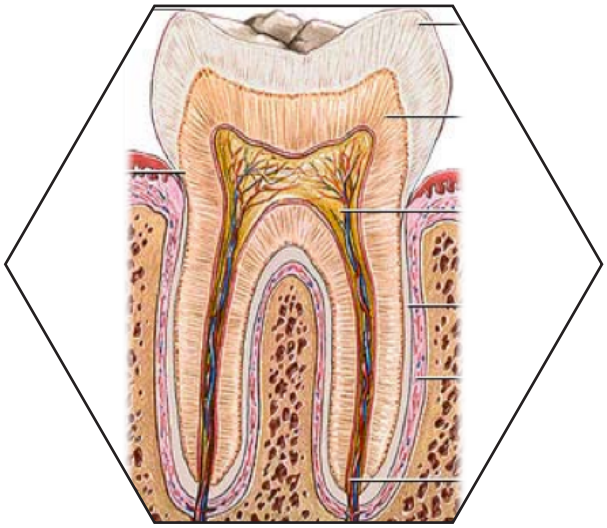

Fall 2011

Throughout its 36-year history, Touro's Lander College of Arts and Sciences in Flatbush (with separate men's and women's schools) has provided cohorts of aspiring high school graduates from well-regarded yeshivas and seminaries with a foundation of academic excellence for professional career growth, in an environment that is supportive of the religious values of its students. Graduates have assumed leadership roles and continue to strengthen Jewish communities throughout the world.

Lander College of Arts and Sciences–Flatbush offers more than 25 majors and preprofessional options, and three joint undergraduate/graduate degree programs in occupational therapy, physical therapy and physician assistant studies with the School of Health Sciences. Honors tracks in biology, the health sciences, political science and psychology are currently offered.

Students are also required to complete a carefully designed core curriculum that emphasizes the development of communications skills, critical thinking and analytical competencies, computer literacy and quantitative reasoning. Enrollment in science courses, notably biology and chemistry, continues to increase, reflecting the career interests of premedical and health science students.

Faculty members continue to earn recognition for outstanding achievements, including Joshua November, Assistant Professor of Languages and Literature, who was selected as a finalist for the Los Angeles Times Poetry Book of the Year Prize in 2011; Karen Sutton, Assistant Professor of History, whose significant Holocaust analysis, The Massacre of the Jews of Lithuania, 1941–44, was published in 2008; and Atara Grenadir, Assistant Professor of Art, whose works were displayed at the Art Expo 2011 show in New York City.

Notable alumni distinctions of Touro's Lander College of Arts and Sciences in Flatbush include: David Greenfield (JD, Georgetown), elected to the New York City Council (44th Council District) in 2010; Dr. Israel Deutsch (MD, Einstein), appointed as Director of Brachytherapy at New York–Presbyterian Hospital/Columbia University; Yossi N. Heber (MBA, Wharton), President, Oxford Hill Partners; Dr. Haim Mozes (PhD, NYU), Associate Professor, Graduate School of Business, Fordham University; Vivian Schneck-Last, Managing Director, Goldman Sachs; and Sara Grossman Wiederblank, who published her fourth novel, Pass or Fail, in 2010. Alumni have published articles in the New York Law Journal, Bloomberg Law Reports, Institutional Investors Journal and other peer-reviewed journals.

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SCOLIOSIS: COMPARATIVE ANALYSIS OF SURGERY VS. BRACE TREATMENT

Jerry Frenkel

INTRODUCTION

The spine, or spinal column, as it is also called, is a complex structure made up of 33 vertebrae (bone segments) arranged vertically in succession from just below the skull to the tailbone. The entire column is broken up into various classes of vertebrae named, from superior to inferior, as cervical, thoracic, lumbar, sacral, and coccygeal. Different forms of spinal deformities that can occur include kyphosis, lordosis and scoliosis (Neuwirth and Osborn 2001).

Scoliosis is defined simply in medical terms as a lateral three-dimensional irregular curvature of the spine. The curvature as seen from the back of the person can be described as an "s" shaped bend or a "c" shaped bend. The spinal curves are measured by degrees of curvature and depending on the severity of the degree of curvature, an appropriate treatment method will be determined. Scoliosis curvatures do not retain a status quo over time. If not treated properly, the curves tend to progressively worsen, causing pain and discomfort to the diseased party. In this paper, different methods that are used in treating scoliosis are discussed including the different types of braces that are used and the various forms of surgeries that are performed in such cases.

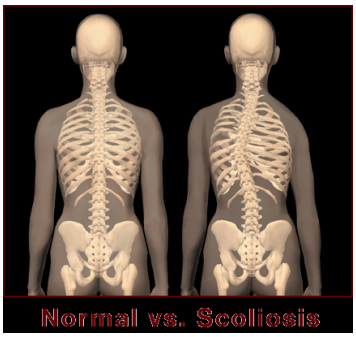

Figure 1:
Normal spine compared to scoliosis.
Source:
http://www.inspireyourbodymt.com/home/2009/9/3/injury-of-the-week-scoliosis.html

CAUSES OF SCOLIOSIS

Although the causes of scoliosis aren't fully understood, most scientists believe that its causes are multifactorial and dependent on the form of scoliosis. Contributing factors may be genetic inheritance or the laxity of ligaments and the deformation of the vertebral bones. The factor contributing to the disease would place it into one of two categories, either structural or functional scoliosis (Wikipedia.org 2010).

If the cause of the disorder is not genetic, it will fall under the grouping called functional scoliosis. This form of scoliosis, which usually appears in people with weak vertebral muscles, may be caused by the patient sitting for long periods of time in a slouched position or from carrying a heavy backpack on the same shoulder too often. Fortunately, functional scoliosis can more often than not be easily treated by toning the back muscles or by making a conscious effort to sit and stand erect with proper posture (Lyons et al. 1999).

Although there may be many factors contributing to functional scoliosis, structural scoliosis is unanimously agreed upon to be caused by direct genetic inheritance. Structural scoliosis, the more common form of the disorder, is the natural progression of the spine adapting a curved position and is the more severe form of the ailment. This mode of scoliosis cannot be treated through good posture and toning the back; rather, a proactive step must be taken to stop the progression and straighten the spinal column (Lyons et al. 1999). While certain types of scoliosis have different ratios of men to women (and some

Jerry Frenkel, B.S. '11, majored in Biology.

forms may even be more prevalent in males than females) due to the genetic factors involved, scoliosis is more commonly found in females than in males. In fact, the more severe the vertebral curve is, the larger the female to male ratio gets.

SCOLIOSIS DIAGNOSIS

There are various ways in which scoliosis can be diagnosed. To a physician, there are certain bodily indications that are easily seen that signify that the patient may have scoliosis. Some examples are asymmetric hips, an elevation of a shoulder, a protrusion of one shoulder blade or a visibly curved spine. These are all indications of a spinal deformity that to any person (not only doctors) are visible to the naked eye. There are a couple of more specific tests that a physician will use to diagnose the patient. The Adams forward bend test is the simplest and most common scoliosis test used by physicians. The patient bends forward with their arms pointed straight down to their toes and the physician examines the spine for any curvatures. If the curve is not clearly visible, using a device called a scoliometer, the physician can look for elevated muscles or a spinal curvature by placing the scoliometer on the back of the patient and measuring the discrepancy between different elevations of the back.

Today, technology has advanced and a more accurate method of diagnosis is used. A combination of X-rays and/or radiographs is used in conjunction with the Cobb angle, which is the angle two right angles make with the curved spine, to diagnose an exact degree of curvature. There are four different types of X-rays that physicians will use along with the Cobb angle to determine the severity the bend. The various X-ray forms range from the classic and simple front/back X-ray to the complicated bending X-ray. Bending X-rays are often used to determine the flexibility of the spine, which could affect the style of treatment that is utilized. Visits and X-rays are taken every couple of months to watch for progression of the curve so that the proper form of treatment can be determined.

TYPES OF SCOLIOSIS

Scoliosis is more much more common in our society than most people imagine. Statistics show that an astounding 1/ 40th of the population have a scoliosis curvature of at least ten degrees. As the severity of the curve increases the ratio decreases but still maintains a high percentage of people, with five people out of every thousand having vertebral curves of over 20 degrees (Dotsky and Lipp 2002). The reason scoliosis is so common is because there is such a wide variety of different types of scoliosis that people can have. No single description of scoliosis can encompass the wide range of curvatures that exist. Origin, time of onset and location of curvature are all variables in classifying what type of scoliosis affects the patient. Categorization, i.e. the shape of the curve and severity, are important because that will determine the form of treatment that a doctor will advise. Some curves exhibit considerable lateral curvature and little rotation while others have extreme rotation and insignificant lateral curvature.

All structural scoliosis deformities can fit into one of two more specific categories, the idiopathic cases and those cases that have an identifiable cause. Non-idiopathic cases can be further subdivided into congenital and neuromuscular scoliosis.

IDIOPATHIC

Idiopathic scoliosis is loosely defined as scoliosis that progressed for apparently no cause. This means that although extensive research has been done, the cause for the onset and progression of the scoliosis still evades scientists and it appears that the deformity is

unrelated to anything else. Approximately 75-85% of people with scoliosis have idiopathic scoliosis in a ratio of 1:8, men to women (Lonstein 2002).

Idiopathic scoliosis is different than congenital scoliosis in that its onset can occur at any point during person's lifetime. In order to discern between the numerous types of situations, specific names are given to each case based on when the onset occurred. Infantile idiopathic scoliosis has its onset between infancy and age 3. While scoliosis in general is more common in women than men, infantile idiopathic scoliosis is more common in boys than girls. Most often, this type of scoliosis will manifest itself as a thoracic curve with left convexity (Lyons et al.1999). If the condition appears between age 4 and 10, it is labeled as juvenile idiopathic scoliosis, while if it occurs from age 11 till the end of the teen years, it is called adolescent idiopathic scoliosis (AIS).

The majority of idiopathic scoliosis cases are considered adolescent idiopathic scoliosis. This may be because even though the onset of the condition began when the person was a juvenile patient, since it only became noticeable at adolescence, it will still be considered adolescent idiopathic scoliosis (Dotsky and Lipp 2002). Logically, it's understandable that AIS is the most common form of scoliosis, because the adolescent growth spurt is the most opportune time for the spine to curve or increase the degree of a curve dramatically. Interestingly, AIS tends to follow a series of specific patterns in forming scoliosis curves; a) thoracic curves which show a right convexity b) thoracolumbar curves which also show right convexity c) lumbar curves which show a left convexity and d) double major curves which show a and c together forming an "S" shaped deformity of the vertebral column (Lyons et al. 1999).

Adult idiopathic scoliosis (onset over 20 years of age), although uncommon, is a possibility. In fact most adult scoliosis cases are idiopathic and structural as opposed to a logical functional cause (due to weaker muscles etc.). This structural deformity is usually a consequence of osteoporosis, degenerative arthritis or another condition that causes deterioration of vertebrae (Arthritis-Symptom.com).

CONGENITAL

Non-idiopathic scoliosis is usually the result of a neuromuscular disease or a congenital abnormality (Lonstein 2002). Congenital scoliosis normally forms when there is an abnormal or warped formation in the spinal cord during embryonic development. "A key feature of congenital scoliosis is the presence of one or more abnormally formed vertebrae. When these anomalies are identified the curve should be classified as congenital, even if the deformity is not apparent until adolescence" (Erol et al. 2002). There are two main causes of congenital scoliosis; cases are classified based on failure of formation, failure of segmentation or a combination of the two (Erol et al. 2002).

Segmentation defects refer to an abnormal separation of the embryo's different spinal segments, called somites, formed during development of the spine in a process called somitogenesis. In this process, segments of tissue called somites are formed in pairs surrounding what will eventually become the spinal cord. When somitogenesis is disrupted even slightly, congenital vertebral defects result.

The second classification of congenital vertebral anomalies are those that occur due to a failure of formation. The most common type of formation anomaly is a hemi vertebra. This is where a portion of the vertebra is missing resulting in a small, triangular shaped "half vertebra" or hemi vertebra. Hemi vertebrae can be sub-classified based on their relationship to the adjacent spine, segmented, semi-segmented and non-segmented. When

several vertebral segments fail to separate bilaterally, a block vertebra results producing fused vertebral bones. Simple hemi vertebrae cause a progression of spinal column curvature of 1-3.5° per year (Harms 2010). These forms of abnormalities in the spine are usually associated with deformities in other organs and organ systems including the heart, kidney and lungs. In fact, a study showed that more than half the congenital scoliosis cases are related to associate health problems (Harms 2010). Congenital scoliosis is rare, but it may require early surgery due to the severity of the spinal deformity involved.

NEUROMUSCULAR

Neuromuscular scoliosis is the other form of the deformity with an identifiable cause. Neuromuscular scoliosis is classified based on two possible origins; neuropathic and myopathic. Neuropathic diseases involve either the upper or lower motor neurons. Both upper motor neuron diseases, such as cerebral palsy, spinal-cord trauma and tumors, and spinal muscular atrophy, and lower motor neuron diseases, such as polio, can lead to scoliosis. Myopathic diseases include arthrogryposis (joint contractures) and muscular dystrophy. Despite the differences, the common factor in these conditions is an inability to provide muscular support to the spinal column or an imbalance of the muscular control of the spine. Realistically, patients who contracted neuromuscular disease before age 10, have a 100% chance of developing neuromuscular scoliosis (Lonstein 2002). Treating neuromuscular scoliosis is more challenging due to all the additional problems in the other systems that come along with the specific disease of the patient. Bracing is a possible approach, but it will only slow the progression, not stop it, while surgery can be complicated due to numerous factors (e.g. most of these individuals have to be on medications which can affect blood clotting). Sometimes, in wheelchair bound patients, surgeons for neuromuscular scoliosis patients will form a base-like structure unlike the regular rods and wire system that are put in for normal standing patients (Lonstein 2002).

TREATMENT OF SCOLIOSIS

The question arises, what is the best method in treating scoliosis? Unfortunately, there is no one correct answer. When the doctor examines a patient for scoliosis, he performs a comprehensive physical examination to get a perception of the patient's health, preferred activities and lifestyle. Only by knowing all these things can a doctor recommend a treatment that is beneficial for the patient. Of course, the specific type of scoliosis that the patient has been diagnosed as having will also affect the form of treatment.

The first step in the treatment process is diagnosing whether there is a need for a remedy at all. There are two routes of activity that a scoliosis curve can take; it could be a resolving curve or a progressive curve. A resolving curve is a form of scoliosis that like its name will probably resolve on its own. These curves that straighten themselves without any action done upon them are commonly found in infantile idiopathic scoliosis. Progressive scoliosis, obviously the more dangerous of the two varieties, is a curve that progressively gets worse unless treated effectively. Treatment of progressive scoliosis can take the form of a brace progression (or body casting in very young patients) for lesser degree curve or surgical stabilization for those curves that bracing alone won't be an adequate solution. Determining the specific treatment for a patient is based on the patient's age, curve flexibility or curve magnitude.

BRACES

TYPES OF BRACES

There are many different types of braces which all serve their own designated functions. Regardless of their categorization they all are meant to achieve the same purpose: to improve scoliosis related deformities, to prevent curvature from progressing and to provide postural comfort and stability. Although braces cannot correct scoliosis curvatures, they can slow or stop the progression, eliminating or at least delaying the need for a surgical remedy. There are four general categories of rigid scoliosis braces known to physicians by their acronyms cervical thoracolumbar sacral orthosis (CTLSO), thoracolumbar sacral orthosis (TLSO), lumbosacral orthosis (LSO), and the bending brace. These braces are more commonly known to the patients by the name of their city of origin (e.g. Milwaukee brace and Boston brace). In each general category of bracing, over the years, technology has advanced and many new braces have come out. All these newer scoliosis braces follow the direction of one of the four general categories but each has their own little nuances that make them different from each other. For example, under the category of the Boston brace we have other braces that follow the Boston brace prototype but are known by other names (i.e. the Wilmington brace and the Miami brace). Regarding treatment, there is no brace that is considered better than the others; all the different forms of braces cater to different people with different curvatures. Each model has a distinct style that will benefit some patients and not others. Sometimes, two brace

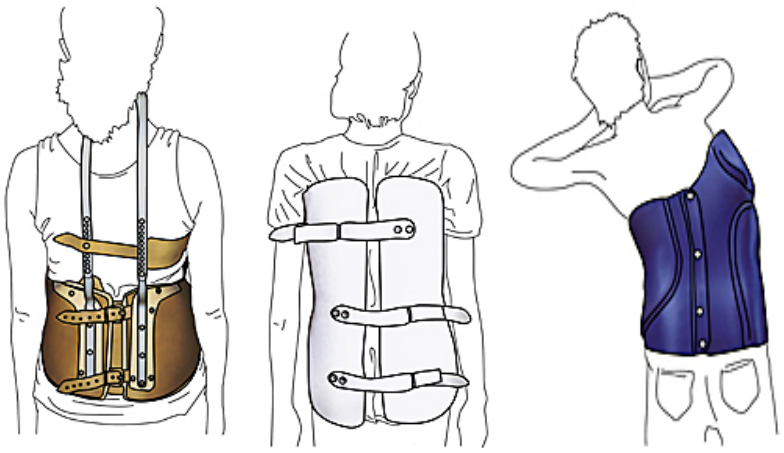

Figure 2: Posterior Views in order from Left to Right: The Milwaukee Brace, The Boston Brace, and The Charleston Bending Brace.
Source: http://www.iscoliosis.com/articles-brace_types.html

models may be recommended for the treatment of a specific patient and the only reason to choose one over the other would be personal preference.

The Milwaukee brace (Figure 3) was introduced in 1946 and first designed by Blount and Schmidt to be worn by patients to "provide comfortable and efficient passive correction and fixation following patients post-surgery". They discovered soon after that this brace was very effective in patients who had not yet gone for surgical procedures. This became the first form of non-operative treatment for scoliosis patients. This brace is a CTLSO brace that is usually given to patients who have high thoracic curves with apexes above T7 vertebrae or patients who have double major curves. It is normally used with

growing adolescents to hold a 25° to 40° advancing curve (Chow 1997). The brace is intended to minimize the progression to an acceptable level, not to correct the curvature.

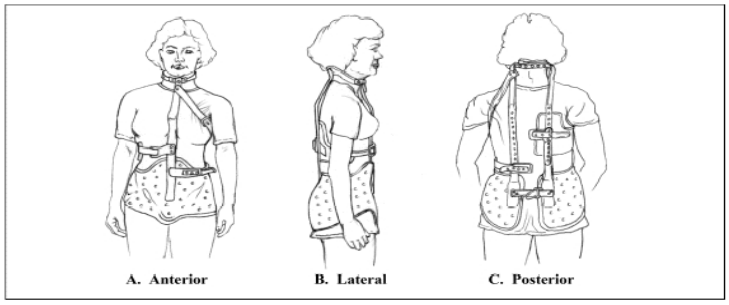

Figure 3: Milwaukee Brace
Source: http://www.ncbi.nlm.nih.gov/books/NBK27217

The Milwaukee brace is commonly worn 23 hours a day and is a full torso brace that is made to custom fit the patient's torso. It extends from the neck to the pelvis and consists of a specially contoured plastic pelvic girdle and a neck ring connected by metal bars in the front and the back of the brace. The metal bars help extend the length of the torso and the neck ring keeps the head centered over the pelvis. Pressure pads, strategically placed according to the patient's curve pattern, are attached to the metal bars with straps. The Milwaukee brace is often characterized by a chin piece that the patient can rest his/her head on. Although the extension of the brace up to the neck is uncomfortable and unfavorable, it is the distinguishing feature that makes this brace useful and effective (Chow 1997). While wearing the Milwaukee brace, patients found it difficult to perform the simple activities of daily living such as putting on socks, tying shoe laces, brushing their teeth and eating spaghetti from a plate, due to the immobility of the spine. Although the brace is difficult to deal with on a daily basis, studies found that the Milwaukee brace is very effective when the patient complies with the proper treatment.

The Boston brace and its sub types are the most commonly used braces in the United States. This brace is usually used by patients with lumbar or thoracolumbar curvatures (apexes lower than T7). Like the Milwaukee brace, the Boston brace has various pads that are placed around the brace to provide specific forces to effectively maintain the curve in the corrected position. The Boston Brace extends from below the breast to the beginning of the pelvic area in front and just below the scapulae to the middle of the buttocks in the back. It is designed to keep the lumbar area of the body in a flexed position by pushing the abdomen in and flattening the posterior lumbar contour.

There are two main varieties of the Boston brace, TLSO and LSO. The difference between the two types and the reason why a patient would wear one over the other is determined by the location of the scoliosis curvature. Curves in the upper lumbar and/or accompanied by minor thoracic curves will wear the TLSO version of the Boston brace. This version extends up to one or both underarms, while the LSO extends only to the lower region of the patient's back. Therefore, patients with curves strictly in the lower lumbar region that show little or no thoracic component will use the LSO version of the Boston brace. Cosmetically, the Boston brace is usually preferred over the Milwaukee brace because it is easily concealed under normal clothes. Like the Milwaukee brace, the Boston is generally worn 20-23 hours a day.

The Charleston bending brace is different from both the Milwaukee (CTLSO) and the (TLSO/LSO) Boston braces in many of its aspects. While the Boston and Milwaukee braces are worn most of the day, the Charleston bending braces are only worn at night. The Charleston brace is meant to be worn at night as it was originally designed for the purpose of increasing compliance in kids and young adults (Hooper et al. 2003). The design of the Charleston brace is similar to the Boston brace but its redeeming

characteristic is that its shape is molded around the patient's body. The brace is bent in the opposite direction of the curve, thereby "over correcting" the spinal deformity, so while the patient is wearing the brace his/her torso is physically being pushed to bend in the opposite direction of the natural curve of the spine. Another reason why the brace is only used at night is because it forces the patient into a bent over position, which would be awkward and prohibitive in daily life. There are many benefits to the Charleston bending brace over the other two, nearly all of which are related to the nightwear component. It allows full, unrestricted musculoskeletal development and opportunity for athletic participation, if desired. Also, it causes fewer and less severe complications. Results can be assessed without the customary long-term follow-up, and decision-making regarding success or failure of the program can be made earlier (Hooper et al. 2003). Patients with single lumbar or thoracolumbar curves are the best candidates for the Charleston bending brace and have the greatest chance of a positive outcome because inadvertently increasing a secondary curve through bracing is not a concern.

The previous three braces discussed were all forms of rigid bracing. Recently, technology has advanced and there is a new system of flexible bracing that is now being used to treat scoliosis patients. Researchers at the St. Justine Children's hospital in Montreal Canada have developed corrective movements for all types of idiopathic scoliosis to "open up" or correct the curves. The patient is braced in that corrective movement and held there 20 hours per day by the elastic bands that make up the brace. The real action in the brace is in the elastic bands. As the patient goes through the movements of the day, they stretch the elastic bands and the bands then resist and pull them back into the corrective movement. This stretch and resist system stimulates the growth centers in the deformed vertebrae and in the neuromuscular system and over time the gentle resistance of the brace and the reprogramming of the body's neuromuscular pattern results in a relatively permanent stabilization or correction of the scoliosis in almost all patients. Unlike rigid braces where patients walk around like a robot, SpineCor allows patients to do virtually any physical activity they want. In fact, exercise and activity are absolutely essential for the SpineCor brace to work.

The brace has a pelvic unit from which strong elastic bands wrap around the body, including the thigh and pelvis, pulling against curves, rotations, and imbalances. The Bolero is the component that wraps around the torso and provides an anchoring point above the pelvis. Attached between the Pelvic Base and the Bolero, the corrective bands, the most important part of the SpineCor, cause the body to be held in the corrective movement. Some of the bands will have high tension on them and others will have virtually no tension but together they work synergistically to bring about the slow but steady stabilization or correction of the scoliosis (Coillard et al. 2010). The SpineCor system is very effective; in fact, a study performed by Coillard et al. (2010) concluded that "the SpineCor orthosis is a very effective method of treatment of juvenile idiopathic scoliosis. Most encouraging perhaps is the fact that the positive outcome appears to be maintained in the long term, and that surgery can be avoided or at least postponed." The SpineCor system was actually the 2010 SOSORT winner and in a study done for the award, 75% of SpineCor users had their curves stabilized and straightened by at least 5 degrees.

Side Effects

In comparison to surgery, bracing causes fewer complications and poses little risk from its treatment but may have an effect on the patient's daily activities and lifestyle. Some side effects include affected pulmonary function, skin complaints, and general impact on daily activities. Studies show that back bracing can affect the pulmonary function and breathing patterns. Shortness of breath is commonly experienced by patients with a brace when climbing stairs or going for a walk or run. This may be caused by the brace being so tight around the body that the lungs have little room to expand and take in air. Obviously, if the patient finds it difficult to breathe, the patient should remove the brace immediately until normal breathing returns. Braced scoliosis patients are usually taught breathing techniques to prevent a decline in lung function. Ironically, some people have found that bracing actually increases their ability to breathe. Scoliosis curvatures may cause the rotation of the spine that will push the ribs to press on the lungs. Bracing, which straightens the rotated spine, will actually decrease the pressure of the ribs on the lungs and make it easier for the patient to breathe (Lyons et al. 1999).

Another common side effect of bracing is that the patient can break out with different skin rashes, sores, blisters or other skin infections. This can be caused by the brace's constant rubbing against the skin during regular daily activities (Scoliosisadvice.com 2010).

Braces may interfere significantly with daily activities, as wearing a brace is often a full-day affair. Although doctors do give patients specific directions on when and how long to wear the brace, generally most physicians instruct the patient to wear it between 18-23 hours a day (depending also on the type of brace). The brace needs to be worn for the full number of hours prescribed by the doctor until the patient finishes growing, although it may be removed for activities such as showering, swimming, and sports. Some braces are only worn at night and do not affect the patient's day at all. Young children with braces often have an intense physical exercise program they go through to improve compliance with treatment and keep the muscles in tone so that the transition period after brace removal is easier (Blackman 2009). Ignoring all the physical effects that bracing can have on a person, there is still an enormous amount of emotional stress that a person can go through in brace treatment.

Surgical Treatment

Some curves are too severe for bracing to be effective and surgery is the only remaining option. In most cases surgery is an elective procedure and very rarely is the surgery a matter of urgency, especially in adolescent idiopathic curves. Urgent surgery is only called for when the spinal rotation is so severe that it is affecting the lungs and breathing capacity or any another organ's functionality. So what is the goal of surgery? "Scoliosis reconstruction is undertaken to improve the curve from a cosmetic standpoint as well as to prevent further progression of the curve. The purpose of scoliosis surgery is not to fully straighten a curve, but rather to obtain reasonable correction of the curve, and to restore balanced posture for the patient" (Walker 2010). In adults, surgery is often used to reduce chronic back pain and discomfort in a progressing curve.

Types of Surgery

Once surgery is agreed upon by the doctor and the patient, there are a couple of different surgical approaches that the doctor may take. The first and most common style of surgery is spinal fusion surgery. In spinal fusion there are still two subcategories, posterior

spinal fusion and anterior spinal fusion. Both use a combination of hooks, screws, wires and rods. The hooks are screwed in and attached together by wires that are anchored in place along the vertebral column. The rods are placed in the anchors and fixed there, so that when tightened or rotated they can straighten the spine. The surgeon then fuses part of the spine using bone grafts, in posterior fusion taken from the top of the pelvis or taken from a rib in the anterior fusion and spread it out along the vertebrae and joints. The bone graft pieces ossify and fuse with the spine creating one solid piece of vertebrae permanently corrected in the straightened position. Although the rods are no longer needed in the patient, removing them would require an added surgery so they are usually left in place (Dotsky and Lipp 2002).

The posterior form of spinal fusion is done through an incision that approaches the spine from the back and runs down the length of the body; a long posterior incision can go

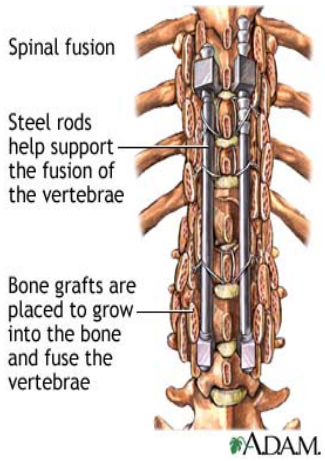

Figure 4: Harrington rods Post-op. Source: http://healthguide.howstuffworks.com/scoliosis-in-depth9.html

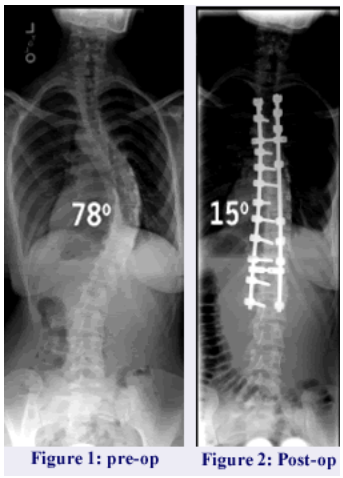

Figure 5: Pre and post-surgery X-Rays. Source:http://www.scoliosis.org/forum/showthread.php?4254-Live-Broadcast-of-Scoliosis-Surgery

from the shoulder blades down to the waist. The original posterior fusion surgeries used Harrington rods (now outdated) which worked by "distraction and compression" to straighten the curve. The issues that arose with the Harrington rods (Figure 4) are that they tended to make the entire spine abnormally flat from the side plane known as "flat back effect". It did not allow a posture where the head and pelvis were perfectly aligned and it did not account for rotational deformity (Figure 5). The more modern systems address these problems of sagittal imbalance and rotational deformities (Modi 2009).

The anterior spinal fusion method approaches the deformity from the front and in recent times has become more common. This method of surgery is a more invasive operation because it entails moving around internal organs in order to reach the spinal column. The incision runs along one side of the abdomen below the ribcage, as opposed to the posterior method, which runs along the length of the back. The anterior method is mostly used in the scoliosis cases that have a curvature in the thoracolumbar or lumbar vertebrae. The discs between the vertebrae are removed, screws are placed into the vertebral bodies, and a rod is attached to the screws like a posterior spinal fusion. The advantage of the anterior spinal fusion is that a smaller segment of the vertebrae is fused leaving more "freely movable" components of the spine. Some people are actually

candidates for an extremely complex surgery in a combination procedure involving both posterior and anterior surgeries.

The newest approach to scoliosis surgery is the thoracoscopic method. This new

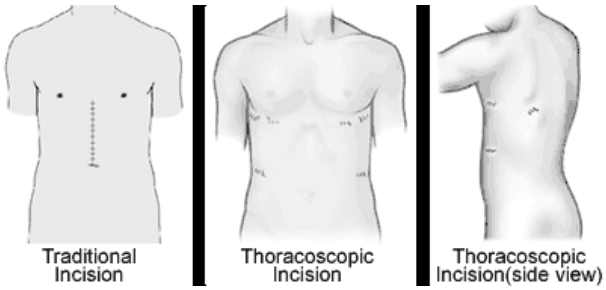

Figure 6 : Thoracoscopic Incision
Source:
http://my.clevelandclinic.org/thoracic/services/video_assisted.aspx

technologically advanced method uses cameras attached to the surgical instruments, inserted into small "peephole" incisions (replacing the long incision and inevitable scar) (Figure 6) on the side of the body by the surgeons. The cameras produce an image that is projected onto the screen in the operating room so the doctor can perform the surgery without exposing the spine. This procedure is not to be confused with thoracoplasty, which is another scoliosis related procedure. Sometimes combined with spinal fusion, this process involves the removal of 4-6 adjacent rib segments that protrude in a 'hump". This is caused by scoliosis related rotation of the rib cage, resulting in a visible protrusion of specific rib segments. This removal of rib will many times serve as the rib graft needed in posterior fusion surgery (Lyons et al. 1999)

SURGICAL SIDE EFFECTS

According to the article "Scoliosis" on the Health Scout web site, for people undergoing scoliosis surgery "complication rates are high, nearly 10%, with any surgical procedure. In fact, 7% of patients require reoperations within 5 years after surgery" (Health Scout 2009). Since the risks of surgery are manifold, people don't really know what to expect post-surgery. Risks and potential complications include the following (Lyons et al. 1999):

- allergic reactions to anesthesia
- bleeding (patients are encouraged to donate blood before the operation for use in possible transfusions)
- postoperative infection of the wound/incision site
- nerve damage (neurologic injury can occur in 1% of patients, which can lead to motor weakness and, in rare cases, paralysis)
- pseudoarthrosis (or failure of the fusion to take place)
- disc degeneration
- lower back pain
- loss of trunk mobility, balance, and muscle strength
- leg and back pain
- dislodging of hooks or broken or loosened rods
- fracture of a fused vertebra

Surgery, like every other method of treatment, has its pros and cons. Advantages of surgery include a stop to the progression, a better cosmetic outcome, and an immediate decrease in the curvature. However, there are many cons to surgery: Once fused, the "arthritis time clock" begins, recovery period is lengthy and painful, many activities become unsafe for the new spinal addition, and like any surgery, it comes with many possible post and perioperative complications.

In comparing the long-term effects of bracing and surgery, we find a landslide of a difference between the two. While bracing has little potential of long-term complications, surgery has many. In every case of spinal surgery there is an irreversible loss of the normal active range of movement in the spinal column. Furthermore, the rigid post-surgical spine puts a lot of strain on the un-fused portion of the vertebral column, which often causes post-surgical degenerative changes and may be a cause for re-operation. Severe back pain after surgery is usually an indicator that a re-operation is needed. Infections and inflammations have been known to appear as early as days to as late as eight years after a spinal surgery. The causes for these infections range from instruments being left in the body (which can be a cause for a second surgery to remove the instruments) to transmission during a blood transfusion and many other reasons in between. Although a rarity, there have been reports showing that the patients received HIV (or other diseases) from blood transfusions because of negligent surgeons. It is possible that rods or anchors can break or loosen, initiating the onset of curvature progression. Although the bulk of surgeries do not have complications that are severe enough to call for a follow up operation, nevertheless, the risks are high.

CRITERIA FOR TREATMENT

Determining the specific treatment for a patient is based on the patient's age, curve flexibility or curve magnitude. The first factor mentioned, age, is really a reference as to whether or not the patient has reached a level of skeletal maturity. Factually, scoliosis increases rapidly during general skeletal growth and most rapidly during the adolescent growth spurt (Lyons et al. 1999). Therefore, doctors must determine in younger patients how far they are from skeletal maturity. Young children, naturally, are significantly far from full skeletal maturity and are therefore almost always braced if their curves call for non-operative treatment. Adolescents must be carefully analyzed as to whether they would be a candidate for bracing based on their skeletal growth. Actual age can also be a factor when it becomes a health issue like being too old to tolerate surgery; most often, though, it is considered for younger patients.

Skeletal maturity is based on a scale called the Risser Sign named after Dr. Joseph Risser. In 1958 Dr. Joseph Risser made an important observation while looking at X-rays of the pelvis of an adolescent with scoliosis. He recognized that as the growth plate on top of the pelvis completed growing, it changed from cartilage to bone. Bone would first appear at the outer border of the growth plate and then

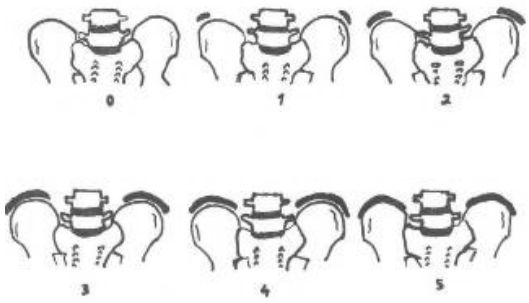

Figure 7:
Risser Signs.
Source:http://journals.tums.ac.ir/upload_files/pdf/11901.pdf

progress inwards as growth neared completion. After two years the X-ray showed that this bone had become like a "cap" on top of the pelvis. This signified that when a bone first appears a prediction can be made that an average growth of 2 years remains. The cap can then be followed like the hands of a clock from the outer aspect of the pelvis inward (Figure 7). Risser divided the appearance of this bony cap into 4 sections; when 25% of the bony cap can be seen on X-ray it is described as Risser 1, 50% is Risser 2, 75% is Risser 3and 100% is Risser 4. The final stage, Risser 5, is when the space between the cap and the pelvis fills in completely with bone (Skeletal Age 2010).

Most physicians agree that by the time "Risser 3" has occurred, the patient has passed the peak of his/her "growth spurt", a period of rapid spinal growth during which scoliosis curves can increase rapidly. To recap, during development, the iliac apophysis first appears laterally and grows medially. The stages are Risser 1 through Risser 5, where Risser 5 denotes that the apophysis has completely fused with the iliac crest and therefore skeletal maturity can be assumed (Skeletal Age 2010).

Curve magnitude is a factor that is equally, if not more, important than determining skeletal maturity. Measured in degrees, the curve magnitude combined with the Risser sign aids doctors in determining the proper treatment for patients. For curves of 20-29 degrees, some doctors will continue observation and if the curve progresses they will then recommend a brace. Bracing cannot totally fix a vertebral curve but only slow down the progression of one (Lange et al. 2009). Occasionally, bracing is used by adults with scoliosis to ward off back pain or just for straightening posture, since surgery may not be recommended for these older patients due to osteoporosis, arthritis or other health reasons. Additionally, younger patients with scoliosis whose conditions are not severe enough to require bracing will sometime turn to back bracing to reduce poor posture or to relieve muscle pain (Aulisa et al. 2010).

When determining whether a patient is a candidate for surgery, the same criteria that are used in bracing are examined, namely the age (skeletal maturity), flexibility of spine and degree of curvature. Age and skeletal maturity can affect a scoliosis surgery done at too young of an age. If a patient goes through a fusion at a young age, the vertebrae might "weld," causing it to no longer grow in length with the rest of the body. That is why surgery is reserved for older teens with progressive curvature degrees over 40 and pre-teens with even more severe curves.

CONCLUSION

In conclusion, determining the proper from of treatment for any scoliosis patient is a multistep process. First, the specific type of scoliosis must be determined by a physician and then the potential treatment options can be discussed based on factors like age and health. Next, in making a decision to begin orthotic treatment for any scoliosis case (juvenile or adolescent), a family has to consider the psychosocial effects, body image concerns and lifestyle changes that may affect the patient. Depending on all these factors, the family and physician will together decide on the proper form of brace treatment that will benefit the patient best. Not all forms of bracing can be used for any patient (although there is overlap) and not all have an equal success rate. If the curvature of the patient is too severe for bracing to be an option, there are still many different surgeries that can be performed. The family also must be aware of all of the concerns and complications that can come with any surgical procedure. Both bracing and surgical procedures in general

have positive effects on correcting the scoliosis curvature and complications are not commonly found in either.

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IS THERE A GENETIC BASIS FOR ALCOHOLISM?

David Aharonoff

ABSTRACT

This paper reviews studies done on the correlation of alcoholism and genetics. Evidence of this correlation can be seen in high heritability of alcoholism. The main methods used in determining genetic risk factors are candidate gene studies and genome wide studies. This review focuses mainly on findings related to specific neurotransmitters and receptors in relation to alcoholism. Evidence has shown that specific neurotransmitters and receptors can play a role in increased susceptibility to alcoholism. The neurotransmitters and receptors discussed in this paper include GABA, glutamate, and endogenous opioids. There is also a discussion focused on mutations of specific enzymes (ADH and ALDH) used to metabolize alcohol and its possible effects on developing alcoholism. When applicable, findings include a potential pharmacological treatment targeting the possible causation for alcoholism. Results from the studies conducted by the Collaborative Studies on Genetics of Alcoholism (COGA) have been discussed as well. COGA findings include specific chromosomes and their relationships with alcoholism, namely 1, 4 , 7, 11, and a possible indicator for increased susceptibility, or level of response (LR).

The World Health Organization (WHO) estimates that alcohol results in 2.5 million deaths each year and in 9% of all deaths between the ages of 15 and 29. The WHO further concludes that alcohol is the world's third largest risk factor for disease burden; it is the leading risk factor in the Western Pacific and the Americas, and the second largest in Europe (World Health Organization 2011). An American survey by Hasin and colleagues (2007) concludes that alcoholism affects 4-5 % of the population at any given time. As a result of these widespread ramifications, alcoholism has been a subject of much research. This paper will primarily deal with the studies of genetic influences that affect alcoholism risk. Locating a genetic link can lead to possible alcoholism prevention of those genetically susceptible and help better treat those who have already developed the problem.

DISCUSSION

In order for researchers to compare and contrast their findings, they must agree on the definition of an alcoholic. Most researchers rely on the definition of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, [DSM-IV] (APA 1994) and the World Health Organization's International Classification of Diseases (ICD-10). The Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association, fourth edition (DSM-IV) defines alcohol dependence, to be equated with alcoholism, as:

> A maladaptive pattern of substance use, leading to clinically significant impairment or distress, as manifested by three (or more) of the following, occurring at any time in the same 12-month period:
>
> 1. Tolerance, as defined by either of the following:
>
> • A need for markedly increased amounts of the substance to achieve intoxication or desired effect.
>
> • Markedly diminished effect with continued use of the same amount of substance.
>
> 2. Withdrawal, as manifested by either of the following:
>
> • The characteristic withdrawal syndrome for the substance.

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• The same (or a closely related) substance is taken to relieve or avoid withdrawal symptoms.
3. The substance is often taken in larger amounts or over a longer period than was intended.
4. There is a persistent desire or unsuccessful efforts to cut down or control substance use.
5. A great deal of time is spent in activities to obtain the substance, use the substance, or recover from its effects.
6. Important social, occupational or recreational activities are given up or reduced because of substance use.
7. The substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance (e.g., continued drinking despite recognition that an ulcer was made worse by alcohol consumption).

The ICD-10 criteria for alcoholism are similar to that of the DSM-IV (Baber 1992). A main difference is that ICD-10 doesn't include a category on alcohol abuse, but rather refers to the same diagnosis as "harmful use." The change is meant to prevent an underreporting of alcohol abuse (Baber 1992). "Harmful use" includes psychical or mental damage due to alcohol dependence (Baber 1992).

A possible indication that a genetic predisposition for alcoholism exists is the fact that an alcoholic's biological offspring are approximately three to five times more likely to develop alcoholism during their lifetime than the biological offspring of nonalcoholics (Cotton 1979). One can argue, however, that the increased rate of alcoholism in alcoholics' biological offspring is due to environmental influences; a positive family history can merely be the result of shared environmental influences. Therefore, behavioral geneticists use twin and adoption studies to identify the separate contributions of genetics and shared environmental factors.

Researchers use analyses of genetically identical monozygotic twins (MZ) and fraternal dizygotic (DZ) twins to measure the heritability of alcoholism. There are eight major twin studies of alcoholism in men, and in all but one of these studies the concordance (same trait in both members of a pair of twins) rate for alcoholism was significantly greater in MZ than DZ twins (McGue1999). For example, in a study by Kendler, Prescott, Neale, and Pedersen (1997) on 8,939 Swedish male twins born from 1902 to 1949, the MZ twin concordance rate is significantly higher than the DZ twin concordance rate. Overall, the studies' concordance rates in monozygotic twins are approximately 47.9%, and in dizygotic twins, 32.8%.

Adoption studies also allow researchers to better separate genetics from environmental influences on the development of alcoholism. Seminal research by Cloninger and colleagues (1987) uses a large-scale adoption study in Sweden to identify genetic and other variables that predict alcohol abuse in adoptees. This study, coined the Stockholm Adoption Study, was done on 862 men and 913 women adopted by non-relatives at an early age in Sweden. The study distinguishes between two forms of alcoholism: Type I is characterized as late onset of alcoholism, after age 25, and marked by frequent psychological dependence; Type II alcoholism is characterized as a relatively early onset of alcoholism, before age 25, and marked by spontaneous alcohol seeking and aggressive behavior. Analysis of data from male

adoptees shows that although Type II alcoholism has an approximate heritability of 90%, Type I alcoholism has a heritability estimate of less than 40%. Many of the key results from the original Stockholm Adoption Study were independently replicated in a second Swedish city, Gothenburg (Sigvardsson et al. 1996). The replication study was carried out on a slightly smaller group (577 men and 660 women) but concludes that the risk of Type 2 alcoholism is increased 6-fold in adopted sons with a Type 2 genetic background compared with others, while Type I alcoholism has only a 4-fold increase in adopted men with Type I genetic backgrounds of alcoholism.

Due to the evidence that alcoholism is heritable, the next step is to identify the possible genetic factors that lead to the heritability. Two basic strategies are used to identify genetic risk factors for alcoholism: candidate gene approach and genome-wide studies (Sloan et al. 2008). The candidate gene approach involves assessing the association between a particular allele (or set of alleles) of a gene that may be involved in the disease (i.e. a candidate gene) and the disease itself (Kwon and Goate 2000). This approach often begins with the knowledge of potential physiological mechanisms that might be related to the endophenotype and makes educated guesses about which of the known genes might be important (Schuckit 2000). The genes most extensively examined by candidate gene studies are those involved in alcohol (i.e. ethanol) metabolism and in neurological pathways responsible for increased risk taking and "reward" stimulation from ethanol (Sloan et al. 2008).

A genome-wide study scans all genomes of individuals and identifies genetic polymorphisms. The two main approaches to genome-wide analysis are association and linkage (Sloan et al. 2008). Association studies examine genetic polymorphisms associated with case or control status, whereas linkage studies investigate the inheritance of specific locations on a chromosome within family lines (Sloan et al. 2008). In association studies, one tests if a given allele contributes to the risk for alcohol dependence. For example, if a researcher hypothesizes that Gene A plays a role in alcoholism, he would expect this allele to differ between the case and the control subjects.

Linkage analysis scans the genome using a type of genetic variation called microsatellites. Microsatellites are nucleotide pair combinations of DNA which repeat themselves and form groupings. Microsatellites can be highly polymorphic, making them useful for comparative genetic studies of organisms. Then, the pattern of transmission of disease (e.g. alcoholism) in families with multiple affected members is compared with the pattern of transmission of certain microsatellites (Foroud et al. 2010). The principal hypothesis in linkage analysis is that alcoholics within a family share many risk alleles; therefore, genes containing alleles that increase the risk for alcoholism reside within chromosomal regions that are inherited by most or all alcoholic family members (Foroud et al. 2010).

A prime example of specific genes related to the risk of alcoholism are those that control the production of the enzymes that metabolize ethanol (i.e. alcohol). Ethanol is metabolized in the liver, where it is converted to acetaldehyde by the enzyme alcohol dehydrogenase (ADH). ADH is responsible for 80% of ethanol's metabolism to acetaldehyde (Gemma et al. 2006). About 10% of ethanol is metabolized by CYP2E1; the percentage increases when ADH is saturated (Gemma et al. 2006). Acetaldehyde is then converted to acetate by the enzyme aldehyde dehydrogenase (ALDH). High levels of acetaldehyde in the blood causes nausea, dizziness, and

headaches; therefore, variation in the genes coding for the ADH and ALDH enzymes are expected to be associated with alcoholism risk (Edenberg 2007).

There are at least six isoenzymes of ALDH (Schuckit 2000). ALDH2*2, an isoenzyme of ALDH, involves a point mutation that results in the exchange of the amino acid glutamate at position 487 of the ALDH protein for the amino acid lysine (Edenberg 2007; Foroud et al. 2010). This mutation acts in a nearly dominant manner to render the enzyme almost inactive (Foroud et al. 2010). As a result, homozygous carriers of ALDH2-2 exhibit highly elevated levels of acetaldehyde, which produces aversive reactions, including flushing, tachycardia and nausea after consuming even a small amount of alcohol (Eng et al. 2007). Studies have shown that ALDH2-2 influences a person's drinking levels, and, consequently, the risk of developing alcohol abuse or dependence (Edenberg 2007; Hurley et al. 2002). There are almost no documented cases of homozygous carries of ALDH2-2 being diagnosed as alcoholics (Luczak et al. 2006). Even people who are heterozygous for this mutation produce an ALDH enzyme with extremely low enzyme activity (Crabb et al. 1989).

Approximately 10% of Asian men and women (e.g. Japanese, Chinese, and Koreans) are homozygous for the mutation of ALDH2*2, although it is not known to be found in any other racial group (Wall and Ehlers 1995). An additional 40% of Asians are heterozygotes for the ALDH mutated enzyme (Wall and Ehlers 1995). Compared with a Chinese man carrying two active ALDH alleles, the odds ratio of an alcoholism risk for a Chinese man carrying one inactive ALDH2-2 allele is 0.33 (Edenberg 2007). Furthermore, although heterozygote ALDH2-2 carriers represent almost half of the general population in their countries, they comprise less than 10% of Asian alcoholics, further supporting the conclusion that even heterozygotes have a relative protection from alcoholism (Murayama et al. 1998).

There are also coding variations in the ADH gene, called ADH2-2 and ADH2-3, which encode highly active enzymes which increase the production rate of acetaldehyde (Li 2000). These variations also reduce the risk for alcohol dependence (Edenberg 2007; Thomasson et al. 1991). For example, a pilot study by Neumark and colleagues (1998) was the first example of association between alcohol consumption patterns and a polymorphism at the ADH2 locus in a Jewish population. The study compares ADH loci in 92 non-drinkers (control) versus 53 heavy drinkers (case). Neumark furthered the study in 2004 by testing the effect of ADH2-2 on alcohol-elimination rates (AER) under experimental conditions. The study is based on the above-cited hypothesis that ADH2-2 increases the rate at which alcohol is metabolized, thereby increasing the production rate of acetaldehyde and producing aversive reactions. The study confirms that the rate of alcohol elimination is significantly associated with the *ADH2* genotype of Jewish males (Neumark 2004).

The evidence that an increase of acetaldehyde can be a deterrent to the development of alcohol abuse has led researchers to develop pharmacological treatments. Disulfiram blocks the enzyme aldehyde dehydrogenase, leading to an accumulation of acetaldehyde following an intake of alcohol (Heilig and Egli 2006). The accumulation of acetaldehyde then causes flushing, shortness of breath, tachycardia and other unpleasant symptoms. The point of disulfiram is not that patients actually experience adverse symptoms, but that that the anticipation of these symptoms helps patients abstain from consuming alcohol (Heilig and Egli 2006). The logic is that an accumulation of acetaldehyde poses a possible medical risk. However,

disulfiram has a limited and largely negative documentation for efficacy (Heilig and Egli 2006). For example, a controlled multisite study of disulfiram treatment of alcoholism in 605 men concludes that although disulfiram may help reduce drinking frequency after relapse, it does not reduce the probability of abstinence from alcohol (Fuller et al. 1986).

Researchers have also done candidate gene studies on genes for receptors in the neurotransmitter gamma-aminobutyric acid (GABA), opioid receptor and components of the pathways for the neurotransmitters serotonin, dopamine, and glutamate (Sloan et al. 2008). A full discussion of each neurotransmitter surpasses the scope of this paper; therefore, but a few neurotransmitters and receptors and their relation to alcoholism will be discussed.

There is evidence supporting a link between the endogenous opioid system and excessive alcohol consumption (Gianoulakis 2001; Herz 1997). This evidence, linking the endogenous opioid system to alcoholism, led to several theories regarding the possible nature of an opioid abnormality in this disorder (Oswald and Wand 2004). The Opioid Deficit Hypothesis postulates that physiological cravings for alcohol may be the result of a deficiency of naturally occurring opiate like substances (Trachtenberg and Blum 1987). Conversely, the Opioid Surfeit Hypothesis maintains that excess (i.e. surfeits), not deficits, in opioidergic activity increases one's propensity to consume alcoholic beverages (Reid et al. 1991).

The first study to suggest a possible link between endogenous opioid and ethanol was conducted by Davis and Walsh (1970), who discovered that morphine-like alkaloids (tetrahydroisoquinolones) are formed in vivo as a result of the interaction between ethanol metabolite, acetaldehyde, and certain metabolites of dopamine. Due to Davis and Walsh's findings, further research sought to demonstrate that these alkaloids could bind to opioid receptors and produce opioid-like effects (Blum et al. 1978; Fertel et al. 1980). However, the pharmacological relevance of these compounds in opioidergic processes remains unclear because their concentrations in brain tissues is extremely low (Oswald and Wand 2004).

The best evidence of the linkage between endogenous opioid systems and ethanol consumption is that pharmacological blockades of opioid receptors reduce alcohol drinking in a dose-related fashion. Naltrexone is an opioid receptor antagonist that reduces alcohol cravings and relapses to heavy drinking, but it does not necessarily produce total abstinence. The initial study done by Altshuler and colleagues (1980) reports on the dose-related effect of naltrexone on decreasing ethanol drinking in 10 of 21 rhesus monkeys who were willing to self-administer alcohol. The treatment results were replicated by O'Malley et al. in 1992. In a 12-week, double-blind, placebo-controlled trial conducted on seventy male alcohol-dependent patients, Volpicelli and colleagues (1992) found that only 23% of alcoholic subjects who were administered naltrexone relapsed to heavy drinking, compared with 54.3% of subjects taking a placebo. Based, in part, on the findings of these studies, naltrexone was approved by the Food and Drug administration (FDA) in 1995 for the treatment of ethanol dependence. Since naltrexone's approval for the treatment of alcoholism, the opioid antagonist has been tested in 29 controlled clinical trials in unselected participants with alcoholism (Pettinati et al. 2006). The study concludes that "19 (70%) of 27 clinical trials that measured reductions in 'heavy or excessive drinking' demonstrated an advantage for prescribing naltrexone over placebo, whereas

only 9 (36%) of 25 clinical trials that measured abstinence or 'any drinking' found an advantage for medication over placebo" (Pettinati et al. 2006).

The effects of naltrexone have led to candidate gene studies on opioid receptors. There are three types of opioid receptors (mu, delta and kappa) that represent respective targets of the major opioid peptides (Herz 1997). Naltrexone acts as an antagonist at opioid receptors, with a relative selectivity for the μ-opioid receptor (Oswald and Wand 2004). There are more than 25 identified allelic variants of the gene that codes for the μ-opioid receptor (O'Brien 2008). In a human laboratory study (Ray and Hutchison 2004), volunteers with Asp40 allele (*OPRM1*) reported greater subjective euphoria at a given ethanol blood level. In a more recent study of heavy drinkers, naltrexone attenuated the increased alcohol stimulation in those carrying the Asp40 allele (Ray and Hutchison 2007).

Additionally, GABAergic mechanisms mediate many of the behavioral effects of ethanol, including its anticonvulsant, sedative-hypnotic, cognitive-impairing, and fine motor skill damaging effects (Kumar et al. 2009). The two classes of GABA receptors are GABA-A and GABA-B. GABA-A receptors are ligand-gated ion channels (i.e. ionotropic) that confer fast synaptic inhibition (Enoch 2007). GABA-A receptors undergo allosteric modulation by several structurally unrelated drugs, most with their own binding sites, including ethanol and benzodiazepines (Enoch 2007). Initial evidence that alcoholism is related to GABA-A receptors is because benzodiazepines show effectiveness in treating alcohol withdrawal (Enoch 2007).

GABA-A receptors are composed of five subunits (Sigel et al. 2006). Most receptors consist of two α (alpha), two β (beta), and one γ (gamma) subunits (Sigel et al. 2006). The GABA-A receptor ion channel is lined by the transmembrane (TM) segments from each of the five subunits that form the receptor (Enoch 2007). Specific mutations in transmembrane domains 2 and 3 of the alpha subunit can abolish or markedly reduce the effects of ethanol on these receptors without necessarily affecting receptor function; therefore, alcohols may bind in a cavity located between TM2 and TM3 (Mascia et al. 2000).

Topiramate, which has a complex effect on GABA-A receptors, is hypothesized to decrease alcohol reinforcement and the propensity to drink (Johnson et al. 2007). A double-blind, randomized, placebo-controlled, 14-week trial of 371 men and women concludes that "topiramate was significantly more efficacious than placebo at reducing the percentage of heavy drinking days," and "topiramate is a safe and consistently efficacious medication for treating alcohol dependence"(Johnson et al. 2007).

GABA inhibits hypothalamic-pituitary–adrenal axis responses to stress whereas glutamate activates the response (Herman et al. 2004). Prime targets of ethanol are the *N*-methyl-D-aspartate (NDMA) receptors in the glutamate system (Spanagel 2009). NMDA is an amino acid derivative that acts as a specific agonist at the NMDA receptor, mimicking the action of glutamate. Acute alcohol exposure inhibits the excitatory action of glutamate at the N-methyl-d-aspartate (NMDA) receptor, whereas chronic alcoholism results in increased NMDA receptor expression so that abrupt withdrawal produces a hyperexcitable state that leads to seizures (Guochuan and Coyle 1998). These finding have led to the formulation of the glutamate homeostasis hypothesis of addiction which suggests that enhanced

glutamate-mediated neuronal excitability during withdrawal and prolonged abstinence contributes to craving and relapse (Kalivas 2009).

Further, "Metabotropic glutamate (mGlu) receptors, which include mGlu1–8 receptors, are a heterogeneous family of G-protein-coupled receptors which function to modulate brain excitability via presynaptic, postsynaptic and glial mechanisms" (Schoepp 2001). In contrast to ionotropic receptors, metabotropic receptors influence the activity of a cell indirectly by first initiating a metabolic change in the cell. Studies provide evidence that activation of group II mGluRs (predominantly presynaptical) by a selective mGlu 2/3 agonist (LY379268) dose dependently blocks the effects of both stress and drug related environmental stimuli on the recovery of extinguished ethanol-seeking behavior (Zhao et al. 2006). A similar study on group I mGluRs (predominantly located postsynaptically) in alcohol-preferring P-rats, a well-defined genetic model of excessive alcohol consumption, shows that infusion of the mGluR5 antagonist 2-methyl-6(phenylethynyl) pyridine (MPEP) in the nucleus accumbens reduces ethanol-reinforced responding (Besheer et al. 2010). Besheer et al. (2010) concludes that the data "confirms the importance of mGluR5 activity in the nucleus accumbens in regulating drug reinforcement and emphasizes the potential therapeutic utility of targeting this receptor system in individuals with genetic risk for excessive drinking."

The interaction of ethanol and glutamate led to candidate gene studies on glutamate. An association study that tested the candidate gene hypothesis of allelic variants of the ionotropic glutamatergic N-methyl-D-aspartate receptor (NMDAR) provides evidence that they are associated with vulnerability to alcoholism (Wernicke et al. 2003). Wernicke and colleagues studied variants of the ionotropic glutamatergic *N*-methyl-d-aspartate receptor (NMDAR the silent G2108A and C2664T polymorphisms of the NMDAR1 and the NMDAR2B genes), in exon 5 of the EAAT2 gene in 702 subjects of German descent, comprising 367 alcohol-dependent subjects and 335 control subjects. Genotype frequencies of the NMDAR1 polymorphism differed significantly between control and alcoholic subjects and the NMDAR2B polymorphism revealed a significantly reduced T allele in Cloninger type 2 alcoholics and in patients reporting an early onset compared with control subjects.

In 1989, the National Institute on Alcohol Abuse and Alcoholism initiated the Collaborative Study on the Genetics of Alcoholism (COGA), a large, systematic effort to identify the genes that predispose to alcoholism. COGA's goal is to elucidate the genetic mechanisms that contribute to a person's susceptibility to alcohol abuse and dependence (Begleiter et al. 1995). COGA generated a dataset of 1,857 families consisting of 16,062 individuals as of March 2010 (Foroud et al. 2010). Due to the genetic complexity of alcoholism, COGA researchers chose an unbiased survey of the entire genome (i.e. genotyping) (Edenberg 2002). Using microsatellite markers, more than 1.2 million genotypes were generated on 2,310 people from families of alcoholics and 1,238 people from control families (Edenberg 2002). This information enables researches to monitor the inheritance pattern of marker alleles within families with alcoholics and therefore helps identify chromosomal regions that show genetic linkage with alcoholic related traits (Edenberg 2002). To be categorized as a "family of alcoholics" the family must comprise at least three first-degree relatives who met lifetime criteria for *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised* (DSM–III–R) (Foroud et al. 2010).

COGA data collected from families with alcoholism are used for both linkage and association analyses (Sloan et al. 2008). In 1998, COGA researchers published their initial linkage findings based on an analysis of 291 markers in 987 individuals from 105 families (Reich et al. 1998). Substantial evidence points to a susceptibility loci for alcohol dependence on chromosomes 1 and 7. In addition, there is suggestive evidence for a protective locus on chromosome 4 near the ADH gene (Reich et al. 1998). At the same time as the COGA findings were published, a second large-scale linkage study of alcoholism, based on a sample of Native Americans, reported suggestive evidence of the existence of a predisposing gene on Chromosome 11p, in close proximity to the DRD4 dopamine receptor (Long et al 1998). Evidence also suggests a protective gene on Chromosome 4p, near the beta1 GABA receptor gene (Long et al. 1998). Although only the Chromosome 4 findings overlapped, the two studies sampled different ethnic groups, and the genes that underlie alcoholism risk might be expected to vary for groups having distinct evolutionary history (McGue 1999). A more recent COGA study by Yang and colleagues (2005) found potential candidate regions on chromosomes 1, 2, and 7 linked with alcoholism susceptibility. Overall, Yang viewed his findings as consistent with Reich's findings (Yang et al. 2005).

Due to prior evidence for the role of the *ADH* genes in alcoholism susceptibility, the COGA investigators did several studies to determine the specific nature of the link (Foroud et al. 2010). In one particular study, COGA researches genotyped 110 DNA markers known as single nucleotide polymorphisms (SNPs) across the seven ADH genes located on chromosome 4q and analyzed their association with alcoholism in a set of families (n= 262) with multiple alcoholic members (Edenberg et al. 2006). The analyses show strong evidence that 12 SNPs located in and around the ADH4 gene are significantly associated with alcoholism (Edenberg et al. 2006).

Evidence of a molecular interaction between ethanol and GABA receptors led COGA researchers to study the link between variation in GABA receptors and alcoholism. Edenberg and colleagues (2004) performed a linkage analyses of 69 single-nucleotide polymorphisms (SNPs) within a cluster of four GABA (A) receptor genes located in chromosome 4p: GABRG1, GABRA2, GABRA4, and GABRB1. The analyses found thirty-one SNPs in *GABRA2* associated with alcohol dependence. Edenberg concludes his study by stating, " the convergence of evidence from different analyses and phenotypes, along with the biological data on its function, provides strong evidence that GABRA2 is a key gene affecting the risk for alcoholism." Interestingly, the GABRA2 is part of the GABA-A α subunit that was mentioned before as a possible binding spot for alcohol.

The genome-wide association studies (GWAS), also known as the whole genome association studies, are examinations of all or most of the genes (the genome) of different individuals of a particular species to see how much the genes vary from individual to individual. Different variations are then associated with different traits, such as diseases. In humans, hundreds or thousands of individuals are tested for single-nucleotide polymorphisms (SNPs). The advantage of GWAS is that it allows a comprehensive test for associations across the genome, rather than testing only one gene at a time.

The genome-wide association studies (GWAS) examine up to a millions SNPs through the human genome in a single experiment (Foroud 2010). The different variations of SNPs between individuals is then associated with different traits, such as diseases. An advantage of GWAS is its hypothesis-free strategy and its suitability for the discovery of novel genetic contributors to disease (Bierut et al. 2010).

This first genome-wide significant association study in alcohol dependence was published in 2009 (Treutlein et al. 2009). The GWAS included 487 male inpatients with alcohol dependence as defined by the *DSM-IV* and 1,358 population-based control individuals (Treutlein et al. 2009). The GWAS tested 524 396 single-nucleotide polymorphisms (SNPs) (Treutlein et al. 2009). The analyses found two closely linked SNPs located on chromosome 2 (Treutlein et al. 2009). However, a recent GWAS of 1,897 European-American and African-American subjects with alcohol dependence compared with 1,932 unrelated, alcohol-exposed, nondependent controls did not replicate the finding reported by Treutlein and colleagues (Bierut et al. 2010). Interestingly, Bierut's GWAS found SNPs genotyped in GABRA2 that overlap with SNPs reported by Edenberg et al. (2004).

The level of reaction to alcohol has been a useful tool to indicate a possible increase in susceptibility to alcoholism. Studies have found a correlation between level of response (LR) to alcohol and the risk of developing alcohol dependency (Schuckit 1999). Numerous alcoholics have reported an ability to consume large amounts of alcohol with relatively little effect from early in their drinking careers (Schuckit 1999). A low LR is usually evaluated by giving alcohol to individuals and determining the intensity of response at a given blood alcohol concentration (BAC), or by indirectly measured through a self-report of a history of a higher number of drinks required to produce a specific effect (Schuckit 2000). A study of 453 sons of alcoholics and controls as conducted over a period of 15 years demonstrated that a low LR was a significant predictor of later alcoholism (Schuckit and Smith 1996).

There are several indications that LR is genetically influenced. For example, a study on 3,810 adult twin pairs reported a higher level of similarity on some aspects of LR in identical twins than in fraternal twins, with an estimated heritability between 40 and 60 percent (Martin 1988). A pilot study (Mazzanti et al. 1999) evaluated 17 men with low LR scores, comparing results to 24 individuals who were clearly higher on the LR scale. The high LR and low LR groups were then evaluated for the patterns of 5 candidate genes relating to serotonin and gamma-aminobutyric acid functions. The 14 men with the LL genotype of the serotonin transporter (5-HTT) polymorphism and the seven with the genotype of the GABAA alpha 6 polymorphism had demonstrated lower LR scores at about age 20, and had significantly higher proportions of alcoholics than the other genotypes for those loci. These studies show that low LR can be a potential indicator to a genetic risk of alcoholism.

Conclusion

In conclusion, evidence has shown that there exists a genetic susceptibility to alcoholism. However, it is clear that there are environmental factors, possible as high as 50% (Dick and Beirut 2006), that can attribute to alcoholism. The main focus of this paper is to show that the genetic component of alcoholism exists as well, though it is clear that alcoholism is a complex disease that involves various mechanisms and pathways. Susceptibility to alcoholism cannot be attributed to one single gene,

neurotransmitter, or receptor. Other possible neurotransmitters that haven't been discussed but yet can play a vital role are serotonin and dopamine (DRD2). This paper is only a brief overview of possible genetic contributions to alcoholism. There is much more research that has been done and more that will be done in the near future.

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HEALTH RISKS OF VERY LOW CHOLESTEROL

Menachem Nagar

ABSTRACT

Cholesterol is a molecule central to all human physiological processes at systemic as well as cellular levels. Cholesterol, combined with Apolipoprotein B as low-density lipoprotein (LDL) has been the focus of scientific research because the molecule has been proven to play a role in the development of cardiovascular disease, a disease of pandemic proportions. Considerable scientific and medical attention has been devoted to identifying the role and management of high levels of total serum cholesterol in order to address this global health burden, creating large scale awareness regarding lowering cholesterol concentration in circulation. However, the same molecule, combined into various lipoprotein moieties, is also involved in 'normal' physiological processes. In this review, an attempt has been made to elucidate some of the physiological events at the other end of the spectrum, when serum cholesterol levels are lower than normal. These health risks may need to be managed by going against the grain and actually raising serum cholesterol levels. Hypocholesterolemia is perhaps as much of a health risk as is hypercholesterolemia. These phenomena emphasize the fact that where cholesterol is concerned, maintenance of optimal systemic levels of cholesterol is more crucial than going towards either end of the cholesterol scale.

INTRODUCTION

Cholesterol has been in the center of many debates concerning its role in the human metabolism. Cholesterol is considered as the main culprit behind modern lifestyle diseases like atherosclerosis, which leads to cardiac arrest, obesity and metabolic syndrome. Although implicated as a causative agent of all of the above, cholesterol is also an important component of cellular membranes and is a mediator of several cellular processes. Cellular membranes owe their fluidity to the presence of cholesterol and lack of cholesterol is likely to have an impact on several physiological processes. Cholesterol is an important precursor for synthesis of steroid sex hormones (Payne and Hales 2004) as well as vitamin D (Lehmann et al. 2000). Cholesterol is an important constituent of cell membranes and, being an amphipathic molecule, it interacts with both lipids in the plane of the membrane bi-layer, as well as with water and other electrolytic substances owing to the presence of an –OH group. Cholesterol is thought to play an important role in maintaining membrane fluidity as well as participate in transient membrane specializations called membrane microdomains or 'rafts' (Sharma et al. 2004).

The role of LDL in the development of cardiovascular disease (CVD), when present in excess in human circulation, has been discussed at length (Stein 2009). The consensus is that very high levels of serum LDL-cholesterol and triglycerides are the causative factors of atherosclerotic plaques, which can eventually cause blockage of cardiac arteries leading to a heart attack. There have been extensive studies on the interrelationships between diet, obesity, serum cholesterol levels and CVD as well as other co-morbidities like diabetes mellitus (Daniels et al. 2009), leading to the formulation of a general assumption that one must slay the cholesterol beast in order to live a disease-free life. However, cholesterol is also an essential component of biological tissues and is also specifically required for several cellular processes.

Therefore, the issue that begs consideration here is "How low can one go with cholesterol?" Given the importance of having an optimal amount of cholesterol present

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in the human system, it is likely that extremely low levels of cholesterol are likely to bring in another set of health morbidities and risk. This review attempts to understand health risks at the other end of the cholesterol range by looking at cholesterol deficiency disorders. Physiological deficits in cholesterol levels can result from a variety of causes and the effects of these deficiencies on various health parameters are discussed in this review.

CHOLESTEROL METABOLISM

Cholesterol can be synthesized by all tissues in the human body, barring enucleated erythrocytes. In addition to synthesis of cholesterol from acetate, cholesterol is also absorbed from ingested food in the form of chylomicrons. Chylomicrons are a class of lipoproteins that are produced by intestinal mucosal cells, the principal component of which is a small protein apoB-48, produced by alternative splicing of mRNA from the apoB gene. Chylomicrons are generated in the intestinal cells after the absorption of free fatty acids and cholesterol and are secreted by the intestinal cells into the lacteals and are redistributed to other organs and peripheral tissues. Chylomicrons are capable of exchanging apolipoproteins with high-density lipoproteins (HDL) to generate mature chylomicrons. Cholesterol from peripheral tissues is exported in the form of HDL, which is brought to the liver via the vascular system. HDL-associated cholesterol and phospholipids are degraded in the liver. Cholesterol breakdown products are excreted in bile as bile salts, which, after storage in the gall bladder, are secreted into the digestive system. Bile salts help in emulsification of dietary fats until they are absorbed in the small intestine.

The liver is also the site of cholesterol synthesis and hepatic cholesterol is secreted into the vascular system in the form of very low-density lipoproteins (VLDL). In the plasma, VLDLs coalesce to form low-density lipoprotein (LDL) particles.

Cholesterol deficiencies can be classified into

a) Inborn errors in cholesterol synthesis or apolipoprotein synthesis
b) Clinically observed hypocholesterolemia of undetermined origin such as in HIV-positive patients (Miguez et al. 2010; Shor-Posner et al. 1993)
c) Cholesterol deficiency induced by surgery and critical illness

THE ROLE OF HDL IN CARDIOVASCULAR DISEASE

Cholesterol is present in serum in three forms: very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL), with each form serving a distinct physiological function. Very low-density lipoprotein is the form that is created by processing of HDL and LDL by the liver into smaller particles meant for excretion. LDL has been implicated as a causative factor principally in atherosclerosis especially when plasma concentrations of LDL are high (Daniels et al. 2009). HDL, on the other hand, functions to transport cholesterol from peripheral tissues to the liver for degradation (Podrez 2010). HDL is chiefly composed of apolipoproteins A I and II (apoAI and apoAII) with apolipoproteins C, D, E, M and A-IV(apoC, apoD, apoE, apoM, apoA-IV) and other enzymes forming the protein components of the particles. Cholesterol is the main lipid component but other phospholipids as well as free fatty acids have also been reported to be included in HDL.

A sum total of 75 proteins have been found to be included in HDL in proteomic studies. These findings suggest that HDL may exist in several distinct forms engaged

in several functions apart from transporting cholesterol to the liver (reviewed in Podrez 2010). In fact, HDL plays a prominent role as an anti-oxidant molecule by preventing oxidation of LDL and acting as a 'sink' for oxidized lipids from LDL (Podrez 2010; Terasaka et al. 2007). The role of HDL as an antioxidant is thought to help in preventing the formation of dysfunctional LDL molecules that may play a role in initiating atherosclerotic plaques (Terasaka et al. 2007). HDL is thought to have a cardioprotective role and help prevent cardiovascular disease. Low HDL is considered as a positive predictor for cardiovascular disease (Schaefer et al. 2010). Patients with mutated apoA-I show normal levels of LDL and serum triglycerides and yet are at greater risk of developing coronary heart disease (Schaefer et al. 2010).

The link between circulating levels of HDL and coronary heart disease has been demonstrated by other researchers as well. In a 16-year follow-up study with 3,026 patients, treatment with bezafibrate reduced the risk of coronary heart disease and death by myocardial infarction by 22% as compared with patients who had been prescribed a placebo (Goldenberg et al. 2009). In this study, patients who showed a greater increase in serum HDL levels in response to bezafibrate were at lesser risk for developing coronary heart disease. The study vindicates the role of HDL in preventing atherosclerosis as well as coronary heart disease. In a Finnish population study of 148 people who presented with low HDL levels, a reduction in HDL particle size was positively correlated with increased carotid intima-media thickness (Watanabe et al. 2006). Increase in the thickness of carotid intima and media, as assessed by ultrasonography, is considered as a reliable marker for atherosclerosis. In this study, people with low serum concentrations of HDL also showed a significant reduction in the HDL particle size and were consequently deemed to be at greater risk for development of coronary heart disease.

Given the diversity of proteins and lipids associated with HDL in addition to cholesterol (Podrez 2010), heterogeneity in size and diversity of functions of HDL is to be expected. In a study that sought to understand the relationship between HDL particle size and coronary heart disease, Arsenault and colleagues found that smaller HDL particle size (which can possibly be attributed to deficiencies in proteins as well as associated cholesterol and other lipids) (Arsenault et al. 2009) was positively correlated with increased risk for coronary heart disease. Patients with smaller sized HDL particles also had greater co-incidence of other unfavorable cardiometabolic factors like elevated waist circumference and higher systolic blood pressure and elevated serum triglyceride levels. The study does indicate that predominance of HDL and large sized HDL particles are indicators of cardiovascular risk, however; analysis of the specific contribution of serum cholesterol was not carried out in this context.

The predominant question that arises from the previous discussion is whether HDL particle size is regulated by cholesterol levels or not. HDL particle size analysis was undertaken in a study of people who were heterozygous for Tangier disease (TD) (Brousseau et al. 2000). Tangier disease is characterized by the presence of orange tonsils, very low levels of HDL and enlarged spleen and liver. The essential mutation in Tangier disease is one that causes a dysfunctional ATP-binding cassette transporter protein ABC1 on chromosomes 9q31. This transporter protein regulates the efflux of cholesterol from peripheral tissues and allows it to complex with apoA-I and apoA-II to form HDL. In patients heterozygous for Tangier disease mutation, HDL particle size was significantly smaller than that seen in controls. Efflux of cholesterol from TD-

heterozygote cells was reduced by 50 % to that of control cells and this reduction in serum cholesterol correlated positively with serum HDL concentration as well as HDL particle size (Brousseau et al. 2000). Cholesterol efflux may also influence the HDL-subtype distribution in Tangier disease heterozygotes (Asztalos et al. 2001). HDL subpopulations can be characterized on the basis of size as well as the predominant apolipoproteins associated with them. In a study of people heterozygous for Tangier disease as confirmed by low HDL levels, the prevalence of large alpha1, pre-alpha1, alpha 2 and pre-alpha2 particles was significantly lower as compared to healthy control subjects. The loss of these specific HDL subpopulations may be the result of reduced cholesterol efflux mediated by the Tangier mutation in the ABC-1 gene (Asztalos et al. 2001). Concurrently, the incidence of coronary heart disease was also found to be greater by 60%, in the heterozygote group as compared to unaffected healthy subjects. Therefore, cholesterol deficiency is likely to influence the particle size as well as the specific nature of HDL particles; it is likely that very low levels of cholesterol in the serum may actually contribute to increasing the risk of coronary heart disease. Although this sounds contradictory to conventional wisdom that cholesterol in the form of LDL IS the 'seed' molecule for formation of atherosclerotic lesions, the lack of HDL particles of appropriate size and functional properties is a serious concern for people with very low levels of serum cholesterol.

CHOLESTEROL AND NUTRITION

Lipoproteins are involved in the transport of vitamin E from the digestive system as well as from the liver to the peripheral tissues. Vitamin E or alpha-tocopherol is known to bind to HDL, specifically HDL-3 (Goti et al. 1998). HDL-3 bound tocopherol can be taken up by hepatocytes (in in vitro studies) and is secreted out in the form of LDL by hepatocytes. Thus both metabolic forms of cholesterol in the form of lipoproteins are involved in the systemic transport of vitamin E.

Cholesterol is known to participate in transient membrane specializations termed as membrane microdomains, along with sphingolipids (Sharma et al. 2002). These microdomains are formed laterally in the plane of the plasma membrane and are especially important for the endocytosis of the folate receptor. Depletion of cellular cholesterol, in in vitro systems has been shown to alter the endocytic pathway (Sabharanjak et al. 2002), recycling time as well as intracellular endosomal destination of the folate receptor (Sabharanjak and Mayor 2004). The overall efficiency of accumulating folic acid in the cytoplasm is also reduced. Although it is unclear whether physiological depletion of cholesterol either by suppression of synthesis or by reduced dietary intake results in similar disruption of microdomains, the role of cholesterol in uptake of water-soluble vitamins is also important.

DEFICIENCIES IN CHOLESTEROL BIOSYNTHESIS

Cholesterol in circulation can be derived from dietary components as well as from de novo synthesis. Cholesterol is synthesized from hydroxymethylglutaryl co-A (HMGco-A) in a series of steps with intermediates like squalene, lanosterol and lathosterol. Cholesterol thus synthesized may also get converted into steroid hormones like testosterone. Incidentally, low testosterone levels in men are also correlated with increased risk of coronary heart disease and show a positive correlation with low levels of HDL as well (Nettleship et al. 2009).

In the biosynthesis of cholesterol, lanosterol is converted via seven steps to lathosterol, which in turn in converted into 7-dehydrocholesterol by the action of lathosterol-5-desaturase. Mutations in this enzyme lead to the accumulation of lathosterol instead of cholesterol, a defect known as lathosterolosis. This defect has been replicated in a mouse model for lathosterolosis. Likewise, the final step in biosynthesis is the conversion of 7-dehydrocholesterol to cholesterol catalyzed by 7-dehydrocholesterol reductase. A genetic mutation of this enzyme has been described as the Smith-Lemli-Opitz syndrome (SLOS), which is characterized by severe developmental abnormalities including mental aberrations (Gondre-Lewis et al. 2006).

Both these defects have been established in mouse models described by Y Peng Loh and colleagues (Gondre-Lewis et al. 2006). In order to examine the effect of abnormal sterol accumulation in membrane trafficking events, these scientists characterized and quantified the occurrence of dense core secretory granules in exocrine pancreatic tissue as well as neuroendocrine cells. In *Sc5d-/-* as well as *Dhcr7-/-* mice, the number of secretory granules in pancreatic tissues was significantly lower than that found in wild type or heterozygous mice. (Gondre-Lewis et al. 2006). Additionally, the fission of dense core granules from the endoplasmic reticulum was seen to be impaired in *Dhcr7-/-* mice, suggesting that lack of cholesterol had interfered with formation of dense core granules at the Golgi. The proportion of immature granules lacking the dense core was higher in the mutant homozygous mice as compared to wild type mice. The accumulation of sterols other than cholesterol is likely to hinder secretory processes. Although alpha-amylase was produced by normal as well as homozygous mutant mice with both genetic defects, the enzyme was found to be constitutively secreted in *Dhcr7-/-* mice, indicating that cholesterol in the granule membrane as well as plasma membrane of pancreatic acinar cells is essential for regulated secretion of digestive enzymes.

This study highlights the importance of having the right sterol in the right cellular membranes. The mutant phenotype can be rescued by culturing cells from these mice in normal LDL-containing serum (or by dietary supplementation in the live animal), which vindicates the requirements of cholesterol in cellular membranes in regulated membrane trafficking events.

Although the lipoprotein measurements in the mouse models have not been reported in this study, HDL deficiency has been shown to be associated with SLOS in children (Behulova et al. 2000). In this study, serum lipoprotein levels were recorded in children suffering from SLOS, ranging from five days to seven years of age. Total cholesterol levels were certainly depressed in these children as compared to normal children but the authors note that the severest symptoms of SLOS were exhibited by children with significantly depressed levels of HDL and HDL-bound cholesterol (Behulova et al. 2000). Developmental abnormalities resulting from cholesterol deficiency in this syndrome usually mean that children are not able to attain adulthood. Heterozygous individuals show normal development owing to the presence of the normal DHCR7 allele.

HYPERCHOLESTEROLEMIA AND SURGICAL AND CRITICALLY ILL PATIENTS

In order to understand hypocholesterolemia, 'normal' levels of cholesterol have to be defined. From studies of primates as well as 'primitive' humans like tribals, the

optimal serum concentrations of total cholesterol was found to be 3.0 to 4.0 mmol/l. Plasma total cholesterol levels ranging from 2.6 to 3.5 mmol/l have been considered as low cholesterol levels in certain conditions (Vyroubal et al. 2008). However, these values show a large overlapping range. In another study, the plasma cholesterol concentration of patients undergoing hepatectomy was judged relative to their own total cholesterol estimations before and after surgery (Giovannini et al. 1999). This study shows that patients whose total plasma cholesterol levels were reduced to 55% of the pre-operative values were judged as hypocholesterolemic and tended to develop fatal health complications.

In some papers, patients with cholesterol levels <150 mg/dl have been considered as being hypocholesterolemic (Miguez et al. 2010; Shor-Posner et al. 1993).

Cholesterol levels may become a useful diagnostic tool in the prognosis of critically ill or surgical patients in the recovery period. Dunham and colleagues have demonstrated an association between the hypocholesterolemic state and likelihood of recovery in patients who have suffered severe injuries (Dunham et al. 2003). In their study of 28 patients maintained on ventilator support, reduction in plasma cholesterol levels was observed in the beginning of the convalescence period. Those patients for whom cholesterol levels rose to the normal population average were able to recover from the trauma and were at reduced risk for sepsis. In contrast, patients with persistent hypocholesterolemia tended to develop secondary infections as well as organ failure and metabolic dysfunction (Dunham et al. 2003). The association between development of sepsis and reduced levels of cholesterol is attributed to the fact that lipoprotein complexes are able to sequester bacterial lipopolysaccharides and prevent toxicemia. Reduced levels of serum lipoproteins are therefore a predisposition towards developing secondary bacterial infections.

Although this study is limited by the number of patients included in the study as well as lack of differentiation between the kinds of lipoproteins (LDL or HDL) involved, it is nonetheless an eye-opener for critical care procedures. A study with animal models wherein sepsis had been induced by irradiation showed that HDL is persistently lowered (for up to 10 days in the recovery period) in individuals who developed sepsis following irradiation (Parra et al. 2007).

Hypocholesterolemia has been shown to be associated with postoperative surgical complications as well. Leardi and colleagues have noted that following invasive abdominal surgery, patients whose total cholesterol levels fell below 105 mg/dl showed the highest incidence of sepsis (Leardi et al. 2000) in the recovery period. Likewise, an association between lowered cholesterol levels and increased incidence of sepsis and multiple organ failure has been reported by Giovannini et. al in a comparative study of 135 surgical patients (Giovannini et al. 1999).

Taken together, these studies indicate that cholesterol homeostasis (balance) in circulation is important for recovery from accidents as well as planned interventions like surgery. Hence, hypocholesterolemia is a risk factor to be considered, perhaps, before opting for invasive surgical procedures. This fact is likely to be most relevant for people bearing known cholesterol metabolism disorders as well as for people undergoing statin-based therapy to reduce cholesterol levels.

CHOLESTEROL AND INFECTION

Cholesterol-sequestering lipoproteins are also likely to play a protective role against infection in non-traumatized and non-surgical patients as well. The sequestration of bacterial lipopolysaccharides (LPS) by LDL to reduce the inflammatory response that is launched to counter LPS has been well characterized. Experiments conducted with knock-out mice that lacked the LDL-receptor gene showed total susceptibility towards septicemia and eventually died (Lanza-Jacoby et al. 2003). This research suggests that LDL bound to bacterial LPS may be endocytosed by cells bearing the LDL-receptor in order to inactivate this exotoxin.

In humans, *Staphylococcus aureus* is a bacterium that not only colonizes and persists in the system in the form of biofilms but can also aggressively invade tissues leading to system-wide sepsis. In fact, resistance of *Staphylococcus aureus* to many antibiotics is a major health issue that results in nosocomial (resulting from a hospital stay) infections (reviewed in Falcone et al. 2009). In the colonized state, when the biofilm reaches a certain bacterial population size, an operon termed as *agr* is switched on, triggering an invasive infectious response. Peterson and others have shown that apolipoprotein B is a natural antagonist of the *agr*-mediated aggressive infection. ApoB binds to an auto-inducing cyclic thiolactone peptide (AIP) and prevents the binding of this protein to its receptor AgrC (Peterson et al. 2008). ApoB is also capable of sequestering other forms AIP2-4 and therefore confers protection against *S. aureus* infections. It is therefore likely that extremely low levels of LDL-cholesterol may actually result in loss of protection from *S. aureus* infections. This is especially a risk factor for people bearing implants such as pacemakers since *S. aureus* biofilms are often associated with such implants.

LDL is a defensive barrier against other bacterial toxins like the *Vibrio vulnificus* cytolysin (Park et al. 2005). LDL has been shown to bind and inactivate the endotoxin by causing oligomerization of the toxin. An important feature of this interaction is that this is a dose–dependent inactivation mechanism (Park et al. 2005). Therefore, in hypocholesterolemic conditions, the reduced availability of LDL is likely to enhance the cytotoxicity of bacterial toxins, a factor that may lead to rapid onset of sepsis. Typically, in trauma patients, when total serum cholesterol levels fall to 50% of the normal population average levels, the risk of sepsis as well as multiple organ failure is high (Vyroubal et al. 2008). These interdependencies highlight the fact that an 'optimal' cholesterol balance is required and low levels of cholesterol are just as detrimental to health as elevated levels of cholesterol.

CHOLESTEROL IN MENTAL HEALTH

Cholesterol, being a membrane component, plays significant roles in neural functions as well. In fact, cholesterol is a major component of the myelin sheath that covers axons of brain neurons (reviewed in Fantini and Barrantes 2009) and is thought to modulate neurotransmitter release and binding to neurotransmitter receptors and therefore downstream events. A major neurotransmitter, serotonin, is important for the emotional well-being of humans and serotonin deficiencies are responsible for depression as well as aggression and suicide.

An association between serum cholesterol levels and serotonin has been established in several studies. People whose serum cholesterol levels are low also tend to have lower levels of serotonin in circulation (Steegmans et al. 1996). In a recent

study, it was seen that low serum cholesterol correlated with serotonin turnover in men. No such conclusive relationship could be demonstrated for women (Markianos et al. 2010). These scientists have postulated that lowered serotonin levels could be responsible for aggressive and violent behavior in men as a response to the evolutionary need to hunt and acquire food.

In a retrospective analysis of familial and personal suicide attempts, Bocchetta and colleagues have proposed a link between low cholesterol and suicidal behavior. A total of 783 patients who were undergoing lithium treatment were analyzed for reported personal or familial (first-order relative) suicide attempts. Based on one-time cholesterol measurements, the researchers found that lower levels of serum cholesterol in men were associated with attempted or completed suicide (Bocchetta et al. 2001), suggesting a neuromodulatory role for cholesterol.

In another study with panic disorder patients, the serum cholesterol levels in patients who attempted suicide were found to be lower than panic disorder patients who had not attempted suicide as well as normal control subjects. The mean total cholesterol levels as well as LDL levels were lower in panic disorder patients who had attempted suicide (Ozer et al. 2004). The study is hampered by limited sample size as well as a large age range of subjects. Extensive analyses will be required to further understand the association between low cholesterol and suicidal behavior.

In a study of 42 people who attempted suicide, a positive correlation was found between low serum cholesterol and low 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (Asellus et al. 2010). These patients were not under the influence of any other drug or therapy and had attempted suicide. The link between low serum cholesterol and depressed serotonergic responses has been suggested to explain these results. Although the cohort size was small with 42 individuals included, it is logically and practically difficult to add to these numbers of people who attempted suicide but missed the bus. Nonetheless, the work does show a correlation between low serum cholesterol and attenuated serotonergic response systems, which, perhaps is responsible for reckless suicidal behavior.

At the cellular level, a drastic reduction in the level of cholesterol is likely to affect the physiology of serotonin receptors (Paila et al. 2008). Paila and colleagues have used an inhibitor AY9944 in Chinese Hamster ovary fibroblast (CHO) cells to create a cellular model of the Smith-Lemli-Opitz syndrome. AY9944 inhibits the terminal step in the synthesis of cholesterol. In this model, signaling via the serotonin 1A ($5\text{-}HT_{1A}$) receptor was found to be reduced (Paila et al. 2008). The attenuation of signaling via this receptor was not explained by reduction in receptor expression or delivery to the plasma membrane. However, signaling via plasma membrane-resident 5-HT1A was reduced in the absence of cholesterol. Although these cells have abundant 7-dehydrocholesterol and 8-dehydrocholesterol, these sterols were not able to interact with 5-HT1A in the same manner as cholesterol and facilitate signaling via receptor-associated G-proteins (Paila et al. 2008). The overall membrane organization as measured by the fluidity of a non-specific fluorescent probe was unaffected in normal as well as SLOS cells used in this study. These results argue for a role of cholesterol in modulating serotonin signaling, albeit in a cell system that is non-neuronal.

In hippocampal tissue, oxidation of cholesterol reduced the binding of agonist as well as antagonist ligands to 5-HT1A receptor (Pucadyil et al. 2005). These results

indicate a physical interaction between cholesterol in the plane of the membrane and the neurotransmitter receptor. Taken together, these results suggest a mechanism for impairment of serotonin receptor dysfunction in situations of low cholesterol availability. Attenuated serotonin metabolism is a hallmark of depression and reduced availability of cholesterol may be a contributory factor.

Cholesterol deficiencies are likely to manifest as changes in behavioral patterns as well. In a one-year follow-up of patients with unipolar or bipolar disorder, a positive correlation between low serum cholesterol and recurrence of manic episodes was noted (Fiedorowicz et al. 2010). The patients were assessed for serum cholesterol along with other parameters, as well as development of psychotic episodes. Patients with unipolar disorder were not affected but those with bipolar disorder experienced manic episodes in the year of follow-up. Depression was not seen in both kinds of patients. Although the difference in mean cholesterol levels of both groups of patients was slim, the persistent deficiency of cholesterol is likely to be influential in neurological responses and behavior. In this study, the prevalence of different fractions of cholesterol throughout the follow-up period was not monitored and perhaps better assessment of the cholesterol status of the patient at the time of occurrence of psychotic / manic events will shed more light on the role of cholesterol (Fiedorowicz et al. 2010). It would be interesting to know whether a sudden and transient reduction in serum cholesterol levels is a trigger or indicator of manic behavior. Since depressive symptoms were not associated with hypocholesterolemia, the study points to other neuromodulatory roles of cholesterol which remain to be investigated.

Dietary intake of cholesterol was shown to reduce dissociative behavior and aggression in a primate study (Kaplan et al. 1994). Cynomolgus monkeys were fed high-fat diets that were specifically low or high in cholesterol. Monkeys who received a low cholesterol diet showed aggressive behavior and tended to be socially alienated.

Serum cholesterol levels may play an important role in addiction as well as de-addiction from cocaine. In a study of thirty-eight cocaine abusers in hospital-based rehabilitation, it was seen that serum HDL levels were depressed in these patients, even in the recovery phase. Serum LDL, HDL cholesterol as well as neuroendocrine responses were assessed two weeks after discontinuation of cocaine abuse. Serum HDL cholesterol was reduced in all patients. The reduction in HDL also correlated with greater experience of a 'high' feeling and euphoria when the same patients were challenged with m-chlorophenylpiperazine (m-CPP). These responses are known to be attributed to altered serotonergic neuronal transmission. In agreement with prior studies, reduced HDL cholesterol was also seen in patients who had prior episodes of aggression (Buydens-Branchey et al. 2000).

The co-incidence of low serum cholesterol and occurrence of mixed manic episodes has also been reported by Cassidy and Carroll (2002). In a study of 174 subjects, patients suffering from bipolar disorder and mixed manic episodes were observed to have serum total cholesterol levels well below the reported national average values. Cholesterol levels were also lower in patients who suffered from pure manic disorders but a pronounced difference in levels of cholesterol was seen in patients who suffered from bipolar disorder. Although it is unclear from this study whether low cholesterol is the trigger for mixed manic episodes or whether the manic episode results in lowered cholesterol levels, the study suggests a link between these two health parameters (Cassidy and Carroll 2002).

Low levels of serum cholesterol have been seen in patients in whom Alzheimer's disease (AD) has been diagnosed. In a study with 138 patients with a confirmed diagnosis of Alzheimer's disease, serum cholesterol levels were found to be lower than normal people of similar average age. Within the AD cohort, the decrease in cholesterol levels correlated with increasing levels of dementia (Tully et al. 2003). Kim and colleagues, in a South Korean study of 291 individuals from a community as well as 79 AD patients, reported that people with AD showed lower levels of serum cholesterol. However, these researchers found no association between progressive dementia and reduction in level of serum cholesterol (Kim et al. 2002). The authors suggest that presence of lower serum cholesterol in AD patients may be a marker of the impaired cognitive state rather than a causative agent.

The molecular mechanisms that result in lowered serum cholesterol, however, appear to be different than just simple reduction in the synthesis of cholesterol resulting from aging. This is indicated by studies which show that treatment with statin drugs (HMGCoA reductase inhibitors) actually reduces the production of the amyloid peptide (deposition of this peptide in plaques on neurons is the primary cause of pathology in AD) and may actually show a neuroprotective therapeutic effect (Buxbaum et al. 2002; Friedhoff et al. 2001). Since a deliberate reduction in the total synthesis of cholesterol is not likely to be the triggering factor for the development of AD, there must be other transport related phenomena associated with cholesterol metabolism and its delivery to neurons.

CHOLESTEROL AND AIDS

Hypocholesterolemia has been noted in patients seropositive for human immunodeficiency virus (HIV). In a study of 94 patients, serum cholesterol and triglyceride levels were assessed along with other parameters in patients who were seropositive and seronegative for HIV (Shor-Posner et al. 1993). Shor-Posner and colleagues found that patients with low total cholesterol (below 150 mg/dl) were seropositive (indicating greater viral load and active virus multiplication in the system) as compared to patients with higher total cholesterol levels who were seronegative, though they were infected with HIV (Shor-Posner et al. 1993). Cholesterol may therefore be required as part of an antiviral mechanism that might possibly slow down virus progression, if not prevent infection.

In a recent study with HIV-positive patients, Miguez and colleagues have reported a startling connection between cholesterol levels and HIV virulence (Miguez et al. 2010). These researchers compared the cholesterol levels of 165 HIV-positive people at the beginning of highly active antiretroviral therapy (HAART) and after 24 weeks. Patients were classified as hypocholesterolemic if their total serum cholesterol levels were below 150 mg/dl. These patients showed detectable viral loads as compared to patients who had higher levels of serum cholesterol. The CD4+ cell counts in the hypocholesterolemic patients were lower than those with higher cholesterol levels implying that the immune response was modulated by hypocholesterolemia. The viral load in hypocholesterolemic patients was seen despite HAART, indicating that cholesterol may be included as a therapeutic molecule in such patients. Thymic output was reduced in patients with low cholesterol levels resulting in very low (<200) CD4+-cell counts in these patients.

CONCLUSION

Cholesterol is an important molecule in human physiology and plays an important role in all processes wherein cellular membrane composition and topography are crucial. Cholesterol is both synthesized in the human body and is acquired from food sources. Excess intake of dietary cholesterol resulting in excessive accumulation of LDL-cholesterol in the vascular system has been identified as the causative factor for cardiovascular disease (Daniels et al. 2009; Ferrieres 2009), leading to the perception that keeping cholesterol levels low has to be a priority in health and wellness management. However, exceedingly low levels of cholesterol also have other detrimental effects on human physiology. Cholesterol is an important modulator of neurotransmitter receptors like serotonin, which are involved in maintaining a healthy neurophysiological state (Paila et al. 2008; Pucadyil et al. 2005). Cholesterol in the form of lipoproteins also helps to fight bacterial infections by neutralizing bacterial toxins and LPS, a function that may be severely compromised in hypocholesterolemic patients suffering from sepsis (Wilson et al. 2003).

Cholesterol is certainly involved in the etiology of cardiovascular disease. LDL-bound cholesterol is capable of acting as the seed molecule for the formation of atherogenic plaques and can be a significant health hazard, moderate to extreme reductions in the levels of total serum cholesterol also represent health hazards.

Mechanisms that result in reduction of total serum cholesterol also seem to have a bearing on the health outcomes. For example patients suffering from SLOS, although lacking in inherent cholesterol synthesis, do not always suffer from depression resulting from serotonergic neuronal dysfunction. Many physiological deficiencies of the SLOS defect can be overcome with dietary intake of cholesterol. However, in other situations like sepsis and critical injury or surgical trauma, cholesterol synthesis alone may not bring up the serum cholesterol to optimal levels in some patients (Vyroubal et al. 2008; Wilson et al. 2003).

Cholesterol is perhaps a unique membrane component that is involved in possibly every membrane turnover event such as exocyotosis and secretion (Gondre-Lewis et al. 2006), neurotransmitter modulation (Paila et al. 2008; Pucadyil et al. 2005) and endocytosis (Sabharanjak and Mayor 2004; Sabharanjak et al. 2002), viral metabolism and cardiovascular health (Miguez et al. 2010).

Research suggests that management of 'optimal' cholesterol levels should be the choice to make rather than aiming for very low levels of cholesterol in circulation.

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THE ROLE OF *STREPTOCOCCUS MUTANS* IN THE FORMATION OF DENTAL CARIES: AN ECOLOGICAL PERSPECTIVE

Jason Yeshaya Friedman

ABSTRACT

The teeth are among the most distinctive and productive features of the human species. It is the longest lasting surface of the body and can be used in research studies many years after death. Yet, in the living individual, the integrity of the teeth is constantly assaulted by a microbial challenge so great that dental caries, or decay, ranks as one of the most widespread medical afflictions. According to studies, dental caries rank third in medical costs, behind only heart disease and cancer (Loesche 1996). This review will attempt to describe what is responsible for dental caries, namely a bacterium called *Streptococcus mutans*. More specifically, it will concentrate on theories regarding the precise role of *S. mutans* and what causes it to flourish at times when bacteria associated with a healthy oral cavity cannot survive. It will further explain how after performing Pure Culture and Mixed Culture studies, the results clearly provided a theory referred to as the "ecological plaque hypothesis." In this theory, it became clear that it was not the mere presence of *S. mutans* that caused dental caries, but rather it was specific environmental factors that allowed *S. mutans* to thrive while rendering the non-pathogenic bacterium insignificant. Based on these updated theories, scientists have been able to find preventative methods to inhibit *S. mutans* even in the environment that normally favors its growth.

INTRODUCTION

Although the oral cavities of most mammalian species contain billions of microbial cells, most scientists have concluded that Streptococcus mutans is the bacterium that is responsible for dental caries, or decay. Over the years, scientists have been researching the precise role of S. mutans and what causes it to flourish at times when non-pathogenic bacteria cannot survive. It is this precise question that this review will attempt to answer. Specifically, it will concentrate on the ecology of the oral cavity and will attempt to prove, via various studies, a theory called the "ecological plaque hypothesis." This theory will appropriately explain the role of S. mutans and will open the door for successful prevention mechanisms.

DISCUSSION

A healthy human being has an estimated total of 100 trillion living cells (Savage 1977). However, 90% of these cells are merely micro-organisms that live in areas such as the intestinal tract, skin, and mouth. In fact, each specific habitat has a particular composition of micro-organisms which, in a healthy individual, remains stable once it's established. This is called microbial homeostasis. The stability of the microbial community in each distinct habitat is due to key ecological factors that are present. These factors include nutrients, pH levels, attachment receptors, and oxygen levels (Marsh 2001). Changes in the environment can severely alter the homeostatic balance necessary to sustain the microbial species, and this can result in disease.

From all the areas which contain these massive numbers of microbial cells, the oral cavity alone contains almost half of these microorganisms, amounting to about six billion microbial organisms (Loesche 1996). There are many properties of the oral cavity that make it an ideal habitat for microbial species. One such feature is the continuous flow of saliva. Saliva greatly enhances the ecology of the oral cavity. The pH of saliva (about 6.95) is ideal for micro-organism growth and its ionic composition provides buffering and reparation of the enamel (Scannapieco 1994). In addition, saliva keeps the mouth warm (around 35°C) and

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moist. Yet, perhaps the most beneficial feature of the saliva in the mouth is its ability to promote the adhesion of microbial species by coating surfaces of the oral cavity with a selective conditioning film.

Although all surfaces in the oral cavity benefit from saliva and serve as habitats for various microbial species, there is one surface in the mouth that has certain features which render it the supreme resting place for the oral microorganisms. This surface is the tooth (Figure 1). The teeth are oral surfaces that have a unique feature which is not seen in any other surface in the body. The tooth surface rarely sheds once it is formed (Bowden 2000). This property facilitates microbial growth, because once biofilm is formed on the tooth, it will not be removed as a result of shedding of the tooth surface.

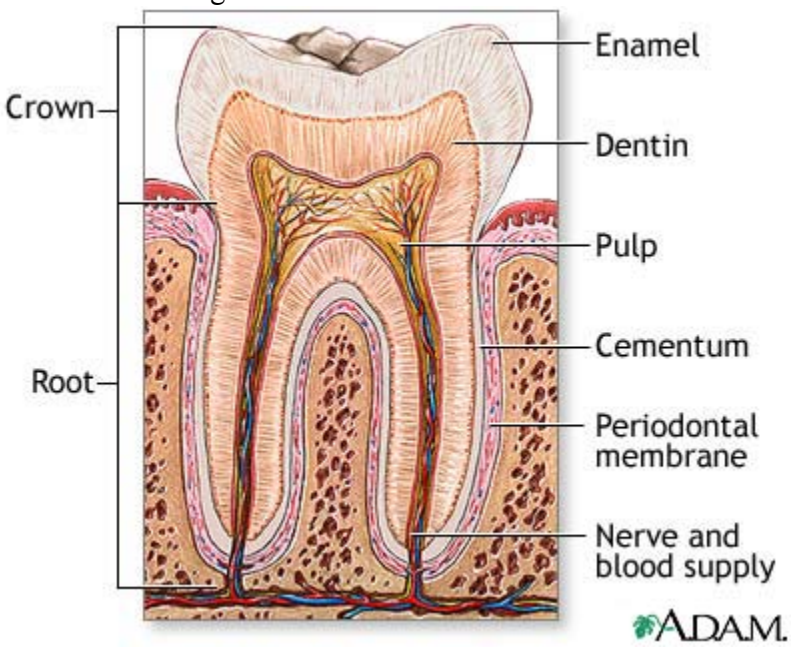

Figure 1: The structure of the tooth.
Source: David Zieve, MD, MHA, Medical Director, A.D.A.M., November 12,

The non-shedding character of the tooth makes its biofilm covering more important when studying microbial species. The extensive formation of biofilm on the teeth is called dental plaque. In healthy dental plaque formation, there is a sequence of events of microbial succession that allow its formation (Busscher and Van der Mei 2000). The first step occurs shortly after the teeth erupt. As mentioned, the host's saliva, along with some bacteria, coats the enamel surface with film. The first round of microbial species adheres to the film, using nonspecific interactions between charged molecules. These organisms grow and create a suitable environment for the colonization of more complex species. The new wave of species adheres to the already attached organisms. This process results in the dental plaque becoming a complex and multi-species biofilm.

Over the years, there have been numerous studies done on the composition of this dental plaque in order to determine which species cause it to decay and facilitate formations of dental caries. Although old hypotheses have been modified and study techniques enhanced, close to all researchers would agree the main species responsible is a bacteria called *Streptococcus mutans* (Figure 2).

The real question is not whether *S. mutans* is responsible for disease. It clearly is. Rather, researchers have been studying the exact role of *S. mutans* in dental caries. The questions scientists have been studying are about the specific triggers that allow *S. mutans* to grow and how to successfully prevent it from doing harm.

The first hypothesis in regards to the role of *S.mutans* was known as the "specific plaque hypothesis." In this conclusion, it was believed that although there is a vastly diverse collection of microbial species present in the plaque, *S. mutans* causes the disease and caries (Emilson and Krasse 1985). It does so by rapidly metabolizing sugar into acid. This process creates an environment with a particularly low pH. Under these conditions, *S. mutans* can continue to grow and eventually wear down the enamel.

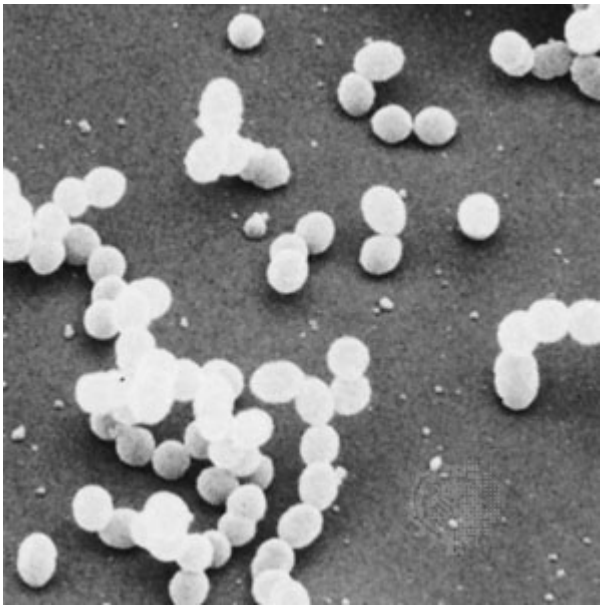

Figure 2: Streptococcus mutans.
Source: Encyclopedia Britannica Online. Web. 25 May, 2011.

However, the "specific plaque hypothesis" did not succeed in painting the full picture. The questions of what triggered *S. mutans* to grow and what allowed it to flourish when bacteria associated with healthy enamel could not do so were still unclear. Further study and research upon the habitats and the different factors that alter the microbial homeostasis led to the formation of an alternative hypothesis which better explains the role of *S. mutans* in dental caries and disease.

One such study is called Pure Culture Study. This study compares selected healthy and diseased stains in a controlled environment. This method provides accurate details on how environment affects the microbial species by independently varying different environmental cues and observing the microbial response. Early Pure Culture studies compared the response of *S. mutans*, the bacteria implicated in disease, and the bacteria of healthy enamel, by introducing them to different sugar and pH environments. The conclusions of these experiments showed that *S. mutan*s had the ability to survive and flourish over a wider range of pH and was in fact at its optimal growth at acidic pH (about 5). In addition, *S. mutans* had a higher rate of sugar uptake and underwent glycolysis more rapidly.

As mentioned, these studies clarified the role of *S. mutans* in dental caries and disease beyond the "specific plaque hypothesis". It confirmed the role of *S. mutans* in disease, but

expanded the theory by explaining how ecological changes are the key to allow the *S. mutans* to flourish as opposed to the bacteria associated with healthy enamel. It showed how differences seen in the composition of plaque are driven by the microbial species' response to environmental changes, resulting in the ability of previously minor components of the microflora to flourish.

To test this hypothesis, and to further explore the impact of environmental changes on the balance of the plaque microbial community, it was necessary to develop a more intricate study. This led scientists to begin a Mixed Cultures Study. In the Mixed Culture Study, researchers exploited the unique advantages of a chemostat to grow mixed cultures of oral bacteria in a range of controlled conditions (Marsh et al. 2006). Due to the fact that a chemostat allows individual parameters to be changed independently, a clear presentation is provided on how specific environmental conditions affect the physiology of the culture.

The reactions to two main environmental conditions were studied in the Mixed Culture experiment. Researchers wanted to see how oral bacteria associated with healthy enamel would react differently to *S. mutans* when presented with large doses of sugar and an environment with low pH. To best study the oral bacteria within these parameters, a chemostat was developed with a system for growing nine oral bacteria at constant temperature (37°C) and pH (7.0). The mixed culture was pulsed with glucose on ten consecutive days, either with or without pH control (Table 1) (Bradshaw et al. 1989).

Table 1: Effects of glucose pulses, low pH, and fluoride on the stability of a mixed culture of nine oral bacteria.

	Percentage Viable Count			
		After 10 glucose pulses:		
Bacterium	Pre-pulsing	With pH control (pH 7)	Without pH control	Without pH control, with NaF
S. gordonii	28.3	25.0	0.2	0.002
S. oralis	15.2	16.9	1.3	4.6
S. mutans	0.3	1.0	18.9	0.2
A. viscosus	0.1	13.1	2.3	0.4
L. casei	0.1	0.2	36.1	36.5
N. subflava	0.1	0.01	ND[b]	2×10^{-5}
V. dispar	9.8	28.7	41.4	57.8
P. intermedia	31.0	5.6	6×10^{-4}	0.5
F. nucleatum	15.2	9.5	2×10^{-5}	0.2
Final pH	7.0	7.0	3.83	4.49

[a] The mixed culture was pulsed on 10 consecutive days to give 28 mmol/L glucose, with or without 1 mmol/L NaF. The pH was either maintained automatically throughout at pH 7.0 ± 0.1 or was allowed to fall for six h following each pulse before being returned to pH 7.0 for 18 h prior to the next pulse.

[b] ND = not detected.

Source: Bradshaw et al. 1989

To understand the table let us simply focus on the first three species. The first two bacteria, *S. gordonii* and *S. oralis* are bacteria associated with a healthy oral cavity. The third strain, the *S. mutans*, is associated with disease. We see in the first column, that before any glucose was pulsed, the non-pathogenic bacteria dominated over the *S. mutans*. This represents a healthy oral cavity. In the second column glucose is pulsed, yet there is pH

control. We see that the non-pathogenic bacteria still remain dominant. This proved that sugar-uptake alone is not the real source for dental decay. In the third column, glucose is again pulsed, but now there is no pH control. We see in this case that *S. mutans* greatly increase in number due to decrease in pH. It was concluded that it was the low pH and not the availability of the carbohydrates itself that was selecting for the unfavorable disease causing bacteria.

The fourth column shows the effect of NaF on oral bacteria. We see that when fluoride is added the pathogenic bacteria are destroyed even under conditions of low pH. This will be discussed later.

This experiment was repeated to find out if there was a "critical pH" in which homeostasis of the oral cavity broke down. To do this, the mixed culture was again pulsed with glucose in three repeating experiments. However, this time the pH was controlled to fall at values at around pH 5.0. Results showed that the microbial community was irreversibly disrupted only when the pH fell below 5.0. The results showed that when the pH was lower than 5.0, the *S. mutans* dominated.

Based on the Pure Culture and Mixed Culture studies, the "specific plaque hypothesis" was clearly not the most accurate assessment of the cause of dental caries. It is not the mere existence of *S. mutans* that causes dental caries and disease. Rather the most accurate hypothesis, based on the above mixed culture studies, became known as the "ecological plaque hypothesis" (Takahashi and Nyvad 2008). This hypothesis suggests that key changes in environmental conditions trigger shifts in the balance of resident plaque microflora. It is these environmental conditions that allow the sites to become predisposed to disease. It is at this time that the *S. mutans*, which has the ability to survive in these adverse conditions which altered the resident microflora (i.e. low pH), contributes to dental caries and disease.

In other words, the ecological plaque hypothesis can be explained as follows. The mouth of a healthy individual always contains some amount of *S. mutans*. However, under normal healthy environmental conditions, there are many more healthy bacteria which can survive, rendering the *S. mutans* as a small percentage, weakly competitive, and clinically insignificant.

Yet, if a person would do something to alter the environment, pathogenic bacteria can become more competitive. For example, if an individual would increase the intake frequency of fermentable sugars, the plaque environment would become much more acidic (lower pH). This low pH environment favors the growth of *S. mutans*, as shown in the above mixed culture experiment. At this time, the *S. mutans* is strongly competitive now that it can render the bacteria associated with healthy enamel as insignificant. This causes the dental caries and disease.

An important aspect of the ecological plaque hypothesis is the idea that disease can be prevented in ways that do not directly inhibit pathogenic bacteria. Prevention strategies can be formed that serve to interfere with the underlying environmental factors that enabled the growth of the pathogenic bacteria in the first place. By finding ways to maintain ecological stability in the oral cavity, we can prevent *S. mutans* from ever attaining the strength necessary to cause harm.

As mentioned, it is the highly acidic (low pH) environment which is favorable to *S. mutans*. Thus, mechanisms that can combat the *S. mutans* viability by also competing in these acidic environments can serve as a beneficial source of prevention (Marsh 2001). Thus, the most common strategy to prevent disease via the ecological plaque hypothesis is the ability to decrease the growth of *S. mutans* even in highly acidic enamel. Studies have showed that

fluoride has such capabilities. Fluoride has the capability to stabilize the environment of the enamel and plaque even during times of low pH, rendering the *S.mutans* ineffective. Thus, Fluoride Therapy became the main prevention strategy that is based on the ecological plaque hypothesis.

Based on a similar experiment to the Mixed Culture Study mentioned above, researchers have determined that fluoride can serve to be inhibitory toward pathogenic bacteria even in environments without pH control (i.e. low pH). To test this theory 1 mmol/L of sodium fluoride was pulsed along with glucose into a mixed culture of three oral bacteria (Table 2).

Table 2:
Effects of fluoride on the stability of a mixed culture of three oral bacteria with 10 glucose pulses on 10 consecutive days with 28 mmol/L glucose and without pH control (percentage viable count)

Bacterium	Pre-Pulsing without Sodium Fluoride	After Pulsing without Sodium Fluoride	After Pulsing with Sodium Fluoride
S. gordonii	28.3	0.2	.002
S. oralis	15.2	1.3	4.6
S. mutans	0.3	18.9	0.2
Source: Bradshaw et al. 1989			

The results clearly show the ability of fluoride to inhibit the competitive capabilities of *S. mutans*. In the first column, at pre-pulsing we see the environment of healthy enamel. The two bacteria associated with health (*S. gordonii* and *S. oralis*) are at much higher concentration than *S. mutans*. In the second column, we see the *S. mutans* become more competitive due to the glucose pulsing and lack of pH control. This is the pathogenic environment seen in the earlier Mixed Culture Experiment. The third column represents the presence of fluoride in the acidic environment. As soon as fluoride is introduced, the *S. mutans* is seen to be inhibited, thus restoring the enamel culture to health (Aioba and Fejerskov 2002). Clearly, the great strides researchers have taken in defining the specific reason for dental caries, via the ecological plaque hypothesis, have allowed scientists to form prevention strategies based on these very principles.

CONCLUSION

In conclusion, it is evident from the research presented that ecological factors in the oral cavity play a large role in the effectiveness of *S. mutans* to cause dental caries. According to the ecological plaque hypothesis, *S. mutans* is always present. However, it is a specific environmental cue, such as low pH, which allows the *S.mutans* to become more competitive and outlive the bacteria associated with healthy enamel. Armed with this knowledge, scientists were able to realize fluoride's capability to prevent dental caries by interacting and inhibiting *S. mutans* even in adverse ecological conditions.

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THE EFFECT OF MELATONIN ON THE OVARIES

Jaclyn Starr

ABSTRACT

Melatonin is a very small molecule whose effects can be both detrimental and beneficial to the ovaries depending on its concentration. Too much of anything is usually not good and so is the case with melatonin. Very high doses can be damaging, but in the right amount melatonin may be able to combat various diseases and increase the chances for fertility in women.

THE PRODUCTION OF MELATONIN

Melatonin is an indoleamine which is produced in the pineal gland. It was first isolated from bovine pineal gland in 1958 and is currently classified as a hormone. This seemingly ambiguous hormone is found in many places in the human body and has many functions. As more research is done on melatonin, more of its importance is being uncovered.

Melatonin is synthesized and released in accordance with how much light reaches the eye. When light enters the eye, the ganglion cells, which contain the photopigment melanopsin, send retinal photic signals to the suprachiasmatic nucleus (SCN) through the monosynaptic retinohypothalamic tract. The ganglion cells which contain melanopsin are sensitive to light with relatively short wavelengths (484 nm) (Macchi and Bruce 2004).

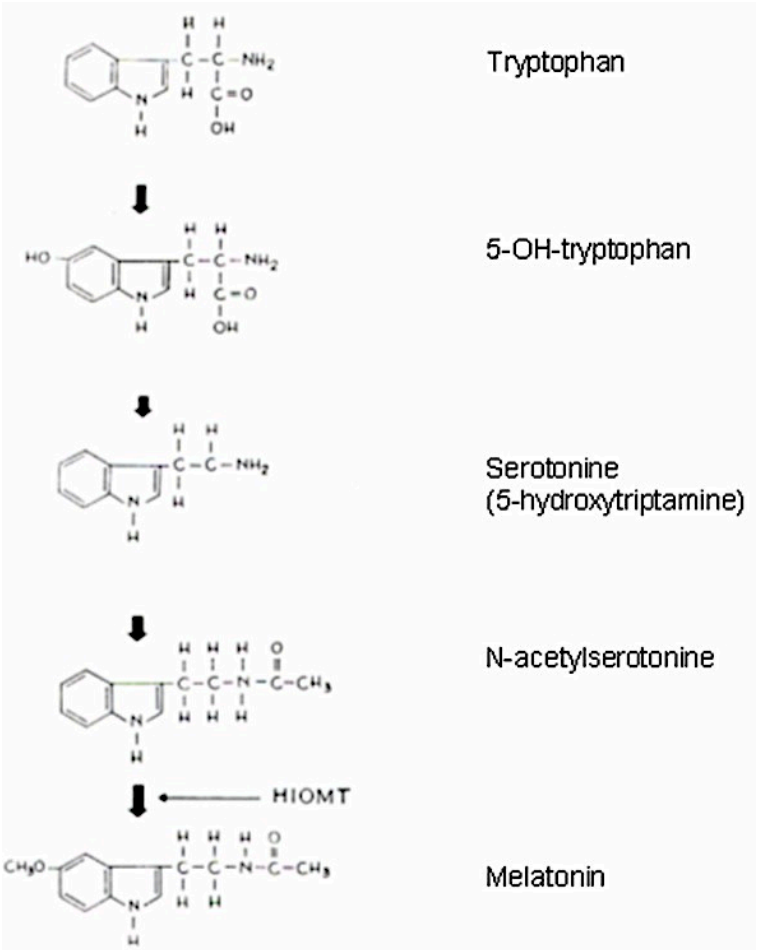

Figure 1: Tryptophan to Melatonin. Source: Macchi and Bruce 2004

There are constant rates of production of melatonin at night, but during daylight hours synthesis is suppressed by light (Macchi and Bruce 2004). The mechanism which causes the light induced suppression and dark induced release of melatonin is due to norepinephrine. Norepinephrine is the input to the pineal gland, and melatonin is the output. During the day, the retinal photoreceptors are hyperpolarized, inhibiting the release of norepinephrine and thus inhibiting the release of melatonin. Conversely, the lack of light at night causes the photoreceptors to release norepinephrine, thereby activating the enzyme N-acetyltransferase which regulates melatonin synthesis (Brzezinski 1997). In addition, melatonin concentrations are affected by exercise and changing posture. During night hours, concentrations of melatonin in the

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plasma and saliva decrease when moving from supine to standing position and vice versa (Macchi and Bruce 2004).

Production of melatonin begins when pinealocytes take up the amino acid tryptophan from the blood. Tryptophan is then hydoxylated and decarboxylated, thus converting it into serotonin. The enzyme N-acetyl transferase (NAT) then converts the serotonin into N-acetyl serotonin. Hydroxyindole-O-methyl transferase (HIOMT) methylates the N-acetyl serotonin, converting it into indoleamine N-acetyl-5-methoxytryptamine, which is melatonin (Figure 1). (Macchi and Bruce 2004). Additionally, studies suggest that melatonin may be synthesized from serotonin in the ovaries. (Itoh et al 1999).

After production, melatonin is released into the capillaries where 70% of it is bound to albumin. Melatonin is metabolized primarily in the liver where it undergoes hydroxylation and conjugation into sulfate and glucuronide (Macchi and Bruce 2004).

A highly diffusible hormone, melatonin is found in the blood, saliva, urine, cerebrospinal fluid (CSF), anterior chamber of eye, retina, gut, and bone marrow, as well as in many reproductive fluids such as breast milk, preovulatory follicles, semen, and amniotic fluid. Three melatonin receptors, MT1, MT2, and MT3, have been identified (Macchi and Bruce 2004; Adriaens et al. 2006). These receptors are localized in lymphocytes, prostate epithelial cells, granulosa cells, preovulatory follicles, spermatozoa, blood, platelets, and the mucosa and submucosa layers of the colon.

Melatonin and its receptors are found in many parts of the human body, indicating that the hormone may have many functions rather than only one main one. The fact that melatonin and melatonin receptors are found in many reproductive fluids and in preovulatory follicles suggests that melatonin plays a role in reproduction.

THE FUNCTION OF MELATONIN

Melatonin has various functions which are mediated either through specific membrane receptors or nuclear binding sites (Tamura et al. 2009). However, unlike other hormones, melatonin is unique in its ability to act as a free radical scavenger. It is an even better antioxidant than glutathione, vitamin E, and mannitol, which are known as effective free radical scavengers. It is one of the more effective antioxidants because it permeates and disperses through cells easily. Therefore it can prevent oxidative damage at any site of a cell (Pandey et al. 2003). Since melatonin acts forcefully upon free radicals, it is a very important hormone (Macchi and Bruce 2004).

Besides melatonin's role as an antioxidant, it plays a part in the immune system as well. Maestroni (1993) showed that suppression of melatonin (which is found in lymphocytes) caused a decrease in thymus and spleen activity. It also caused a decrease in the primary response of antibodies to T-dependent antigens. These results were reversed with administration of melatonin. In addition, constant administration of melatonin caused an increase in helper T cell activity and in IL-2 production (Macchi and Bruce 2004). High estrogen levels cause suppression of cell mediated immune response. Melatonin acts as an anti-estrogen, improving immune response (Sanchez-Barcelo et al. 2005).

Another important function of melatonin is its role in cardiac activity. When humans sleep at night, heart rate and blood pressure are lowered. It is also during the night that melatonin is produced. This concurrence seems to demonstrate a link between melatonin and a decrease in heart rate and blood pressure. Studies have shown that pineal extracts lower blood pressure in humans and in rats. But when exogenous melatonin was administered, heart rate and blood pressure only went down when given during the day. When melatonin

was administered at the time of endogenous melatonin production, only systolic blood pressure was lowered. Since melatonin has a beneficial effect on our cardiac system, it is more common for a stroke to occur in the morning when melatonin levels are low. Coronary disease patients have low melatonin levels, making melatonin's role in cardiac function even more plausible (Macchi and Bruce 2004).

Melatonin is released in a circadian rhythm. Therefore, it has been used to help regulate sleep in people with insomnia by entraining their circadian rhythm (Dubocovich 1988). In addition to helping regulate sleep, melatonin also regulates seasonal reproduction in photoperiodic animals (Macchi and Bruce 2004). These types of animals only reproduce during specific seasons of the year. Melatonin regulates reproduction in photoperiodic animals because it has a progonadotropic effect on these animals during certain seasons by increasing follicular stimulating hormone (FSH) concentrations and luteinizing hormone (LH) pulses (Dair et al. 2008).

MELATONIN AS AN ANTIOXIDANT

The cells in our body undergo a process called apoptosis, which is programmed cell death. This occurs in order to renew the cells in our body, eliminate defective cells, and maintain homeostasis. Apoptosis can be induced by growth factor deprivation, radiation induced DNA damage, activation of death receptors, and oxidative stress. Overall, it is a beneficial process, but only if it occurs when necessary. Excessive apoptosis can lead to an array of diseases such as Alzheimer's, Parkinson's, ischemia, stroke, and AIDS (Pandey et al. 2003). One of melatonin's most important features is its ability to act as a free radical scavenger, thus protecting against oxidative stress. Much research has been done on the use of melatonin as an antioxidant since it is a safe molecule and is therefore an excellent candidate for use in treatments for diseases which are caused by excessive apoptosis, which is due to oxidative stress (Pandey et al. 2003).

Research was done on rat hepatoma cells in order to see if serum deprivation induced oxidative stress, leading to apoptosis. Serum deprivation did induce oxidative stress, but treatment with antioxidants such as vitamin E and melatonin prevented apoptosis. One hundred (100) μM of melatonin were added to the medium six hours before serum deprivation. Oxidative stress caused by serum deprivation induced production of radioactive oxygen species (ROS), but with melatonin pre-treatment, the level of ROS was lowered (Figure 2) as seen by the significant reduction in nuclear condensation and cellular blebbing (features of cellular apoptosis). To ascertain that the pre-treated hepatoma cells not only survived but remained functional, they were re-plated in complete media. Most of the cells divided; they doubled three times in seventy two hours. In contrast, serum-deprived cells (without melatonin treatment) died within ninety six hours (Pandey et al. 2003).

Another antioxidant, glutathione, is a tripeptide which acts as a free radical scavenger by reducing oxidative stress. In the latter experiment, glutathione levels were tested in the serum deprived cells and in the melatonin treated serum deprived cells. In the untreated cells, glutathione levels were decreased, but in the melatonin pre-treated cells, glutathione levels remained the same (Pandey et al. 2003). This study demonstrates that melatonin not only has an anti-apoptotic effect as an antioxidant, but also has a protective effect over other antioxidants.

MELATONIN AND WOMEN

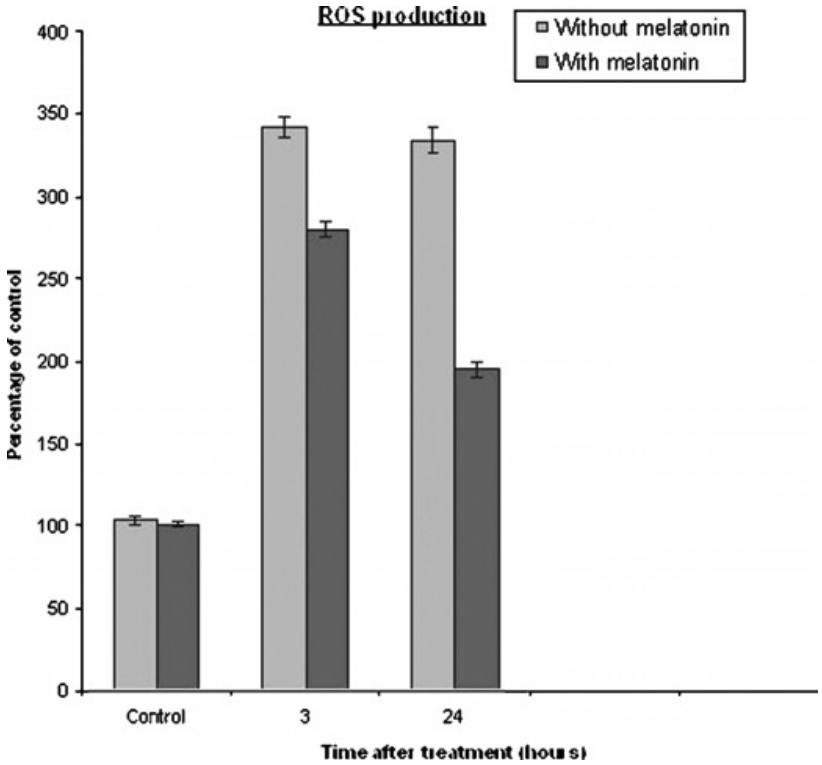

Figure 2: The effect of ROS on cells treated with melatonin versus control cells. Source: Pandey et al. 2003

Melatonin affects the female reproductive system by acting upon the ovaries both directly and indirectly. It acts indirectly on the ovaries by acting on gonadotropin releasing hormone (GnRH), thereby effecting the release of gonadotropins (GnRH is responsible for the release of LH and FSH from the anterior pituitary gland) (Romeu et al. 2010). Melatonin acts on the ovaries directly through its antioxidant and anti-apoptotic properties, as well as its regulation of LH mRNA in the ovaries (Tamura et al. 2009).

One indication that melatonin is involved in reproduction is that melatonin levels decrease at the start of puberty. This may hint to melatonin's role in reproduction because high levels of melatonin are known to inhibit ovulation (Macchi and Bruce 2004). Secondly, the concentration of melatonin in human preovulatory follicular fluid is three times more than in the plasma and melatonin and its precursors are found in human ovary extracts. For example, NAT and HIOMT are found in human ovarian homogenates (Itoh et al. 1999). In addition to melatonin itself, MT1 and MT2 receptors are also found in ovarian extracts such as ovarian granulosa, luteal cells of humans, and rat ovaries. The binding site for the receptors has been detected in the granulosa cell membrane.

There are two possibilities for the source of the melatonin found in the female reproductive system. It is possible that it is synthesized in the ovary and then released directly into the follicular fluid, or it may be derived from the circulation. The latter seems more likely because when infertile women were given a 3 mg tablet of melatonin (taken orally), there were higher levels of melatonin in the follicular fluid (Tamura et al. 2009).

High or low melatonin levels in rats can affect their ovaries. In an experiment done to determine the effects of melatonin on rat endometrial morphology and embryo implantation, results indicated that melatonin had a positive effect on the endometrium by allowing for better implantation of embryos (probably by raising progesterone levels) (Soares et al. 2003).

Rats are non-photoperiodic, just like humans. Melatonin was administered to the rats in large doses in the afternoon of their proestrus phase (the phase proceeding ovulation). As a result, the surge in LH which normally causes ovulation was prevented, not allowing for ovulation. In another experiment, when rats were given exogenous melatonin, ovarian weight

was decreased. But, when the rats were subjected to prolonged light, decreasing the high melatonin levels, an increase in ovarian weight was observed (Soares et al. 2003).

This antigonadotropic effect was seen not only when melatonin levels were too high, but also when melatonin levels were too low. When melatonin production was suppressed in rats, either by pinealectomy or light, precocious puberty, ovarian atrophy, chronic anovulatory state, permanent estrous condition, and hyperprolactinemia were observed. Pinealectomy induced a prolonged estrous stage in the rats, possibly by raising estrogen levels and lowering progesterone levels (Dair et al. 2008). Moreover, when rats underwent a pinealectomy, there was an increase in atretic follicles in the ovaries (Tamura et al. 2009). Atresia is hormone controlled apoptosis; approximately ninety nine percent of ovarian follicles undergo this degenerative process; the rest proceed to the preovulatory stage (Manabe et al. 2008). Pinealectomy of rats in another experiment resulted in overgrowth of the ovarian stroma (Romeu et al. 2010).

From these experiments on rats, it seems that high levels of melatonin have antigonadotropic effects (indirectly affecting the ovaries) resulting in a lack of ovulation, and low levels of melatonin cause an increase in atretic follicles and decrease in ovarian weight. But it should be noted that high levels appear to improve the endometrium for implantation by having an effect on hormones such as estrogen and progesterone. It is also possible that in humans, high melatonin levels are detrimental to fertility because infertility may be a result of low estrogen levels and high melatonin levels (Macchi and Bruce 2004).

In women with stress induced, exercise induced, or functional hypothalamic hypogonadism, melatonin levels are higher than normally present. Additionally, women with functional amenorrhea, have high melatonin levels which are inversely related to their estrogen levels, suggesting a relationship between high melatonin concentrations and hypothathalamic-pituitary-gonadal hypofunction which can cause temporary infertility by preventing menstruation in women. In one study, exogenous melatonin and progestagen were given to women; as a result LH secretion decreased, not allowing for ovulation. Although the luteal phase increase in progesterone was blocked, FSH and estradiol were not affected (Macchi and Bruce 2004).

The relationship between melatonin and estrogen was also studied in breast cancer patients. High melatonin levels decreased the amount of estrogen, and low melatonin caused an increase in estrogen. In breast cancer patients, tumors are sometimes estrogen-dependent. These women were treated with estrogen inhibitors such as tamoxifen, which is a drug that acts as an estrogen receptor antagonist and agonist. Melatonin acts as an anti-estrogen as well, but in a different manner; it interacts with the estrogen receptor signaling pathway. Melatonin binds to its own receptors and causes a decrease in expression of estrogen receptors, thereby blocking estradiol from binding to the estrogen receptors (Sanchez-Barcelo et al. 2005). Therefore, infertile women may have high levels of estrogen which could possibly be treated with administration of melatonin.

Low levels of melatonin in human ovaries may also be unfavorable by affecting the ovaries directly. Many studies provide evidence that follicular cell death during atresia occurs through apoptosis. Many women who are having ovarian failure such as premature ovarian failure, polycystic ovary syndrome, oophoritis, or unexplained infertility, may have these disorders due to a large number of follicular cell deaths via apoptosis. In order for apoptosis to occur, the cell's mitochondrial membrane must become more permeable, so as to release pro-apoptotic factors. Melatonin has been shown to directly inhibit the permeability of transition pores (a protein pore), causing anti-apoptotic effects.

An experiment was done in which mice were injected with either xenogenic anti-ovarian antibodies or allogenic anti-ovarian antibodies. One hour before antibody injection, melatonin was administered (5 mg/kg). In the control mice, exogenous melatonin had no effect on the ovaries, but in the mice which had been injected with xenogenic anti-ovarian antibodies, melatonin reduced the negative effects caused by the antibodies; it improved oocyte quality and caused a decrease in the number of follicular cell deaths by apoptosis. Melatonin reversed the antagonistic effects of anti-ovarian antibodies on the mouse ovaries, but it did not protect against the larger immune reaction of the xenogenic antibodies on a systemic level. On the other hand, melatonin administration in mice injected with allogenic anti-ovarian antibodies had no effect (Voznesenskaya et al. 2007).

In addition to providing anti-apoptotic factors, melatonin in the follicles can scavenge free radicals such as radioactive nitrogen species (RNS) and ROS, as mentioned above. The capability of melatonin to act as a free radical scavenger is especially important because ovulation stimulates a local inflamatory response, causing inflammatory cells such as macrophages and neutrophils to produce free radicals such as ROS and RNS, which induce apoptosis in ovarian cells. Higher melatonin levels in ovarian follicles offers the ovarian cells a greater chance of maturing and developing (Tamura et al. 2009). Therefore, if a woman's melatonin levels are too low, there may be an increase in the percentage of follicular deaths which would result in a lower number of follicles that have the potential for ovulation. Clearly, melatonin levels must be maintained within a certain range, since very high and very low levels can prevent ovulation, leading to infertility.

Very few follicles mature and become capable of releasing their ovum for fertilization purposes. The maturing follicle is filled with follicular fluid which contains water, electrolytes, serum proteins, and steroid hormones secreted by granulosa cells. Melatonin affects sex steroid production at different phases of follicular growth by regulating the steroidogenic enzyme, which activates gene expression in thecal and granulosa cells. Melatonin has been proven to alter granulosa cell steroidogenesis and follicular function. As follicles mature, they shift from dependency on FSH to LH. The process by which follicles are selected to continue maturing may be linked to the timing of mRNA expression encoding LH receptors in granulosa cells. Melatonin may directly affect the ovaries by regulating LH mRNA expression; melatonin treatment (10 pM-100 nM) increased the LH receptor mRNA expression in granulosa cells (Tamura et al. 2009). This confirms that higher levels of melatonin in the ovaries can be beneficial, but what concentrations are too high?

Research was done in order to find a melatonin concentration that would protect female oocytes without being toxic. This experiment was specifically performed to find a way for young women who are undergoing treatments such as chemotherapy and radiation to

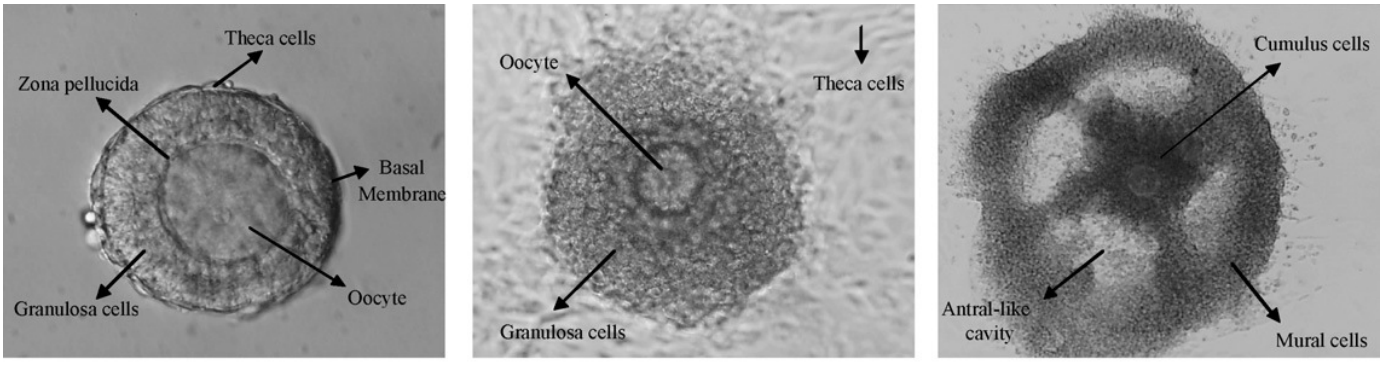

Figure 3: The granulosa cells of the ovaries, which each house an oocyte, go through three major phases: the follicular, diffuse, and antral stages. Source: Adriaens et al. 2006

retain fertility. Mouse follicles were cultured in vitro and different concentrations of melatonin were added to each. Ovulation was then stimulated. Two (2) mM of melatonin was found to be toxic (this is about four thousand times more than physiological melatonin concentrations in the follicular fluid); it affected follicular differentiation leading to a decreased amount of antral follicles (Adriaens et al. 2006).

One (1) mM negatively influenced oocyte development, and 100 µM influenced steroidogenesis. Ten (10) µM was the highest concentrations that had no negative effect on the follicular system. This dosage could possibly be used to protect female ovaries. Additionally, progesterone levels increased significantly in follicles with 100 µM and 1 mM of melatonin. After ovulation was stimulated, progesterone levels increased in all cases except for with 2 mM melatonin. In the control groups, estradiol increased exponentially, but in follicles with 1 or 2 mM of melatonin, estradiol amounts were much lower. As seen by the results in Figure 4, large doses of melatonin caused a reduction in the number of granulosa

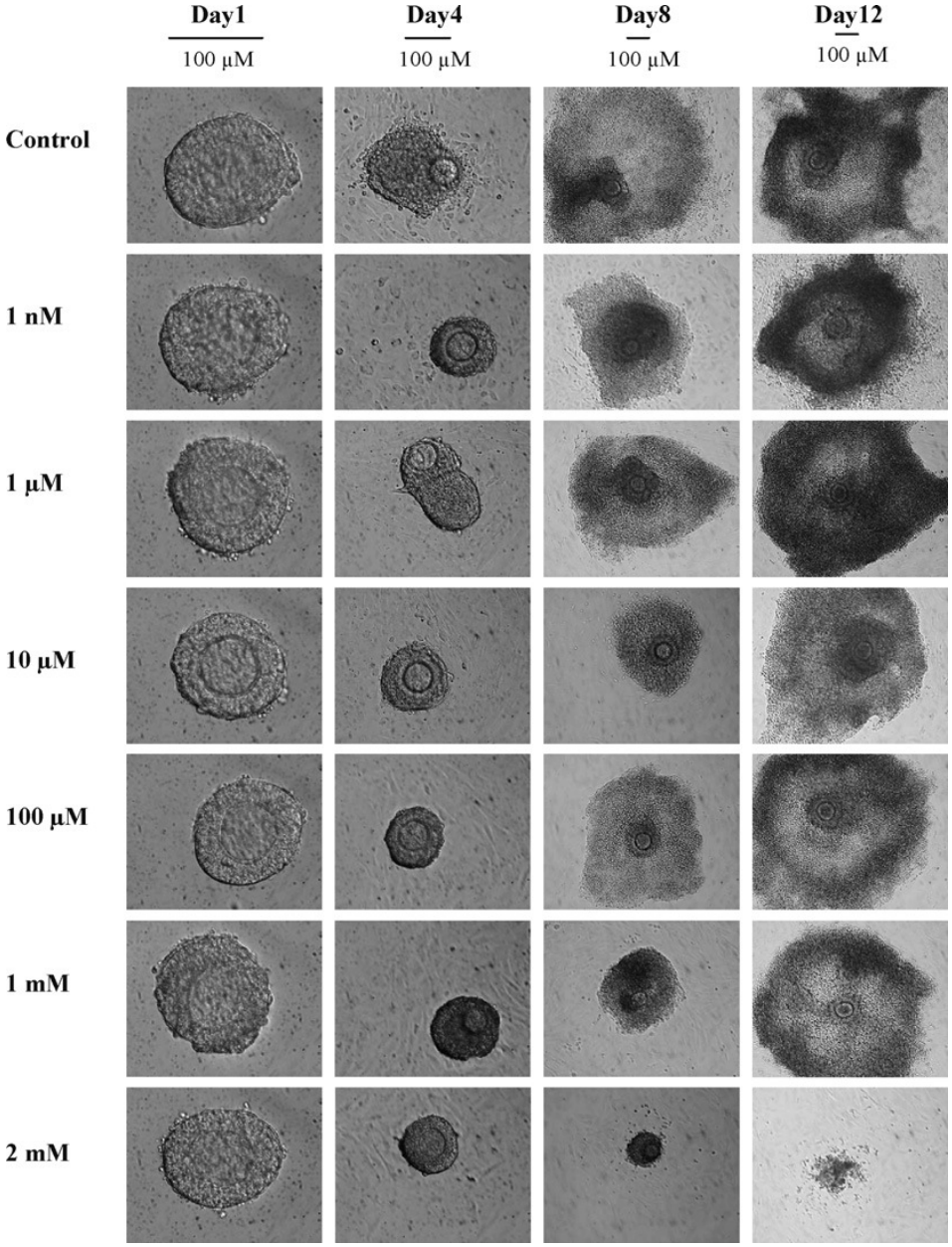

Figure 4 : Morphological changes of follicles over a 12 day period under different melatonin concentrations Source: Adriaens et al. 2006

cells. But by day twelve, most of the follicles reached the antral stage, with concentrations of up to 1 mM melatonin (Adriaens et al. 2006).

This experiment only used three mice (six ovaries) for evaluation; this is a very small control group making its validity questionable. Everyone has varying levels of hormones, such as estrogen, progesterone and melatonin, in circulation so a larger number of ovaries should have been used in this study. On the other hand, this study was done well because they used concentrations of melatonin spanning physiological and pharmacological follicular fluid concentrations (1nM-2mM). According to this study, there would be no health hazard (in regards to fertility) for women to take 3 mg tablets of melatonin (the normal dosage found over-the-counter).

In another experiment, three week-old mice were whole-body irradiated with gamma-radiation. Gamma radiation causes an increase in atresia of ovarian follicles. The purpose of this experiment was to observe if melatonin provided a protective effect against radiation. One group of mice was treated with 10 µg of melatonin before radiation, and another was treated with 100 µg. After radiation, the ovaries were taken out and examined. The number of follicles (including normal and atretic) was about one hundred and twenty five in the largest cross sections; in the irradiated mouse ovaries, the number of follicles was reduced to about one hundred and three. Primordial follicles were the most sensitive to radiation; melatonin treatment seemed to protect these follicles from radiation. Ten (10) µg of melatonin did not have a large protective effect on the primary follicles from radiation, but 100 µg did display a protective effect on them. But lower melatonin concentration did have a protective effect on preantral and antral follicles. Different concentrations of melatonin have protective effects against radiation at different stages of follicular development (Kim and Lee 2000).

As opposed to most of the previous studies mentioned, one study found that melatonin increased follicular atresia. Research was done to study the effects of pineal indoles (including melatonin) on gonadotropin-induced ovulation in mice. To stimulate ovulation, mice were injected with PMSG (pregnant mare serum gonadotropin) and hCG (human chorionic gonadotropin). Then various pineal indoles, at a dose of 0.2 mg/25 g body weight, were administered. After the mice were sacrificed, the ovaries were removed and examined. Although ovarian weight and quality remained the same, the number of atretic follicles appeared to increase in the mouse ovaries which had been injected with melatonin. Plasma levels of estradiol and progesterone were decreased (Chan and Ng 1995).

The two latter experiments discussed (on gamma radiation and on pineal indoles) both used young mice which were prepubertal. Puberty in humans causes a decrease in endogenous melatonin production, as well as other hormonal changes. When examining whether melatonin has protective effects on follicles for the purpose of helping prepubertal females, using prepubertal mice is appropriate. Otherwise, mature mice should be used for experimentation because hormone levels and interactions would be more similar to that in mature women.

Future Research on Melatonin

As seen by the experiments and research done on melatonin, it is apparent that the indoleamine affects the reproductive system. It appears that melatonin is beneficial in high concentrations when it is present in the ovaries because it directly decreases apoptosis and acts as a free radical scavenger. Therefore, low melatonin levels in the ovaries are detrimental to fertility. On the other hand, high melatonin concentrations in the blood can inhibit ovulation by inhibiting the surge in LH. It is necessary to determine the concentrations of

melatonin that will be most beneficial in increasing ovulation by protecting and helping oocyte maturation, without being too high to be antigonadotropic. In addition, research should be done to see if melatonin can be directly administered into the ovaries (in order to decrease apoptosis) without too much of it entering circulation and possibly inhibiting ovulation (by inhibiting the release of GnRH).

Moreover, more research needs to be done on finding a concentration of melatonin that has protective properties on oocytes (by acting as free radical scavengers) without having detrimental effects. This would be beneficial to young women undergoing chemotherapy or radiation treatments whose fertility in the future might be at risk. Ten micromoles of melatonin is a dose that should be experimented with further.

Additional work should be done on the relationship between melatonin and estrogen. A few of the experiments done showed that low levels of melatonin, as a result of pinealectomy, raised estrogen levels and caused an increase in atretic follicles. On the other hand, other research indicated that high levels of melatonin and low estrogen levels were detrimental to fertility.

Melatonin could also be used as a supplement for treatments of various disorders since it is generally safe (Brzezinski 1997). As an antioxidant and mitochondrial membrane permeability transition pore inhibitor, melatonin may be a good candidate to help in the management of cancer, Alzheimer's disease, diabetes, and viral infections (Tamura et al. 2009). Research has shown that melatonin can fight against tumors by reducing vascular endothelial growth factor (Romeu, et al. 2010). It is also permeable enough to disperse throughout cells and can even penetrate the nucleus where it can protect DNA from oxidative damage, reducing the risk of cancer (Meki et al. 2001).

CONCLUSION

Melatonin has a relationship with estrogen and other hormones, has an anti-apoptotic effect, and regulates LH mRNA. With these properties melatonin can be beneficial as it may help increase fertility in women who are infertile, prevent damage in diseases where there is too much apoptosis, and possibly protect the ovaries from radiation. Although more research has to be done on melatonin and the doses which work best in each case, it seems that the hormone will play a role in various therapies in the future.

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DID THE FDA PROPERLY ASSESS THE SAFETY OF OLESTRA AS A FOOD ADDITIVE?

Leah Lebel

INTRODUCTION

Olestra, a fat-substitute comprised of sucrose that has been esterified with fatty acids (Blume 1995), has been the subject of much controversy ever since its creation. Olestra is not absorbed (Mattson and Nolen 1972) because it cannot be hydrolyzed by pancreatic lipases (Mattson and Volpenhein 1972) or taken up across the enterocyte microvillus membrane (Freston et al. 1997), and thus, cannot be utilized for energy. Olestra has physical and organoleptic properties similar to those of traditional triglycerides (Jandacek and Webb 1978) and is emulsified together with triglyceride (Freston et al. 1997), yet it passes through the colon and is excreted unchanged (Fallat et al. 1976). It therefore adds no fat, sugar, or calories to the diet (Thomson et al. 1998)

There is a noteworthy advantage of replacing fat with olestra: olestra is far less energy-dense than its triglyceride counterpart and can, at least in theory, be a valuable asset to a weight-loss regimen. Olestra can serve to satisfy one's cravings for fatty-foods without containing the dense energy that triglycerides provide (Eldridge et al. 2002). By causing a reduction in energy intake for consumers, olestra, when incorporated with other calorie-saving methods, has the potential to cause weight loss when replaced with more energy-dense products (Rolls and Bell 1999).

After Procter & Gamble, the makers of olestra, obtained their first patent on olestra in 1971 (Nestle 1998), olestra underwent a more than 20-year struggle until it was finally

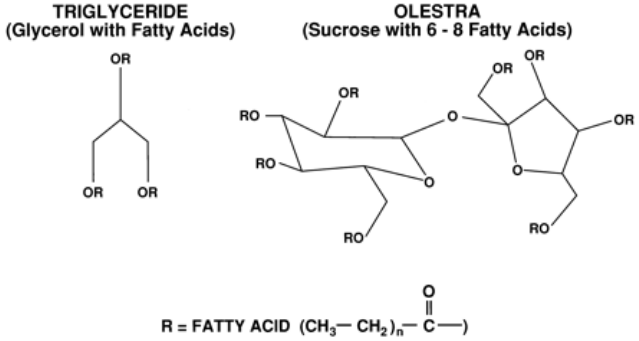

Figure 1: The chemical structure of olestra as compared with traditional triglyceride
Source: http://extoxnet.orst.edu/faqs/additive/olstru.htm

approved in January 1996 (Nightingale 1996). Much of the controversy surrounding this food additive stems from its versatility, as olestra can withstand high heating temperatures and can be used in virtually any food(Eldridge et al. 2002), and from its potential to be consumed in large quantities. Olestra presents a potential danger in that it is a macro-additive; a serving of olestra-containing food has several grams of the additive, rather than milligrams (as artificial sweeteners and many other additives have). This fact presented an unprecedented decision for the FDA—it was now choosing to approve something that would be consumed in potentially substantial quantities. Nevertheless, on January 25th 1996, the FDA released its ruling that olestra was to be approved for use in savory snacks. The FDA did, however, require that vitamin losses typically experienced from consuming olestra (particularly fat-soluble vitamins) be added to compensate for these losses. Thus, vitamins A,

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D, E, and K are added to olestra during manufacturing. Initially upon approving olestra, the FDA, still unsure about long-term safety at approval time, required a warning notice for all products (Nestle 1998) stating that "This Product Contains Olestra. Olestra may cause abdominal cramping and loose stools. Olestra inhibits the absorption of some vitamins and other nutrients. Vitamins A, D, E, and K have been added" (Center for Science in the Public Interest).

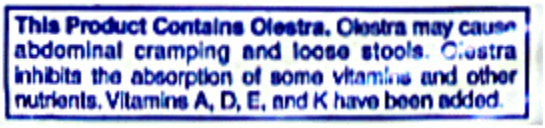

Figure 2: Sample of the warning label originally required on all olestra-containing products
Source: http://www.carboh.com/olestra.phtml

Much consumer controversy resulted from olestra's approval (Miller 2001). From olestra's entry into the market, there have been tens of thousands of adverse anecdotal reports from olestra consumers. People claimed they had suffered severe diarrhea, fecal incontinence, and/or abdominal cramps hours after eating olestra chips. Procter & Gamble, however, claimed that there is no proof that olestra is the cause of these effects (Center for Science in the Public Interest).

The Center for Science in Public Interest (CSPI) in particular was infuriated with olestra's passing into market and constantly collected data in an attempt to convince the FDA to withdraw their decision. Many other groups and independent challengers also believe that olestra should not have obtained FDA approval. They challenged the studies conducted by Procter & Gamble, pointing to the fact that some studies conducted by Unilever, Procter & Gamble's competition, show that olestra, when consumed at high levels, causes gastrointestinal problems in up to 30% of subjects, point to the large marketing campaigns used to promote the product, and claim that significant losses of fat-soluble vitamins, and particularly carotenoids, can increase the risk of chronic diseases. Yet because critics could not prove that carotenoid losses were harmful and because the product had a label warning of GI distress potential, no action was taken to remove the product from the market (Nestle 1998). According to Porterfield, "the PDA [sic] granted approval [of Olestra] acknowledging that some patients may experience abdominal cramps and diarrhea if they eat too much" (Porterfield 1997).

In 2003, the FDA chose to eliminate the requirement for a warning label, stating that it "was no longer warranted as post-market studies showed that olestra caused only infrequent, mild gastrointestinal effects" (FDA removes olestra warning 2003).

Michael F. Jacobson, the executive director of CSPI, notes, "P&G's own studies prove olestra causes diarrhea, cramps and other symptoms. If that weren't enough, the FDA has more than 20,000 complaints about olestra in its files--more than it has for all other food additives in history combined" (FDA removes olestra warning 2003). In a post marketing surveillance study, .4% of consumers were hospitalized, and suspected olestra to be the cause for their hospitalization (Allgood et al. 2000). Other consumers pointed to other side effects such as headaches and blame olestra as the cause (Blume 1995). Nonetheless, there is no direct proof that this is the case. As Procter and Gamble noted in their defense, it is quite possible that there was another unrelated cause to all these ailments and that experiencing ailments at some time after consuming olestra was a coincidental occurrence.

Despite this, many people began to question the FDA's approval of olestra. Many ask: how has the FDA been able to responsibly pass into market a product that may not be safe for such large segments of the population who may not realize the effects of olestra on their overall health? Olestra causes side effects of great magnitude; diarrhea, after all, can cause

complications including weight loss, dehydration and electrolyte imbalances (Tortora and Derrikson 2006). Moreover, Procter and Gamble contributed funds to the FDA, just weeks before the FDA actually approved olestra (Nestle 1998). Thus, many wonder if olestra is safe and if the decision was based solely on fact.

This study attempts to determine whether the FDA took the necessary scruples to avoid prematurely allowing olestra into market. An analysis of the standards utilized by the FDA, followed by a comparison of those standards with the research is warranted.

WHAT ARE THE CRITERIA UPON WHICH THE FDA MAKES ITS "SAFE" OR "UNSAFE" RULINGS?

ADDITIVE REGULATION GUIDELINES WHICH THE FDA ADHERES TO:

In an article aimed at explaining the requirements and procedures involved in the FDA's additive-approval process, Rulis and Levitt (2009) depict many of the factors that would explain what Procter and Gamble, the company that manufactures olestra, would have had to undergo to get olestra approved for consumer use. The article notes the fact that the FDA requires that additives undergo a strict approval regimen and employs an "unsafe until proven safe" mentality: "New food additives are presumed to be unsafe for their intended use unless and until they are proven 'safe'" (Section 3; 2008). The additive in question must meet with the FDA's standards of "reasonableness to cause no harm." Clearly, "reasonableness to cause no harm" is a subjective term and thus in the article's appendix, the author elaborates on just what "reasonableness" is defined as. Section 409(c)(3) of the FD&C act explains that the food additive in question requires a "fair evaluation of the data" and that the concept of safety involves the question of whether a substance is "hazardous to the health of man or animal." Primarily, calling a product "safe" requires proof of "a reasonable certainty that no harm will result from the proposed use of an additive" (House of Representatives, Report No. 2284, 85th Congress, 1958, as qtd. in appendix 10 of Rulis and Levitt 2009). Thus, in assessing the safety of olestra, the FDA would have been expected to determine (1) whether olestra could potentially be hazardous to the health of man or animal and if the substance in question has a reasonable certainty to cause harm based upon the data available prior to olestra's approval date on January 25th 1996.

The authors of the articles also reference the House of Representatives Report No 2284, 85th congress, 1958 statute 21CFR 170.3(i)) which elaborated upon the term "safe" to mean "a *reasonable certainty* in the minds of competent scientists that the substance is not harmful under the *intended conditions of use*" (italics added). It is important to note that, in this ruling, there must be a reasonable certainty only. In other words, the product in question is not guaranteed to be safe, but rather, it must present itself as reasonably harmless to health. Rulis and Levitt adeptly point out that this wording does "[result]in decisions inevitably being made without absolute certainty" (appendix 10).The burden to provide proof of safety lies with the petitioner (Rulis and Levitt 2009), and therefore Procter and Gamble would have had to demonstrate to the FDA that there is reasonable certainty that the item causes no harm in the minds of competent scientists. Hence, the questions that remain for this research paper to address is (2) were the competent scientists company based (and thus possibly biased) or were they independent researchers and (3) how credible are the studies upon which the scientists were basing their decision? A critical review of the literature available until olestra became approved is warranted in order to assess the meticulousness of the scruples taken before the FDA's decision was finalized. At least in theory, the research should have been able to persuade the FDA of olestra's reasonable safety.

Another inquiry to the above is what is olestra's "intended condition of use"? Rulis and Levitt's journal article then goes on to explain that "[there are] a range of technical functions in food…as outlined by Food Ingredients, as outlined in a brochure produced jointly by the FDA in collaboration with the international Food Information Council." The article then goes on to list some of these technical functions, and the one that best fits olestra would be the second one, or, "to improve or maintain nutritional value," which includes the point of "lowering the calorie or fat content of foods." Hence, reducing the energy consumed would be considered a sufficient benefit of the product's use. The purpose of reducing the energy content of a specific food would, in turn, be to lower the weight-gain by the consumer who chose to consume the food with the additive rather than the one without. In an age where obesity is reaching epidemic proportions (World Health Organization 1998), this is an important task to be accomplished. Over a long period, even modest caloric-reduction benefits can translate into significant weight loss. In light of this, olestra's use serves a very important function to lower the skyrocketing obesity rate. Thus, it is probably safe to infer that the "purpose" or benefit seen by the FDA for olestra's use would be to produce a weight-loss effect in the obese, thus opening a fourth underlying question that would provide the justification, or lack thereof, of ruling for olestra's approval:(*4*) whether olestra actually causes weight reduction in those who would benefit from weight loss. Section three of the article then goes on to state that "once approved, food additives must be safe for everyone—children and the very young; teenagers and adults; the elderly; pregnant and lactating women." This fact obviously raises a fifth question: how does olestra consumption affect those who may experience negative consequences resulting from a lower energy consumption (such as pregnant women who require a higher caloric intake to support a growing fetus or children reaching puberty who also require a higher energy intake to support growth spurts and maturation) and can these subgroups easily meet these increased energy needs while consuming lower-calorie versions of fat such as olestra. The FDA would have had to assess this (5) and would have had to provide special labeling so special-risk subpopulations can be properly informed, if necessary that they must avoid the product. At present, no food labels are featured on the package of olestra-containing products. Also, along with the question of whether olestra promotes weight loss, is a further question: does it lower the health risks associated with Obesity (6), such as the low-density lipoprotein (LDL)or serum triglyceride levels. (*7*) Does the added vitamin K promote coagulation and hinder the effects of warfarin therapy? (8) How does olestra interact with lipophilic drugs?

HOW MUCH DID THE FDA APPROVE AS SAFE?

CALCULATED ESTIMATED DAILY INTAKE (EDI):

In FDA's assessment of an additive's safety they consider the probable intake of the additive, the cumulative effect of all uses of the additive, and the relevant toxicological data needed to establish its safety (Rulis and Levitt2009). The FDA determined that the estimate for daily chronic intake was a mean of 3.1 grams per day, with the 90th percentile consumption at 6.9 grams per day. Even among the highest consumer levels (teens and men) the estimate for daily chronic intake was as low as 11.0 grams per day at the 90th percentile level. Primarily, *7* grams per day would be considered, according to the FDA, the average chronic consumption level. Because a 2-ounce bag of chips contains about 20g of olestra, the chronic consumption level would be in effect from one consuming merely about .*75* ounces daily (Prince and Welschenbach 1998), or about 8 chips daily.

Potential Problems and Benefits Associated with Olestra Consumption

What Procter and Gamble Submitted to the FDA for Olestra's Approval:

According to an article published in the Journal of the American Dietetic Association (Prince and Welschenbach 1998), Procter and Gamble provided data in five categories: the estimated daily intake (EDI), toxicological effects, olestra's effect on drug absorption, nutritional effect, and gastrointestinal effect. This study will provide both the studies submitted to the FDA before olestra's approval and the numerous studies from independent research pursuits that occurred subsequent to olestra's approval to provide an independent and comprehensive evaluation and answer not only if the FDA's decision was appropriate, but what effects of olestra that had not been found that may have been available had the FDA waited longer before approving olestra.

Toxicological Effects:

From studies performed on several animal species including rats, pigs, rabbits, mice, hamsters, and dogs, the FDA decided that the data supported the petitioner's conclusions for olestra's non-toxic and non-carcinogenic properties and that olestra is neither metabolized nor absorbed by the body(Prince and Welschenbach 1998). Many people pinpoint the fact that no human studies were conducted before FDA approval. While the reasoning behind this remains unanswered, the FDA had obviously believed that testing olestra on animals was sufficient for determining the safety for the general population's consumption.

Olestra's Potentially Negative Effects on Carotenoid Concentration, 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) and Drug Absorption

Hof and Weststrate (1995)concluded that, "Even at low doses, SPE[sucrose polyester] strongly reduces plasma carotenoid concentrations." "Sucrose Polyester and Plasma Carotenoid Concentrations in Healthy Subjects," concluded that, "Even at low doses, SPE strongly reduces plasma carotenoid concentrations. Studies suggest that carotenoids have provitamin A activity as well as antioxidant properties (Faulks and Southon 1997) and cause a reduced incidence of hypertension and cataracts (Thomson et al.1998). On the surface, therefore, this finding would appear to merit "careful consideration in its relevance to assessing the long-term health effects of SPE-containing consumer foods" (Hof and Weststrate 1995). A subsequent study, however, posits that although olestra would have an effect on potentially beneficial phytosterols, this effect would be miniscule and nutritionally insignificant (Cooper et al. 1997). Moreover, there is no scientific proof that carotenoids contribute to health and wellbeing. Therefore, the FDA determined that the assertion of olestra's negative impact on dietary carotenoid level did not warrant attention and did not deem it appropriate to force the company to add compensatory carotenoids to olestra-containing snacks (Thomson et al. 1998).

Another possible issue with olestra is that it increases fecal excretion of 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) by eight to ten fold. In turn, this reduces the elimination half-life of TCDD by about 5 years. The article's author states that these compounds may diffuse across the intestinal wall along the concentration gradient of the chemical between lumen fat and blood fat. This, according to the author, "would be expected

to increase the capacity of the lumen to take up lipophilic contaminants in the blood" (Geusau 1999). The results of this could be problematic but more needs to be assessed before conclusions can be drawn from this study.

Olestra is a lipophilic substance and therefore has the potential to affect the bioavailability of lipophilic drugs (Prince and Welschenbach 1998). In a 1990 study performed on rats, the researchers found little possibility for severe drug malabsorption due to olestra's presence. At first, the researchers hypothesized that "any effect that a nonabsorbable lipid, such as olestra, might have on the absorption of an oral drug will result primarily from interference with incorporation of that drug into the intestinal mixed-micelles by solubilization of the drug in the nonabsorbable lipid. The more lipophilic the drug is, the higher the potential for it to be solubilized by the lipid. The lipophilicity of a drug can be described by its oil-water partition coefficient…" (Miller et al. 1990) In other words, a highly lipophilic drug has far more potential to be affected by the presence of olestra simultaneously in the GI tract than a drug with modest lipophilicity. While Miller et al. (1990) found that "there is little potential for olestra to reduce absorption of oral drugs in general," it is up to competent doctors to recognize this and inform patients when olestra may affect the drugs the patient is taking. In theory, a patient's treatment can be hindered in the rare instance where olestra does inhibit a lipophilic drug's absorption and therefore a drug's efficacy is reduced. However, this is unlikely, particularly when adhering to the acceptable daily intakes established by the FDA. Another study performed on humans was also reviewed by the FDA, yet in this study, the experimental group's result data does not differ from the control group's (Prince and Welschenbach 1998), and therefore the FDA concluded, based on this study and the one conducted by Miller et al. (1990), that "olestra does not interfere with the absorption or bioavailability of lipophilic drugs…when administered at the 18g/day level" (Note that this is above the FDA approximated toxicity level). Still, it is important to underscore the fact that there is no direct proof that olestra does not hinder drug absorption and more research is warranted to assess the correlation (although minute) found by Miller et al.

GASTROINTESTINAL EFFECTS OF OLESTRA:

The FDA required two 56-day studies to determine the gastrointestinal effects plus a 4-week multicenter study on patients with inflammatory bowel disease, ulcerative colitis and Crohn's disease. Tests for those in the 4-week study were used to determine if olestra's presence in the GI tract aggravates already damaged epithelium. There was no statistically significant increase in bowel permeability, but the FDA decided that the study was too narrowly-ranged to rule out detrimental effects that olestra may have on gastrointestinal diseases (Food additive petition 7A3997 volumes 192-194 submitted Feb 1993 and volumes 183-184 submitted January 5th 1993 as cited in Prince and Welschenbach 1998).

Clinical trials following olestra's approval pointed to side effects experienced by some and sparked adverse publicity about olestra. This prompted Procter and Gamble to establish a panel of experts to independently analyze the studies relating to olestra and digestive system function. Procter and Gamble agreed to submit the panel of experts' findings regardless of their conclusions. The panel convened in September 1996 and "analyzed data from preclinical and clinical studies pertaining to the chemistry of olestra, its mechanism of action, and its potential for gastrointestinal effects" (Freston et al. 1997). An article published in *Regulatory toxicology and pharmacology* reviewed and analyzed olestra's effects on gastrointestinal functions and the symptoms experienced as a result of olestra consumption as determined by this board (Freston et al. 1997).

From the results of clinical trials, the board found that in a study in which subjects of the experimental group were consuming 18g of olestra daily rather than triglyceride, no statistically significant difference between the two groups with respect to bloating, belching, heartburn, nausea, cramping, or diarrhea was found. However, in two studies that involved consumption of 20 grams of olestra or more per day, "there was an increase in the number of symptom episodes and the total number of days on which symptoms were reported" (Freston et al. 1997). Another striking revelation about these two studies is that symptoms were reported several days after olestra ingestion. This allows for possibility that other studies of very short durations may not have had enough time elapse after olestra consumption for symptoms to appear, and therefore might have under-reported symptoms.

The group established by Procter and Gamble also reviewed studies of children. In the three studies that this board reviewed, no significantly worse incidence of symptoms has occurred in studies performed on children than on those performed in adults.

Obviously, consideration must be taken in realizing that the group of reviewers was funded by Procter and Gamble. Although this did not necessarily influence the findings of the group, it may have done so.

Post marketing surveillance for olestra via an established toll-free number reported 85 reports during the first 9 weeks. There were 65 incidences in diarrhea, 64 of cramping, and 16 of nausea among others. These are pertinent side effects that warrant attention (Freston et al. 1997). It is not known how much olestra the reporters of side-effects consumed. Not necessarily were these symptoms caused from olestra, and it is likely that at least some of the reports may have been misattributed to olestra. Nonetheless, the overwhelming number of people who suspected side-effects from olestra should necessitate more research in this area.

Other studies, including two sponsored by Procter and Gamble, including one which fed 20 to 32 grams of olestra, which, granted, is more than the FDA recommends, showed significant dose-related increases of diarrhea, loose stools, and an increase the severity and frequency of GI symptoms (Jacobson and Brown 1998). A rechallenge study confirmed this (at the 20g consumption per day level) and a similar study conducted over the course of 5 days found a significant increase in adverse GI effects at the 34g/day level.

In 1998, researchers conducted an experiment in a movie-setting (Cheskin et al. 1998) whereby subjects who were randomly selected to consume olestra chips were compared to those who were not. Of the more than 1,000 people studied, there was no statistically significant difference in gastrointestinal effects of the two groups. This serves to support the FDA decision. Note that in this study, the amount of olestra consumed by many subjects exceeded four servings. This supports the widened gap between the amount approved by the FDA (around 8 chips) and the amount consumers are actually ingesting. An important observation of this study is that consumers were given a 13-ounce bag of chips, but no measures were taken to check how much, or if any, olestra-containing (or regular) chips were actually consumed. This slightly invalidates the conclusion because the experimental group may not have consumed the chips.

In 2000, the Annals of Internal Medicine published a study that tested for steatorrhea, the presence of above-normal amounts of fat present in feces, in individuals who consumed olestra (Balasekaran et al. 2000). The purpose of the study was to determine the effect of olestra consumption on fecal fat excretion. "If olestra increases the amount of measurable fecal fat," the researchers hypothesized," physicians may suspect the malabsorption syndrome in patients who consume olestra and may subject them to unnecessary diagnostic tests" (Balasekaran et al. 2000). The researchers found that "by all three analytical methods [which

they used], apparent concentration of fecal fat was higher when the ten participants were consuming olestra chips than when they were consuming conventional chips" (Balasekaran et al. 2000). The mean number of bowel movements per day was significantly higher when subjects consumed olestra. "Olestra consumption increased stool frequency, wet stool weight, percentage of stool solids (measured by lyophilization), and fecal output of both water and solids. The output of fecal solids increased by an average of 36.2 g/d, which almost equals the quantity of Olestra ingested." (Balasekaran et al. 2000) In addition, fecal fat output increased as consumption of olestra did, a fact that seems to indicate the possibility that olestra causes loose or greasy stool. The article also mentions that "when stool from participants who were ingesting olestra was stained…more than 100 orange-red fat droplets were found per high-power field. Drop diameters ranged from 6 to 120 µm. The size and number of the fat droplets were not noticeably different after acidification and heating, which suggests that olestra was excreted intact rather than as a fatty acid" (Balasekaran et al. 2000). Nonetheless, steatorrhea, although uncomfortable, is not necessarily harmful and this would not have hindered the FDA's approval of the product.

Overall, there seems to be an increase in olestra-related side-effects in some studies where the amounts of olestra that the experimental group's subjects consumed exceeded the amount recommended by the FDA.

Does Olestra have Other Benefits Such as Lowering Low Density Lipoprotein (LDL) Cholesterol Levels or Serum Triglyceride Levels?

Studies have shown that olestra does lower LDL levels when olestra replaces 60g of saturated fat in the diet (far more than the FDA approved as safe) (Glueck et al. 1982). LDL levels were reduced without a significant reduction in High-Density Lipoprotein (HDL) levels. It is important to note that this change in cholesterol level may not necessarily be due to olestra's presence in the diet (and the possibility that it inhibits cholesterol absorption), but rather may be a mere result of lower saturated fat consumption (Miller et al. 1990).

Another study in which 50 grams of olestra was substituted for an equal weight of fat showed that olestra consumption has no effect on plasma triglyceride levels (Fallat et al. 1976). Serum triglycerides are also decreased after a meal containing olestra as compared with a meal containing triglyceride of equal amounts (as contrary to above where there was no significant relationship). Coronary blood flow was greater after olestra consumption above (Cook et al. 2000).

In general, there are health benefits of consuming olestra; however, these benefits appear when consuming olestra in exorbitant amounts. The FDA has not proved such large quantities as safe.

Does the Added Vitamin K in Olestra Affect Patients Receiving Warfarin Therapy?

Moderate intake of olestra does not seem to affect patients who are receiving long-term warfarin therapy despite the added Vitamin K in olestra (Beckey et al. 1999; Holbrook et al. 2005).

Are the Studies that were Reviewed Before FDA Approval Company-based or Otherwise Researched?

Petitioners must show the FDA proof of safety; the FDA does not have its own allocated funds for independent evaluation. Thus, the research is provided by the petitioner

seeking to get the additive approved (Nestle 1998). This addresses question three above: were the "competent scientists" company based?

Is Olestra's Intended Function of Use, Weight Loss, Likely?

There is mixed data regarding whether or not substitution of olestra for fat will result in weight loss or gain of weight. In theory, substitution of a higher caloric product with a lower caloric product would result in fewer calories consumed, and thus weight loss over the long term. Studies have demonstrated that when subjects consume lower-calorie, yet similar-appearing (portion-wise) meals, they do consume fewer calories from their meals (Bell and Rolls 2001). If one can consume the same serving size of a lower-calorie product and thereby save oneself over fifty calories per serving, thousands of calories per month, and possibly tens of thousands per year duration can theoretically be saved(Rolls and Bell 1999). In practice, however, this is may not be the case because consumers may compensate for their caloric deficits or consume more because they feel that it is a "diet" product.

The FDA approved olestra in 1996. Perhaps by examining the studies done prior to the approval date, a better analysis of the limited data available to the FDA can be obtained.

Studies Prior to or during Olestra's Approval

In a study conducted in 1994, researchers found that when lean males were fed 55 grams of sucrose polyester (far more than the FDA approved) during meals or throughout the day, they did not consume more during subsequent meals or throughout the following day. Hence, the males consuming sucrose polyester, as compared with those consuming triglyceride consumed a reduced total fat and energy intake over the 2-day study. There were no reported increases in hunger ratings (Burley et al.1994).In a subsequent study the same group fed their experimental groups of lean males the same 55 grams of sucrose polyester to replace triglyceride, but this time replacing it specifically at lunch or evening meals. The results were consistent with those from the previous study; energy intake and subjective hunger ratings (both following the meal and during the next day) of those consuming sucrose polyester were not significantly different from the control group consuming triglyceride. Again, this resulted in a decreased energy intake over the 2-day study (Cotton et al. 1996a). Other studies, such as the two studies done by Hulshof et al. (1995a and b), one by feeding normal weight adults buttered croissant preloads after a duration of food-deprivation which ranged from .25-4.75 hours and one by giving subjects high-carbohydrate preloads, seemed to agree that, when fat is replaced by a polyester substitute, a caloric deficit (and thus eventually potential weight loss) would result.

A study performed in weanling pigs, an animal with a GI tract similar to small children, suggested that olestra is not absorbed in children (like adults) (Dahler et al. 1996). Young children, however, do seem to compensate for the caloric deficit created by substituting full-fat foods for olestra-containing counterparts. In a 1993 study, 29 small children (ages 2-5) were studied to determine the compensatory effects of consuming olestra. The children were fed diets of either 10% fat or 10% non-fat triglyceride substitute. Children who consumed fat-substitute wound up consuming more on a subsequent day and the total energy intake difference was only approximately 100 kJ(kilojoules) over 2 days. Children adjusted their energy intake to replace the energy deficit from consuming the fat substitute rather than actual fat. Therefore, the use of fat-substitute did not produce a noticeable caloric deficiency in these children(Birch et al.1993).Eldridge et al. (2002)propose a rationale for this compensation: "Young children may regulate energy intake more precisely because of their high energy demands relative to body size. Food consumption in children may also be

influenced more by physiological factors than in adults in whom external factors may play a greater role." Nonetheless, the same principle is evident when olestra is fed to another group requiring high-energy needs per kg of body weight: lean young men. Apparently, it is not only children, but also other groups with higher caloric needs, that compensate calories lost by a higher consumption later on. Other authors reported that when olestra was substituted for fats in diets of lean men, the subjects completely counteracted the effect of the non-caloric replacer they were fed (namely, it created an energy deficit) and consumed more, balancing the energy intake to match the subjects who consumed the full-fat energy counterparts over course of the study (Rolls et al. 1992). This addresses question five (5) of this paper—the FDA would not have had to place targeted group warnings to those who would experience adverse effects from caloric reduction because the members of these subgroups compensate for the imposed caloric deficits.

Studies Following Olestra's Approval that May have Aided the FDA had they been Available Earlier

In a long-term study (9 months) performed by Bray et al. (2002) on overweight and obese men, subjects randomly received either a diet of 33% fat (diet A), a diet of 25% fat (diet B), or a diet of 25% fat plus 8% of their diet from a fat substitute (diet C). Diet C contained the palatability of enough fat equivalent to Diet A. Essentially, although it appeared to the subjects given Diet C that they were consuming the amount of fat present in Diet A, they were actually consuming a fat percentage resembling that of diet B, yet due to the presence of fat-substitute, made the fat content appear (or taste) larger than it actually was. Diet A serves as the control in the study; diet B mimics a traditional low-fat diet whereby fat content is reduced by 8% of total daily caloric intake, and diet C resembles the traditional diet but is actually reduced fat. While subjects of all groups were required to consume their diets, all groups were entitled to consume more of fixed-composition foods if desired. All groups lost weight over the first three months of the study, but over the next six months, members of the control (diet A) maintained the weight they reached after the three weeks, members of diet B (the low-fat diet without substituted olestra for the missing fat content) regained some weight and those consuming Diet C (the low-fat diet plus olestra to substitute for the fat) continued to lose weight (Bray et al. 2002). This study, although done after olestra was FDA approved, seems to indicate that over the long-term, when olestra replaces a portion of the diet's fat content, weight loss results. A similar study performed on women displayed that when subjects have access to products which are normally restricted on diets (such as olestra-containing potato chips) they feel less deprived and are more likely to comply with their diets (Bolton et al. 1996).

How would olestra's possible compensatory mechanism differ when olestra is found in liquids? Rolls et al. (1992) conducted a study whereby creamed soup (high-fat and non-fat (made with olestra)) was fed to subjects. This study was done on both normal-weight and obese-weight subjects and follow-up intake (the next meal) did not differ. Consequently, those consuming olestra-containing soup consumed a lower energy level over the course of the study, indicating that olestra consumption in liquid form does not promote compensatory overeating.

In another study, in groups of both normal weight and obese people of both genders, researchers substituted a tenth of some of the subjects' diets' fat at breakfast and dinner with olestra while some consumed traditional triglyceride. These results concur that total energy and fat intake was lower for subjects consuming olestra rather than triglyceride because,

although subjects did compensate for a percentage of the caloric deficit they assumed when consuming olestra, they nevertheless did not compensate for the total caloric reduction (Hill et al.1998).

Some presumed that the availability of a fat-free potato chip product would translate into a belief for consumers that they can eat as much as they desired without consequences. Because olestra provides no energy, foods made with olestra are considerably less energy dense than the full-fat options or other reduced-fat alternatives in which fat energy is substituted with carbohydrates. "There are concerns that consumption of reduced-fat foods, such as olestra, can lead to unrestrained eating if consumers believe these foods are calorie free. There is some research that consumers do not substitute equal amounts of high-fat with reduced fat foods; but rather, they may eat larger portions of reduced fat products." (Satia-Abouta et al. 2003)

Many worried that consumers would use the availability of fat-modified food products as a rationale to eat more of the reduced-fat food; they may falsely assume that it won't significantly affect weight-gain (Rolls and Miller 1997). In 1998, The American Journal of Clinical Nutrition published a study designed to test whether the knowledge that a product is fat free would result in more chips consumed by subjects. Ninety-five subjects were divided into groups of those informed about the fat-free product and those uninformed. The results demonstrated that indeed, along with knowledge that a product is "dietetic," comes a desire to consume more of that product. Unrestrained subjects consumed significantly larger amounts; however, because the product was lower in fat and calories, they consumed fewer calories as compared with the full-fat chip eaters (Miller 1998). This study seems to indicate that the FDA's approval would be worthwhile. Yet it's important to note the side effects encountered during the study among those consuming fat-free chips. The author states "There were no significant differences in the occurrence of illness or malaise related to the consumption of the two types of potato chips" (Miller 1998). This may have been the case when referring to headaches, cold, and flu symptoms, but there were five complaints of menstrual discomfort and eight reports of vertigo and dizziness from those consuming fat-free chips and no complaints for those consuming regular chips. Additional studies may be in order to determine if olestra is a potential cause for these symptoms. None of the effects or symptoms hindered subjects from continuing to participate in the study. Thus, although they may have been present, they were probably not very severe. Also, because the study was only ten days in length, it is not known if there are any long-term side effects. The study found: "No significant changes in body weight occurred in any group as a result of consuming either type of potato chip over the 10-d periods." This seems odd considering the fact that those consuming fat-free chips were ultimately consuming fewer calories from the chips. It is possible that the caloric savings were modest, and thus the product was not eaten over a long enough duration for the net calorie savings to appear as pounds lost (or not gained as compared to those eating regular chips)and that that changes in weight would have become apparent had the study continued over a longer period of time. It is also possible that the subjects were compensating the calories saved due to a stimulated appetite or subsequent hunger caused by the fat-free chips (in a similar mechanism to aspartame and other macronutrient substitutes). In either case, the study demonstrated that at least, over the short term, no significant weight loss results from the substitution of olestra in chips for fat and that indeed, consumers compensate the amount and eat more when aware that the product contains fewer calories (Miller et al. 1998).

In a study designed to measure the relationship between olestra consumption and changes in dietary intake, serum lipids, and body weight, participants were required to fill out

a thorough food questionnaire; researchers characterized subjects according to both weight and olestra intake. Subjects were "normal weight," "overweight," or "obese" and either had no olestra intake (0g/d), very low intake (0-.4g/d), low intake (.4-2g/d), or moderate/high intake (2 or more grams daily). In one ounce of a snack food containing olestra, there are eight grams of olestra, and thus those were classified as "moderate/high intake." This group was eating only one quarter or more of a serving per day, which essentially translates into only five chips per day. Only two percent of the sample fell into the high intake category. The sample included fewer than 1200 participants; thus, no more than twenty-four participants were considered to consume high intakes of olestra. As compared with a baseline clinic visit (before olestra had entered the market), carbohydrate intake increased by 37g/d and total energy increased by 209 kcal/day among high olestra consumers. Among non-olestra consumers, however, a decrease of 87 kcal/day and a carbohydrate intake decrease of 14 g/d were evident. The researchers concluded that there was no statistically significant relationship between the amount of olestra consumption and serum lipids or body weight. The article attributes the lack of significant findings to "the study sample [which] limited the ability of this study to detect significant effects" (Satia-Abouta 2003).

These findings, however, cannot be taken at face value; BMI, and, correspondingly, weight (since BMI is essentially a height-weight ratio),is not the most accurate measure of one's body fat (Burkhauser and Cawley 2008). Indeed, there are a number of factors, unrelated to body fat, which may affect a person's weight, and thereby affect his/her height-weight ratio, and therefore his/her BMI. For example, salt may cause a person to retain more water (Kumar and Berl 1998), making his/her body a heavier weight. Because the researchers only measured body weight, and not body fat, it cannot be assumed that excess body fat accompanied the excess weight gained from participants who consumed the chips. Yet this water-weight is short-lived and will disappear thereafter. Particularly because olestra is used for heavily-salty snacks, consumption of olestra would translate into increased sodium consumption as well. Thus, water retention may have caused the weight gain experienced by people in the study, and not the fat-substitute, olestra.

There is also concern that olestra may adversely affect those with binge-eating disorders: "Clinical experience has shown that persons with bulimia and other binge-eating disorders are increasingly using fat-free foods during binge episodes. Because persons with bulimia are frequently mortified by their abnormal eating behavior, the use of fat-free foods allows them to feel less guilt and provides some self justification [sic] for their behavior because they are bingeing on "healthful" foods" (Hampl and Sheeley 1998). In fact, to some bulimic people, the after-effects of consuming olestra may be considered a form of purging, as many Americans perceive olestra to have a laxative effect. (Hampl and Sheeley 1998). Apparently, this idea is believed, despite the fact that research results from a study have stated otherwise (Peters et al. 1997). Regardless of whether or not this concern is an accurate one, this prediction only became apparent after the FDA approved olestra, and hence, even if true, would not have been available to deter the FDA from allowing it to pass. More research is required in order to make definite claims for the possible danger of the binge-eating disordered population with olestra on the market.

Cotton et al. found that, in general, the lower the percentage of fat in the diet remaining after the replacement of triglyceride with olestra, the more likely caloric compensation will occur(Cotton et al. 1996b). Therefore, modest substitutions of fat in the diet would result in lower compensation and greater likelihood for weight loss.

In a post marketing surveillance study (Patterson et al. 2000), heavy consumers of olestra (consuming more than 2.0g/d) had experienced a minor weight loss effect (.55 kg/year) as compared with an even smaller weight-loss effect for those who were non-olestra consumers (.01 kg/year). In this study, olestra and weight loss seem to be insignificantly correlated. In fact, this serves to underscore the fact that, even with a large replacement of fat for olestra, large weight loss reduction does not necessarily result. Obviously, because this is merely a surveillance study, little is known about the people's eating habits excluding olestra. It is possible that consumers of olestra ate more calorie-dense foods, and thus olestra did not cause weight loss despite a significant caloric reduction. Also, it is possible that those who do not consume olestra do not eat regular chips either and are generally more health-conscience.

In sum, data on weight loss of olestra presents conflicting results from other studies. There is no reliable conclusion that can be drawn from the plethora of diverging statements and conclusions.

REITERATION OF ANSWERS TO THE EIGHT QUESTIONS ABOVE:

To recapitulate the answers to the eight questions posited: (1) Olestra can be potentially hazardous to the health of man or animal if consumed in amounts higher than those established as the toxicity level by the FDA. (2) The scientists whom the FDA used to evaluate olestra were company based. (3) A case-by-case analysis of individual studies was conducted throughout the research paper.(4) No conclusive statements can be made about olestra's cause of weight loss (or lack thereof). (5)Olestra's possible weight loss effect does not endanger subgroups that would be threatened by caloric deficiency because compensation occurs in groups who need the calories to maintain and/or gain weight. (6) Olestra seems to cause other benefits of weight loss such as lowering LDL cholesterol level and lowering serum triglyceride level when consumed in amounts greater than those stated as safe by the FDA. (7) Olestra's vitamin K addition, when olestra-containing products are consumed within FDA allowed amounts, does not interfere with warfarin therapy. (8) When consumed within FDA's proposed limits, olestra does not significantly interfere with lipophilic drugs.

CONCLUSION AND DISCUSSION

In light of the fact that FDA only approved olestra in the amount of approximately 18 grams or fewer per day, and in acknowledging that no statistically significant harmful data resulted from studies testing such small amounts of olestra, it is clear that the FDA did do a thorough assessment of the product when calculating the allowed amounts for consumption during data review. However, there seems to be a considerable mismatch between the amount of olestra the FDA approved for safe consumer use and what's actually being promoted as a safe limit and consumed. The stated serving size on a package of olestra-containing Lay's Light chips contains the amount of olestra that exceeds the amount approved by the FDA as safe. Moreover, in many of the studies reviewed (particularly those which resulted in side effects), it is clear that some people consume far more than one serving of chips. In order to avoid future side-effects experienced from olestra, there should be an emphasis placed on clearly informing the public of just how much olestra-containing product is at the safety level. Thus, better communication is vital in order to avoid potentially hazardous results.

Some may argue, due to much inconclusive or hazily concluded research (particularly papers stating that more research is necessary before making any claims), that olestra had been approved prematurely to obtaining assurance of the product's safety. While it is true that numerous studies have been conducted since olestra's approval which may have shed more light on this controversial product, the FDA may have had to weigh the possibilities of

damaging effects that might have emerged from a study conducted later against a glaring negative consequence of the delay—a possible increase in obesity that could have been lessened if olestra had been approved earlier. This is especially salient, as research bringing clear results may have taken a very long time to conduct; in fact, conclusive data from long-term studies and determination of effects are still underway.

While it is true that the FDA perhaps did approve olestra hastily and should have waited a bit longer until more published studies indicated the harmfulness (or lack thereof) of the product, it is important to remember that it is *not* the FDA's job to be assured of consumer safety; they merely have to possess a reasonable certainty that a product does not cause harm. From the data, indeed there was a reasonable certainty. At a later date, when conclusive data can be drawn from the multitudinous conflicting opinions and study outcomes, the FDA can reassess and once again determine whether olestra's market circulation is appropriate for the general population and the safety of the American consumer.

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GASTROESOPHAGEAL REFLUX DISEASE: AN OVERVIEW

Mayer Eckstein

All living organisms, including humans, require nutrients in order to survive. The way our bodies absorb these nutrients is through the digestive system. This occurs when the swallowed bolus travels through the esophagus to the stomach. From the stomach it travels to the small intestine, then to the large intestine, and it is then excreted via the rectum.

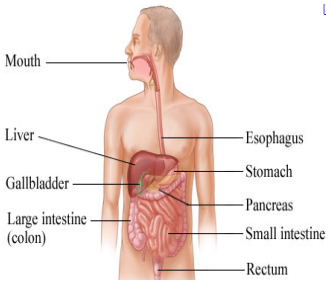

Figure 1: The Digestive System. Source: www.health.com

The question is: how does the body break down the large food particles into the nutrients we need? The answer to this is the stomach. Composed of a muscular bag, the stomach breaks down large food macromolecules. Food is released into the stomach via the lower esophageal sphincter (LES), a round muscle that controls the entrance to the stomach. Acid, released by the parietal cells of the stomach, breaks down the food into their nutrient components. The parietal cells release H^+ and Cl^- via a protein pump. These ions combine in the lumen of the stomach (otherwise they would destruct the parietal cells themselves) and form hydrochloric acid (HCl), giving the stomach a pH of ~2. The acid helps break down the food into smaller molecules. The stomach lining is protected by a mucus sheath secreted by mucus cells in the stomach lining. In addition to this, cell division replaces the stomach lining every three days (Campbell and Reece 2008).

This mixture of acid and food known as acid chyme cannot be excreted, as the acid would burn through the lining of the intestinal walls. In order to bring the pH back towards a neutral *7*, the liver and pancreas come into effect, secreting different molecules into the small intestine. The liver makes bile salts that are stored in and secreted from the gallbladder. These salts function as buffers that resist the change in pH. The pancreas secretes bicarbonate (HCO_3^-). Together, these secretions work to bring the pH level back up towards neutral (Pepitone 2010).

There are many well-known disorders associated with the digestion process, the most common of which is gastroesophageal reflux disease, more commonly known as GERD. There are six major factors that are the primary cause for GERD: impaired esophageal motility, defective mucosal defense, lower esophageal sphincter (LES) dysfunction, reflux of gastric contents, delayed gastric emptying, and hiatal hernia (DeVault and Castell 1999). Impaired esophageal motility occurs when the esophagus has trouble moving the food down and normal peristalsis does not occur. Defective mucosal defense can cause irritation to the esophagus, eliciting a burning sensation as the acid makes its way past the mucosal defense. Lower esophageal sphincter (LES) dysfunction is when the esophageal sphincter does not close properly and allows acid chyme to flow back up the esophagus. Reflux of gastric contents is similar to LES dysfunction; however, the movements and convulsions of the stomach play a major role. Delayed gastric emptying causes a back up in the stomach, which can lead to a greater chance of reflux as well as upper abdominal pain (Lewis et al. 2007). "Hiatal hernia occurs when the upper part of the stomach moves up into the chest through a small opening in the diaphragm….Some doctors believe a hiatal hernia may weaken the LES and increase the risk for gastroesophageal reflux" (Nazario 2009).

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The symptoms of GERD vary depending on the individual. The most common adult symptoms are pyrosis, regurgitation, non-cardiac chest pain, and trouble swallowing (dysphagia).

Pyrosis, commonly referred to as heartburn, is described as a "burning, tight sensation that is felt intermittently beneath the sternum and spreads upward to the throat or jaw" (Lewis et al. 2007). Pyrosis occurs when any of the six factors that can cause GERD disrupt the normal digestive system. GERD then causes an inflammation in the chest, giving a burning sensation.

Regurgitation, another sign of GERD, usually occurs due to the buildup of undigested food near the LES. Due to the inability of digesting these food particles, the body rids itself of them by vomiting up the undigested food.

Dysphagia, or the inability to swallow, is a more complex problem then it may appear. "Patients are at a high risk of aspiration due to food or liquids going the wrong way into the lungs" (Logemann 1998). Dysphagia can also result in dehydration, malnutrition, and renal failure. Some symptoms of dysphagia include the inability to control food or saliva in the mouth, difficulty initiating a swallow, coughing, and choking. The most common symptom of dysphagia is the inability to swallow food, which the patient will describe as 'becoming stuck' or 'held up' before it either passes into the stomach or is regurgitated (Logemann 1998).

The symptoms of GERD in children are different than those of adults. Children with GERD may have any one or more of the following: colic-like symptoms, excessive vomiting, bad breath, refusal to eat, swallowing problems, excessive burping after nursing, nighttime coughing. Although most babies have the GERD symptom of mild vomiting, commonly known as 'spitting up,' most of them will outgrow it once they get a few months older and are able to sit up properly. Pediatric GERD is also a common cause of weight-gaining problems in infants (Winter 2008).

GERD can also cause some lesser common diseases. Among them are diseases such as Barrett's esophagus, esophageal ulcers, esophageal adenocarcinoma, erosion of teeth enamel, and esophageal strictures. Although these diseases are less common, their severity is not lessened by their rarity (Lewis et al. 2007).

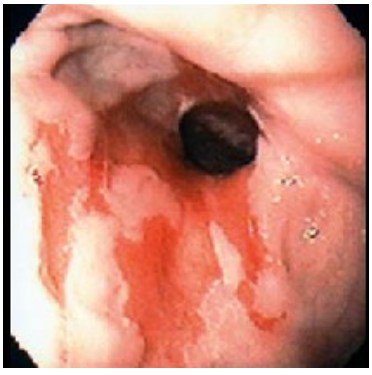

Figure 2: Barrett's esophagus. Source: www.medscape.com

Barrett's esophagus is usually caused by chronic reflux but is also sometimes caused by ingesting a corrosive substance. The acid in the reflux damages the columnar epithelium tissue in the lower esophagus. The changed form of cells is called "premalignant," because, although they are not cancerous themselves, they significantly raise the risk of esophageal cancer. Barrett's esophagus has other symptoms that evolve from the changed tissue, including chronic heartburn, trouble swallowing (dysphagia),

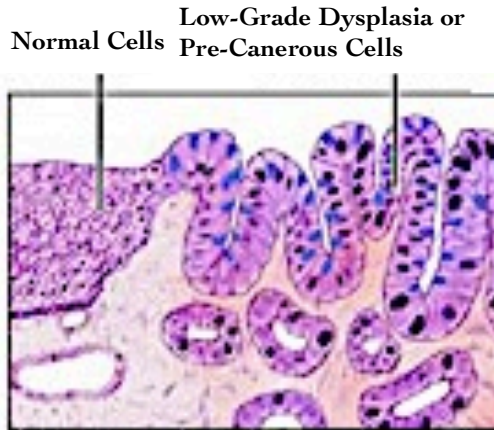

Figure 3: Change in Epithelial Tissue due to Barrett's Esophagus. Source: www.mayoclinic.com

regurgitating blood, noncardiac-related pain, and weight loss (Mayo Clinic 2011).

Another complication that GERD causes is the formation of ulcers. An ulcer is an erosion of a mucus-covered membrane and, if in a highly acidic area, can be very painful. An esophageal ulcer is a hole in the lining of the esophagus corroded by the acid chyme refluxed back up through the LES. Esophageal ulcers are usually located in the lower section of the esophagus, which is closer to the LES. Esophageal ulcers cause pain that is felt behind or just below the sternum, similar to heartburn symptoms. Chronic and severe recurrences of esophageal ulcers can cause esophageal strictures, a narrowing of the esophagus after the thicker scar tissue layer forms. Symptoms of esophageal ulcers can include heartburn, inflammation of the esophagus, the vomiting of black or bright-red-colored blood, and bloody or tarry foul-smelling stool (due to oxidized iron from hemoglobin) (Sameul 2008).

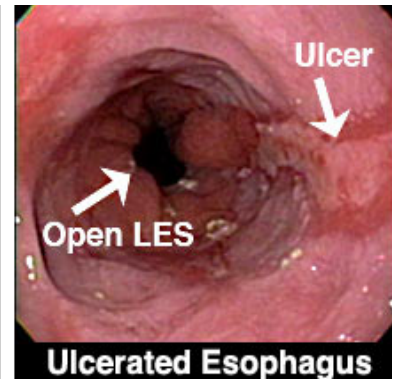

Figure 4 Esophageal Ulcer. Source: stomach-ulcer-symptoms.com

GERD can also cause esophageal adenocarcinoma, a form of esophageal cancer. Most commonly occurring in Caucasian men over the age of 60 (Chou and Gress 2006), adenocarcinoma comes from glandular cells at the junction of the esophagus and stomach, right above the LES. The most dangerous aspect of this disease is that most people diagnosed with esophageal cancer are already in the later stage of the disease. This is because significant symptoms do not usually appear until half of the inside of the esophagus is obstructed, by which point the tumor has already grown big. Because of this, in some severe cases, parts of the esophagus are removed due to the spread of the cancerous cells. Another problem of late recognition is that the cancer can spread and infect the rest of the body, spreading the disease to the entire digestive system, including the liver and pancreas. However, under chemotherapy, the tumor may shrink enough for it to be removed in a standard operating procedure. The risk of esophageal cancer is much higher for a patient with Barrett's esophagus. This is because Barrett's esophagus changes the tissue in the esophagus to a pre-malignant stage (Mayo Clinic 2011).

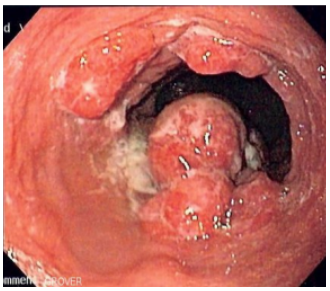

Figure 6: Esophageal Cancer Source: disease-picture.com

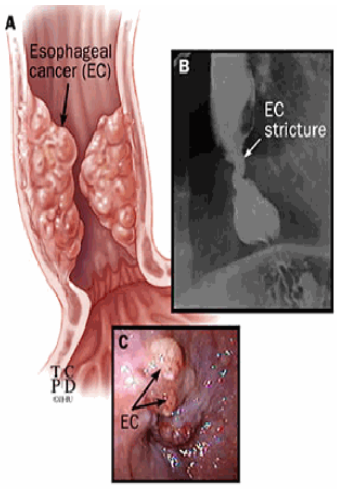

Figure 5: Esophageal cancer (A) with corresponding barium swallow x-ray (B) and endoscopic view (C). Source: disease-picture.com

The symptoms of esophageal adenocarcinoma vary greatly depending on the severity of the disease. Among the most common symptoms are: dysphagia (difficulty swallowing), odynophagia (painful swallowing), substantial weight loss (due to reduced appetite and poor nutrition), pain (usually a burning, heartburn-like feeling), husky, raspy, or hoarse sounding cough, (as a result of the tumor obstructing the airway). The presence of the tumor may disrupt normal peristalsis of the esophagus, leading to nausea, vomiting, coughing, and a

feeling of 'something stuck' in the throat. If the ability to swallow becomes too restricted, a stent can be surgically placed in the esophagus. The stent widens the esophagus, easing swallowing (Lewis et al. 2007).

Another side effect of GERD is the erosion of dental enamel. The main mineral of enamel is hydroxylapatite, which is a crystalline calcium phosphate. High acidity levels in the mouth allow bacteria to thrive and enter into the crevices of the enamel, causing cavities. GERD plays a major role in the pH level of the mouth. Usually the alkalinity of the saliva alone is enough to counter and neutralize the ingestion of acidic products. However, when a patient suffers from GERD, the acidity level in the mouth becomes much greater and overpowers the basic saliva. This usually occurs at night when the patient is sleeping in a supine position and the acid chyme is able to make it all the way back up into the oral cavity (Fried 2010).

Figure 7: Tooth Erosion. Source: www.colgate.com

The erosion of enamel is very serious because as the enamel continues to become less mineralized, it is unable to prevent the encroachment of bacteria, and the underlying dentin becomes affected as well. When dentin, which normally supports enamel, is destroyed by decay, enamel is unable to compensate for its brittleness and breaks away from the tooth easily. This leads to the complete destruction of the upper layers of the tooth, requiring either a replacement crown or a dental implant (Fried 2010).

The chronic occurrence of GERD can cause esophageal strictures. Esophageal strictures are when the esophagus narrows, making it hard to swallow. If a person suffers from GERD, the acid reflux inflames the esophagus. When the esophagus heals, scar tissue grows thick to prevent reoccurrence. This causes the tissue to pull and tighten, which can lead to difficulty swallowing. During healing, a change in texture of the esophagus also occurs. Instead of consisting of very soft tissue, the strictures cause the walls of the esophagus to harden. This can cause severe pain when swallowing (Logemann 1998).

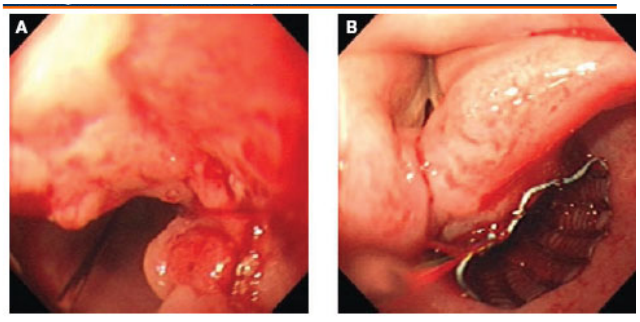

Figure 8: Esophageal Stricture. Source: www.medscape.com

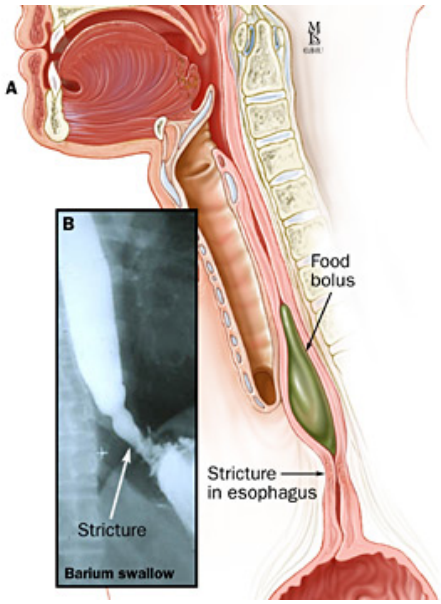

Figure 9: Esophageal Stricture.
Source: www.hopkins-gi.org

Esophageal strictures may also be cancerous. As mentioned above, (see Barrett's esophagus) the change in the tissue of the esophagus can spread and infect other cells and cause those cells to become cancerous. Symptoms of esophageal strictures include heartburn, choking, coughing, shortness of breath, frequent burping and hiccupping, a bitter or acidic taste in the mouth, pain or trouble swallowing, the vomiting of blood, and unintentional weight loss (Drugs.com 2011).

There are a few major ways in which a doctor can diagnose a patient with GERD. Among the most commonly used methods are esophageal pH monitoring, X-rays (barium swallow), esophagogastroduodenoscopy (EGD), and esophageal manometry.

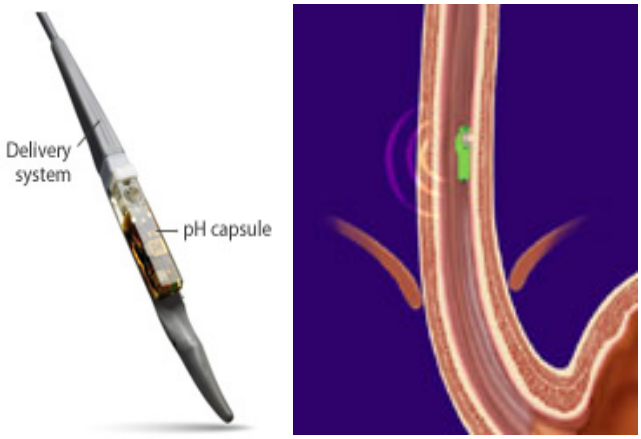

Figure 10: Esophageal pH monitor.
Source: www.givenimaging.com

Esophageal pH monitoring measures the acidity level in the esophagus. The latest technological way to test the pH involves the use of a Bravo pH monitor. The procedure of the Bravo capsule is as follows:

You will be asked to sit or lie back while the physician places the capsule into the esophagus. After the capsule is in place, suction is applied, drawing a small amount of tissue into the capsule. The capsule is then locked into place. The placement procedure is simple to perform and well tolerated by most patients. The capsule begins measuring the pH levels of the esophagus immediately, transmitting pH measurements wirelessly to a small receiver worn on your waistband or belt. The receiver houses three symptom buttons, and you will be asked to press the corresponding button when you experience heartburn, regurgitation, or chest pain during the procedure… The disposable capsule will spontaneously detach and pass naturally through a bowel movement a few days after the test (Given Imaging).

A physician may also utilize X-rays to determine if the patient has GERD. The patient is first given a radio contrasting solution that improves the visibility of internal structures. The most commonly used radio contrast for this procedure is barium sulfate. Barium sulfate is insoluble when mixed with water, and in the X-ray, reveals the internal structures in a cloudy white color. The X-ray can reveal the movement of acid chyme backing up toward the esophagus. Although barium is a heavy metal, and its water-soluble compounds can be highly toxic, the low solubility of barium sulfate protects the patient from absorbing harmful amounts and is secreted out of the body along with regular fecal matter (Lerner 2003; Fallon and Shratter 2012)

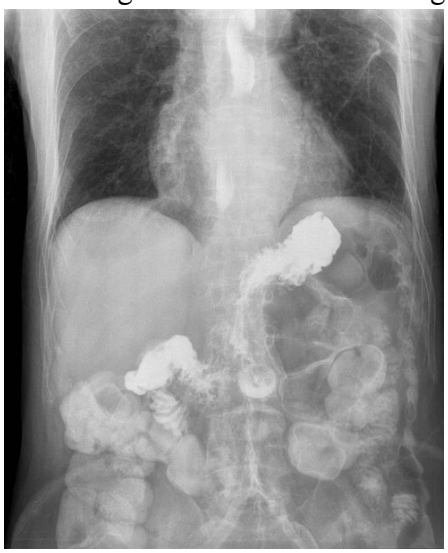

Figure 11: Barium Swallow X-Ray Source:http://en.wikibooks.org/wiki/Medical_Physiology/Gastrointestinal_Physiology#Graft_v. _Host_Disease

Another way a doctor may test for GERD is with an esophagogastroduodenoscopy (EGD). More commonly referred to as an endoscopy, it is a 15-20 minute, minimally invasive procedure that images the esophagus. Endoscopy involves passing an endoscope, a long, flexible black tube with a light and camera on the end, through the mouth to examine the esophagus, stomach, and small intestine. The camera sends a live feed to a monitor. In some cases a biopsy may be taken together with an endoscopy. The biopsy usually takes a small sampling of 1 to 3 mm of tissue from the esophagus via a forceps inserted into the endoscope. The sample is then sent to a laboratory for further histological testing. There are some possible complications with an endoscopy, such as the possibility of tearing the esophagus or stomach. However, the procedure has less than a 1 in 1000 chance of a serious complication occurring (Barrettsinfo.com).

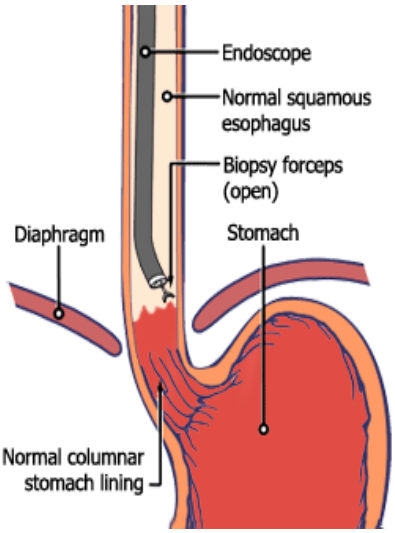

Figure 12: Endoscopy Procedure. Source: www.barrettsinfo.com

Esophageal manometry is another method for determining if a patient has GERD. A manometry is used to determine if the peristalsis of the esophagus is functioning correctly. During an esophageal manometry, a thin, pressure-sensitive tube called a catheter is passed through the mouth into the stomach. Once in place, the tube is pulled slowly back into the esophagus. When the tube is in the esophagus, the patient will be asked to swallow. The pressure of the muscle contractions are measured along several sections of the tube. The data is graphed by a computer, and the results show the peristalsis of the esophagus. Abnormal peristalsis can be a determining factor in diagnosing a patient with GERD

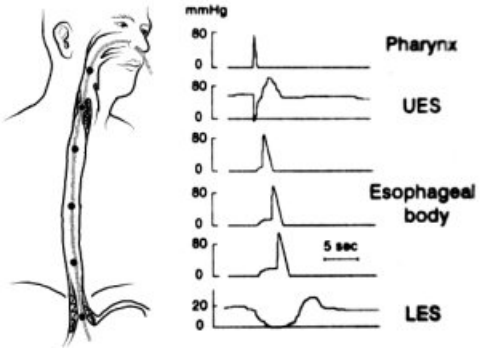

Figure 13: Esophageal Manometry Graph. Source: www.ctsnet.org

(Longstreth 2011).

There are various treatments a doctor can use to treat a patient with GERD. There are three general categories of treatments: medication, life style changes, and surgery. There are five main classes of drugs a doctor prescribes for GERD: H_2 receptor blockers, H^+ pump inhibitors, antacids, foaming agents, and prokinetics.

H_2 receptor blockers work by acting as anti-histamines at the H_2 receptors. This suppresses the acid secretion, which reduces gastric juice volume, decreasing reflux reoccurrence. A few common H_2 receptor blockers are cimetidine (Tagamet®), famotidine (Pepcid®), nizatidine (Axid®), and rantidine (Zantac®) (HealthCentral.com).

Doctors also prescribe H^+ pump inhibitors. H^+ pump inhibitors prevent the secretion of H^+ ions from the parietal cells. This slows the formation of hydrochloric acid in the lumen of the stomach, effectively raising the pH in a short period of time. H^+ pump inhibitors include esomeprazole (Nexium®), lansoprazole (Prevacid®), omeprazole (Prilosec®), pantoprazole (Prononix®), and rabeprazole (Aciphex®) (Cohen 2007; Lewis et al. 2007).

Antacids are another category of treatment used to relieve GERD. Antacids neutralize gastric acid by acting as buffers to help raise the pH, reducing acidity in the stomach. Although some antacids were found to actually raise the pH level in the stomach, all antacids reduced acidity in the lower esophagus, relieving the symptoms of GERD. Antacids also promote ulcer healing and reduce reoccurrence. There are two types of antacids: absorbable and non-absorbable. Absorbable antacids usually contain sodium bicarbonate ($NaHCO_3$) or calcium carbonate ($CaCO_3$) and undergo complete neutralization. The most common absorbable antacids are Alka-Seltzer® ($NaHCO_3$), Maalox tablets® ($CaCO_3$), and Tums® ($CaCO_3$). Among the most common non-absorbable antacids are Maalox liquid® ($Al(OH)_3$ and $Mg(OH)_2$), Mylanta® ($Al(OH)_3$), and Milk of Magnesia® ($Mg(OH)_2$). Another common antacid is Pepto-Bismol® ($C_7H_5BiO_4$) (Brown et al. 2006).

Both classes of antacids share common side effects in addition to their individual side effects. A common shared side effect is a problem with reduced acidity, which results in the inability to digest and absorb essential nutrients. Low acidity can also lead to infections; high acidity usually kills ingested bacteria, so when the pH becomes more basic, bacteria are able to survive. Too much absorbable antacid consisting of sodium (Na) can increase blood pressure as well as cause kidney disease. High levels of carbonate (CO_3) can cause kidney stones, and the high level of CO_2 output can distend and stretch the intestinal walls. Although the side effects of non-absorbable antacids are fewer, high levels of $Mg(OH)_2$ can lead to hypermagnesium, causing cardiovascular and neurological complications. An excess amount of $Al(OH)_3$ can cause formation of insoluble $Al(PO_4)$ complexes, leading to decreased phosphate levels in the blood (Decktor et al. 1995).

A doctor may also suggest the use of foaming agents to relieve GERD. Foaming agents coat the stomach and esophagus, preventing the acid chyme from reaching the mucosal layer. Common foaming agents include Gaviscon® and sucralfate (Carafate®) (NDDIC 2008).

Another treatment for GERD involves the use of prokinetics. Prokinetics are drugs that increases the frequency of contractions in the small intestine, thereby making them stronger. Prokinetics also assist in strengthening the LES, and helps empty the stomach faster. Metoclopramide (Reglan®) and bethanechol (Urecholine®) are the two most common prokinetics (Lewis et al. 2007).

Instead of drugs, or in addition to them, doctors also recommend various life style changes to prevent reoccurrences of GERD. Doctors suggest changes that lower the chance of reflux. These include, but are not limited to, eating smaller meals, sleeping with the head

raised, wearing looser clothing to relieve stomach pressure, losing weight to reduce intra-abdominal pressure, quitting smoking in order to increase the LES's competence, and avoiding foods known to cause reflux, including fatty foods, chocolate, peppermint, caffeine products, alcohol, and acid based products (NDDIC 2008; Eckstein 2012).

In the most serious of cases, when a hiatal hernia or esophageal stricture is involved, and if conservative therapy fails, surgery may be recommended. The surgery is referred to as a Nissen fundoplication. During the surgery, the upper section of the stomach is wrapped around the LES in order to strengthen it. Nissen fundoplication is a minimally invasive surgery known as a laparoscopy, which decreases complications and the overall cost of hospitalization (Lewis et al. 2007).

Endoscopic radiofrequency is another procedure used. "During this procedure a balloon-tipped four-needle catheter, called a Stretta device, delivers radiofrequency energy to the smooth muscle of the LES. The radiofrequency energy induces collagen contraction, which helps form a barrier against reflux" (Lewis et al. 2007).

GERD is a common illness, affecting about a third of the American population, according to the International Foundation for Functional Gastrointestinal Disorders (IFFGD). In most cases it is easily manageable and with proper care, reoccurrence can be prevented. In the most severe cases, GERD may cause life-threatening illnesses such as esophageal adenocarcinoma. Doctors use various methods for diagnosing and treating patients. Treatment may be as simple as life style changes or as complex as surgery. If one suspects that he or she is suffering from GERD, a competent medical doctor should be consulted.

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ALTEPLASE: THE CLOT-BUSTER

Mayer Goldberg

INTRODUCTION:

The human body is defined by many complex and inconsistent characteristics. For example, the body forms blood clots in traumatic events, but blood clots are likewise associated with hazardous or fatal conditions. Platelets are small cells, derived from the precursor megakaryocytes, which are responsible for blood clotting to prevent bleeding from a ruptured blood vessel. Platelets fasten to each other and release a coagulation factor that solidifies around the ruptured area to prevent blood loss. A subordinate quantity of platelets can result in excessive bleeding. However, high platelet numbers can progress into blood clots, which may potentially be dangerous because they produce a condition known as thrombosis. Thrombosis precedes harmful conditions such as stroke, myocardial infarction, pulmonary embolism, and other conditions associated with the disruption of circulatory blood flow.

Depending on the severity of the condition, there are different treatments available for thrombosis. Some patients may require surgery for an illness caused by thrombosis. In other instances, there are thrombolytic or fibrinolytic drugs that can be used as medicinal therapy to dissolve blood clots. Thrombolytic drugs disintegrate blood clots by converting plasminogen into plasmin. The plasmin, a serine protease, actually does the blood clot dissolving. The most important thrombolytic drug categories include tissue plasminogen activator and streptokinase (Klabunde 2005). While there are two significant families of thrombolytic medications that can be utilized in thrombolytic therapy, alteplase, a recombinant tissue plasminogen activator, is the preferred medication for treatment of acute ischemic stroke, myocardial infarction with ST elevation, and, in some rare cases, pulmonary embolism.

DISCUSSION:

THROMBOLYTIC THERAPY FOR STROKE

For approximately a decade, stroke has been the third leading cause of death in the United States following cardiac diseases and cancer. Roughly 137,000 Americans die from stroke every year (Center for Disease Control 2009). Since stroke can cause brain cell damage or cell death, patients often exhibit sudden numbness or paralysis of the face, legs, or arms. Thus, stroke is the primary cause of permanent disabilities. There are two general classifications of stroke: ischemic strokes and hemorrhagic strokes.

ISCHEMIC STROKES

Almost 90% of strokes are ischemic strokes, caused by decreased blood flow to the brain (Swanson 1998b). Whenever normal blood flow to the brain is interrupted, the neurons begin to die within minutes. Since brain cells require oxygen for survival, and they acquire oxygen from red blood cells, they begin to decline in function (see figure 1). There are two subcategories of ischemic strokes: thrombotic and embolic. A thrombotic stroke is caused by the formation of a blockage, thrombus, in one of the arteries supplying the brain with blood. A thrombus forms from arteriosclerosis, which is created by an accumulation of fatty deposits. An embolic stroke occurs when the body carries a blood clot from an alternate origination to a blood vessel in the brain. For example, atrial fibrillation in one of the two upper chambers in the heart can proceed to the formation of a blood clot that will travel elsewhere in the body. Thrombolytic drugs are utilized in ischemic strokes to bust open the thrombus or embolus. In

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the 1980s, the first drug developed for the treatment of stroke was streptokinase, but this medication was abandoned after it caused unacceptable incidence of cerebral hemorrhage (Saver and Lutsep 1995). Alteplase was developed, and research found it to be a safer and more efficient choice for thrombolytic therapy in ischemic stroke patients. An alternate study showed the efficacy of alteplase administered to acute ischemic stroke patients up to four hours after the onset of symptoms.

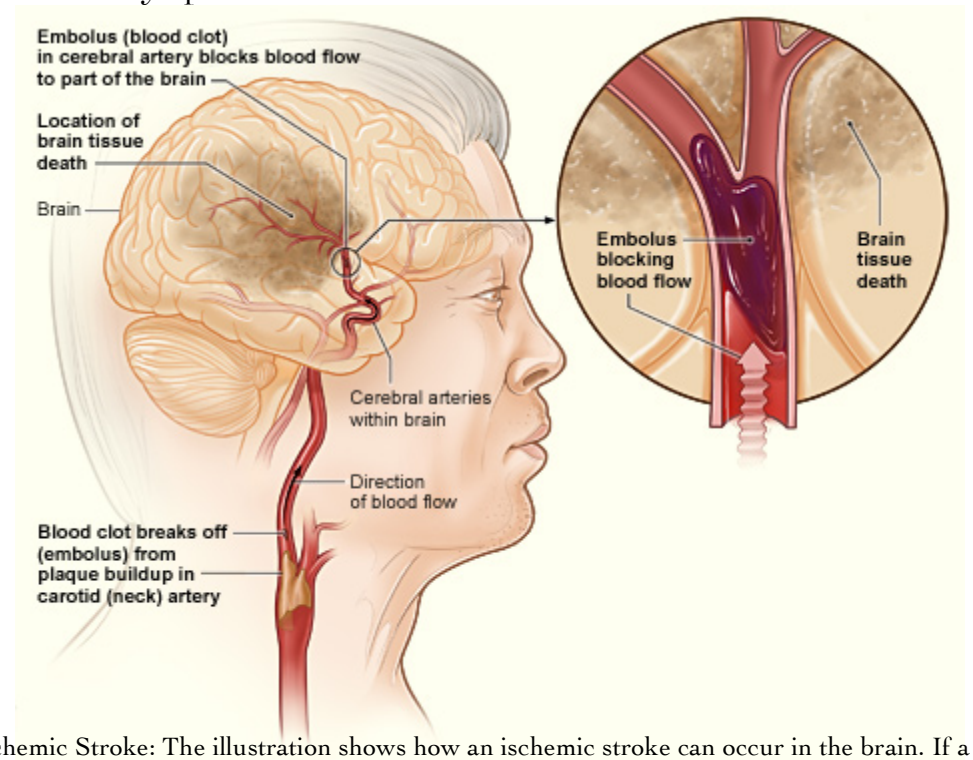

Figure 1: Ischemic Stroke: The illustration shows how an ischemic stroke can occur in the brain. If a blood clot breaks away from plaque buildup in a carotid (neck) artery, it can travel to and lodge in an artery in the brain. The clot can block blood flow to part of the brain, causing brain tissue death. Source: http://www.nhlbi.nih.gov/health/dci/images/stroke_ischemic.jpg

Patients were admitted or excluded from the alteplase clinical trial based on various elements. Some of the inclusion criteria for the acute ischemic stroke trial were the ability to have the study drug administered between 3 and 4.5 hours after the onset of stroke symptoms and the presence of stroke symptoms for at least 30 minutes with no significant improvement. Some rejection criteria were intracranial hemorrhage, seizures at the onset of stroke, and serious head injury within the previous three months. For a complete list of selective and dismissive criteria see table 1(Hacke et al. 2008).

A total of 821 patients enrolled in the trial and were randomly assigned to either the alteplase group or a placebo group. The alteplase group had 418 patients, and the placebo group had 403 patients. Approximately 82 patients were administered the drug or a placebo between 3 and 3.5 hours, 384 patients obtained it between 3.5 and 4 hours, and 322 received it between 4 and 4.5 hours. Of the remaining patients, treatment time for 12 patients from the alteplase group and 15 patients from the placebo group were not obtainable. Additionally, one patient from the alteplase group and five patients from the placebo group were administered treatment after 4.5 hours. The trial result was a ratio of 41.6% to 36.7% in favor of the alteplase group. Although the results indicated more symptomatic intracranial hemorrhage

Table 1: Major exclusion and inclusion criteria.

Main inclusion criteria
Acute ischemic stroke
Age, 18 to 80 years
Onset of stroke symptoms 3 to 4.5 hours before initiation of study-drug administration
Stroke symptoms present for at least 30 minutes with no significant improvement before treatment
Main exclusion criteria
Intracranial hemorrhage
Time of symptom onset unknown
Symptoms rapidly improving or only minor before start of infusion
Severe stroke as assessed clinically (e.g., NIHSS score >25) or by appropriate imaging techniques*
Seizure at the onset of stroke
Stroke or serious head trauma within the previous 3 months
Combination of previous stroke and diabetes mellitus
Administration of heparin within the 48 hours preceding the onset of stroke, with an activated partial-thromboplastin time at presentation exceeding the upper limit of the normal range
Platelet count of less than 100,000 per cubic millimeter
Systolic pressure greater than 185 mm Hg or diastolic pressure greater than 110 mm Hg, or aggressive treatment (intravenous medication) necessary to reduce blood pressure to these limits
Blood glucose less than 50 mg per deciliter or greater than 400 mg per deciliter
Symptoms suggestive of subarachnoid hemorrhage, even if CT scan was normal
Oral anticoagulant treatment
Major surgery or severe trauma within the previous 3 months
Other major disorders associated with an increased risk of bleeding

* A severe stroke as assessed by imaging was defined as a stroke involving more than one third of the middle cerebral-artery territory. NIHSS denotes National Institutes of Health Stroke Scale in which total scores range from 0 to 42, with higher values reflecting more severe cerebral infarcts.

Source: Hacke et al. 2008

in the alteplase group than in the placebo group, the overall outcome of the trial showed a more favorable result for the alteplase group in the 4.5 hours range. This indicates that supplying alteplase intravenously can clinically better the condition of ischemic stroke patients up until 4.5 hours following the incipience of symptoms (Hacke et al. 2008).

Hemorrhagic Strokes

Hemorrhagic strokes take place following a rupture or leak from a blood vessel in the brain (Figure 2). There are two subdivisions of hemorrhagic strokes: intracerebral hemorrhage and subarachnoid hemorrhage (Swanson 1998a). In an intracerebral hemorrhagic stroke, a blood vessel bursts causing blood seepage into brain tissue. The sudden increase in pressure within the brain can cause cellular damage. Furthermore, the brain cells beyond the ruptured blood vessel are damaged due to the interruption of blood flow and oxygen delivery. A subarachnoid hemorrhagic stroke is caused by leakage from a blood vessel into the space between the surface of the skull and the brain. Hemorrhagic strokes are not treated with thrombolytic therapy because of the major risk of further hemorrhaging.

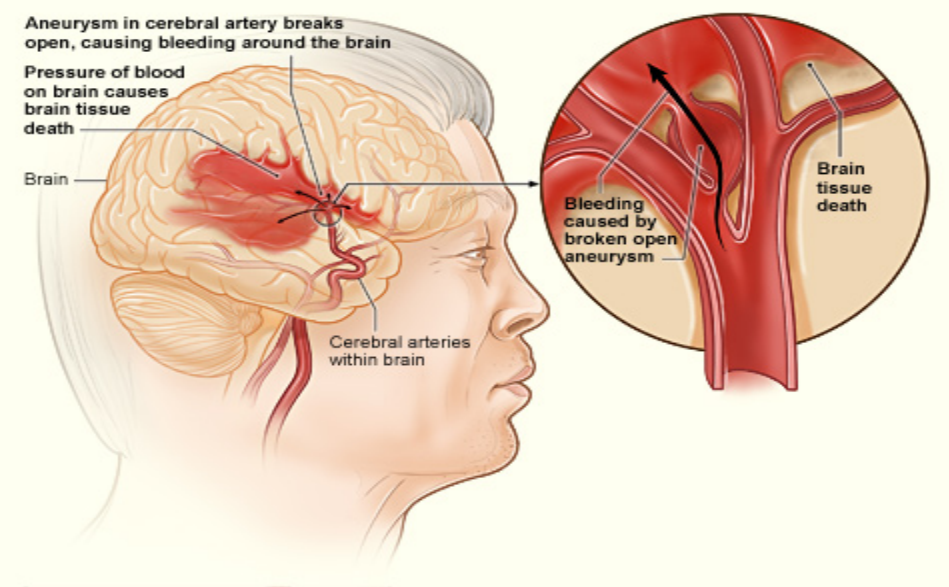

Figure 2: Hemorrhagic Stroke: The illustration shows how a hemorrhagic stroke can occur in the brain. An aneurysm in a cerebral artery breaks open, which causes bleeding in the brain. The pressure of the blood causes brain tissue death.
Source: http://www.nhlbi.nih.gov/health/dci/images/stroke_hemorrhagic.jpg

Thrombolytic Therapy for Myocardial Infarction

Major heart disease is the leading cause of death in the United States. According to the CDC, nearly 48% of all deaths reported in 2010 were cardiac related. Heart attack, also known as acute myocardial infarction, happens when there is an interruption of the blood

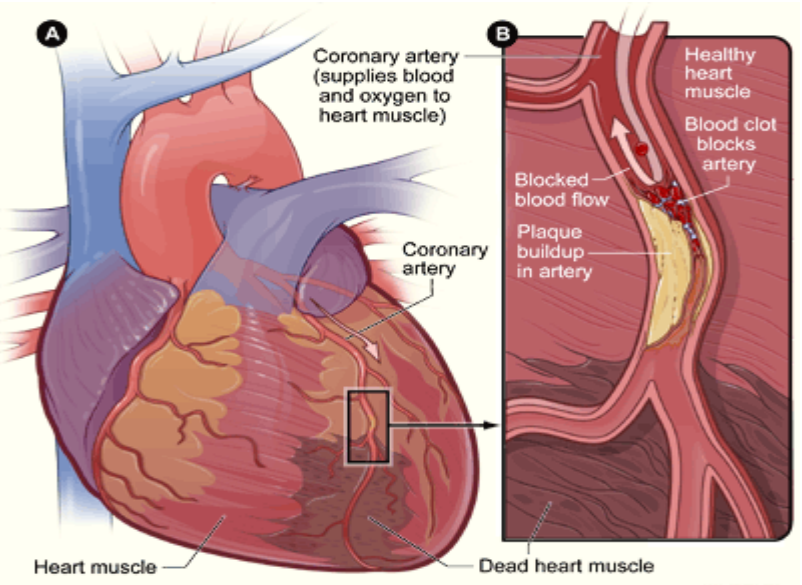

Figure 3: Heart with Muscle Damage and a Blocked Artery: Figure A shows a heart with dead heart muscle caused by a heart attack. Figure B is a cross-section of a coronary artery with plaque buildup and a blood clot.
Source: http://www.nhlbi.nih.gov/health/dci/images/heart_coronary_artery.gif

flow to the heart, causing the cardiac muscles to die (Pub Med Health 2010a). Many heart attacks are caused by blood clots forming an obstruction in one of the coronary arteries, which carries blood and oxygen to the heart tissues. The blockage disrupts the oxygen flow to heart tissues, causing heart muscle cell death (Figure 3). Atherosclerosis, a principle cause of blockages, is the accretion of plaque that adheres to the coronary artery walls and forms an occlusion. The plaque may tear, and blood platelets may fasten to it forming a thrombus (Pub Med Health 2010a). A myocardial infarction (MI) can be classified as a non-ST elevation MI or an ST elevation MI based on electrocardiogram changes (Cleveland Clinic 2009).

MYOCARDIAL INFARCTION WITH NON-ST SEGMENT ELEVATION

A non-ST elevation MI does not exhibit changes on an electrocardiogram. Additionally, the extent of damage is minimal since the artery is only partially obstructed (Cleveland Clinic 2009). According to the American Heart Association, there is a close correlation between non-ST elevation MI and unstable angina. Unstable angina is a condition caused by a diminished supply of blood flow and oxygen to the heart; as research indicates, it can be an introduction to a heart attack (Pub Med Health 2010b). Non-ST elevation MI and unstable angina are treated by anti-platelet agents, anti-thrombin agents, or anti-ischemic agents (Jevon et al. 2008). Although the treatments for non-ST elevation myocardial infarction and unstable angina are similar, there is a different course of action taken for a myocardial infarction with ST elevation.

MYOCARDIAL INFARCTION WITH ST SEGMENT ELEVATION

An ST-elevation MI is caused by the prolonged obstruction of blood supply that affects a large portion of the heart. Thus, ST segment changes on an electrocardiogram are clearly visible and are easily detected (Cleveland Clinic 2009). If ST-elevation MI is detected early enough, thrombolytic treatment can be an essential part in its treatment as thrombolytics restore blood flow, thus decreasing casualties. Thrombolytic treatment is the preferred treatment for ST segment myocardial infarction, because it is the most effective at achieving reperfusion. Thrombolytic treatment can effectively restore blood flow when administered within 12 hours of symptom onset, but maximum benefit is obtained when administered promptly (Hilleman et al. 2007).

The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries, known as the Gusto-I trial, was conducted to compare the efficacy of streptokinase and alteplase in ST segment myocardial infarction. Additionally, the study determined whether earlier and sustained reperfusion with alteplase improves the survival rate in people with acute myocardial infarction. A total of 41,021 patients within six hours of the onset of acute myocardial infarction with ST segmentation were randomly assigned to one of four thrombolytic treatments: streptokinase, streptokinase with intravenous heparin, accelerated tissue plasminogen activator, or tissue plasminogen activator with intravenous heparin. The primary follow-up and end point of the trial was 30 days (Hacke et al. 2008).

After the 30 days, the results indicated that patients receiving accelerated tissue plasminogen activator had a more significant reduction in mortality during the 30-day period than those administered streptokinase and any other combined strategies. There was, however, an increase of hemorrhagic stroke episodes in accelerated tissue plasminogen activator patients above the others. Still, the overall endpoint of death or permanent disability was lower in patients administered accelerated tissue plasminogen activator than in patients administered any other combination. A Gusto-III trial was soon established to determine if

reteplase, a newer recombinant tissue plasminogen activator, was better than alteplase. More than 15,000 patients, all within six hours of the onset of symptoms, entered the trial. The 30-day mortality rate for reteplase did not exhibit any additional survival benefits in acute myocardial infarction (Hacke et al. 2008).

THROMBOLYTIC THERAPY FOR PULMONARY EMBOLISM

A pulmonary embolism is an occluded artery that disrupts the blood flow to the lungs. Deep vein thrombosis, the most common cause of pulmonary embolism, occurs when an embolus that originated in a deep thigh vein travels up to the lungs and blocks the blood flow (Fischbein 1981). Pulmonary embolism is an extremely dangerous condition, since it may cause damage to lung tissue due to a diminished oxygen supply. Additionally, it can damage other organs due to a lack of oxygen and, in some severe cases, may even cause death. Pulmonary embolism affects men as well as women. If the pulmonary embolism is caused by deep vein thrombosis, patients may exhibit symptoms such as swelling of the leg, pain or tenderness in the leg, or a discoloration of the skin on the affected area. Pulmonary embolism is responsible for 10% of all deaths, and an initial diagnosis is not straightforward. In fact, in 70% of all pulmonary embolism patients, it was not clinically suspected but confirmed after an autopsy (Davidson 1999) (Figure 4).

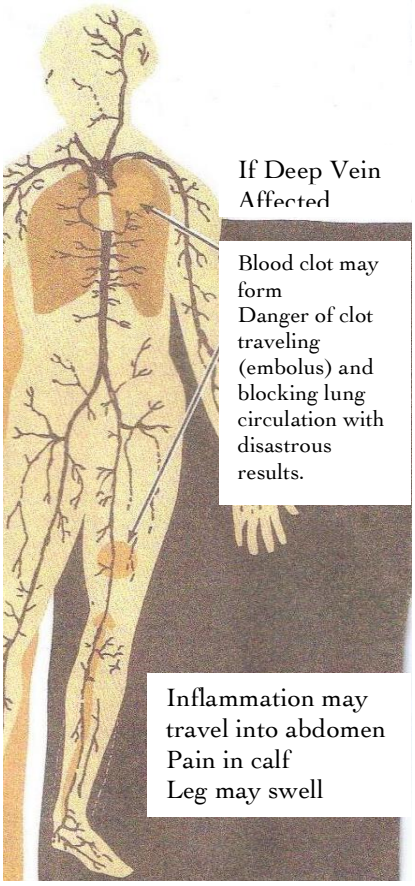

Figure 4: Deep vein thrombosis can cause pulmonary embolism
Source: Fischbein 1981

A majority of patients with pulmonary embolism are treated with anticoagulation medication. Anticoagulation therapy is beneficial in the treatment of pulmonary embolism, because it provides prophylaxis against further thromboembolic events while the body's own fibrinolytic system gradually lyses the embolus. Since dissolving the clot is the treatment for pulmonary embolism, many have wondered if thrombolytic therapy would be even more efficient as thrombolytic drugs dissolve clots more rapidly than endogeneous fibrinolytic activity can. There was a study comparing the efficacy of recombinant tissue plasminogen activator and anticoagulants in pulmonary embolism. As part of the study, 790 stable patients with pulmonary embolism were randomly assigned to either the heparin (anticoagulant) group or the alteplase (recombinant tPA) group. While 169 patients received recombinant tissue plasminogen activator, 550 patients received heparin alone. The overall 30-day mortality rate for patients administered tissue plasminogen activator compared to those receiving heparin was 4.7% to 11.1% respectively. Additionally, during the in-hospital phase, recurrence of pulmonary embolism was more frequent in patients taking heparin. In fact, the ratio of recurrence was 7.7% to 18.7% in favor of alteplase (Arcasoy and Kreit 1999).

SYNTHESIS OF THROMBOLYTIC DRUGS

ALTEPLASE

Alteplase is beneficial in thrombolytic treatment because it is easily synthesized using recombinant DNA technology. The composition of alteplase consists of a purified

glycoprotein consisting of 527 amino acids. It is synthesized using a complementary DNA from a human melanoma cell line. Alteplase is manufactured by secretion of an alteplase enzyme into a culture medium. After secretion of the enzyme, the antibiotic gentamicin is added to the culture causing fermentation. Gentamicin, however, is not present in the recombinant tissue plasminogen product. The product also undergoes lyophilization, which is the process of rapidly freezing and dehydrating in a vacuum (Genetech 2005).

Streptokinase

On the other hand, streptokinase is a drug easily synthesized from a β-hemolytic streptococcus culture. However, there is a major drawback to using streptokinase. Most people have had a streptococcal infection at some point in their lives, so they are likely to have built up antibodies against streptococcal bacteria. Streptokinase is produced from streptococcal bacteria, so the circulating antibodies are likely to neutralize its effect on the clots (Finkel et al. 2009). Furthermore, once administered to a patient, streptokinase cannot be used again for some time due to its antigenic property. Thus, the antigenic property of streptokinase is one of the most significant detriments to the drug.

Mechanism of Action

Alteplase

Alteplase has a mechanism of action that makes it fibrin specific and thus favorable for thrombolytic therapy (Figure 5). First, alteplase attaches to fibrin on the surface of a clot and initiates fibrin bound plasminogen. Then plasmin is cleaved from the plasminogen associated with the fibrin, fibrin molecules are broken apart by the plasmin, and the clot dissolves. Although alteplase has a low attraction for plasminogen in the plasma, it activates plasminogen attached to fibrin in a blood clot (Klabunde 2005). Alteplase's fibrin selectivity is what makes it therapeutically useful. Thus, administering alteplase in low doses lyses only the wanted clot without degrading other proteins.

Streptokinase

Streptokinase is a protein cultured from the broth of streptococci bacteria. Since streptokinase is not a protease, it contains no enzymatic activity and has a different mechanism of action than alteplase. Streptokinase forms an active complex with the plasminogen (Figure 6). The active complex activates and releases the plasmin to dissolve the clots (Klabunde 2005). However, streptokinase is not fibrin specific and, therefore, its

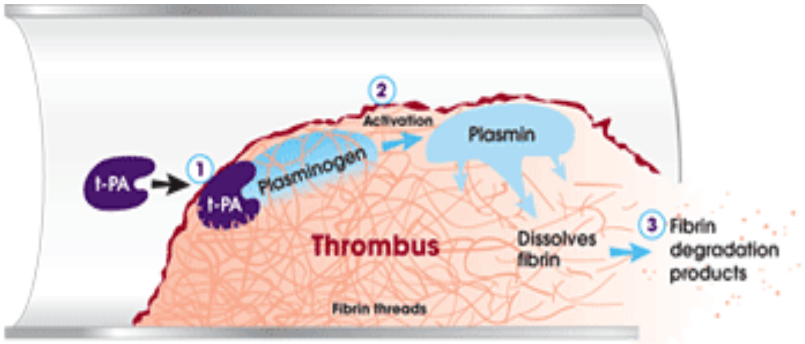

Figure 5: (1) Recombinant t-PA (alteplase) binds to fibrin in thrombus (2) converts entrapped plasminogen to plasmin (3) that initiates local fibrinolysis.
Source: http://www.cathflo.com/images/moa.gif

associated plasmin lyses circulating and non-circulating plasminogen. Streptokinase decays the blood clot as well as important clotting factors, such as Factors V and VII (Finkel et al. 2009).

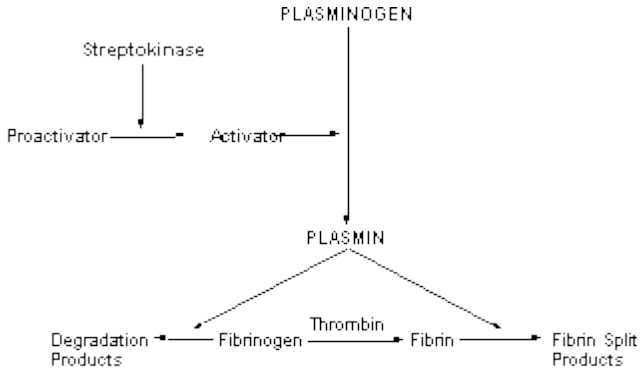

Figure 6: Streptokinase and its effect on the fibrinolytic system.
Source: http://www.anzcp.org/CCP/Pharmacology/streptokinase_files/image002.gif

PHARMACOKINETICS OF THE THROMBOLYTIC DRUGS

Pharmacokinetics is the pharmacological research of drug movement within the body. Moreover, it studies the mechanism of absorption, span of drug impact, and chemical modifications the body experiences. On certain occasions, a drug will be administered intravenously to ensure that the distribution of medication is instantaneous and efficacious. The half-life of a medication is the moment it takes a medication to decrease to half its original potency. Consequently, half-life forecasts the length of time it will take for a drug to leave the blood plasma. Therefore, if a drug contains a relatively brief half-life, the body disposes and eliminates that drug quickly. In contrast, when a medication has a prolonged half-life it lingers in the blood plasma for an extended period of time. Thus, half-life is a good indicator for drug dosage, because it measures the length of time it takes for the body to dispose of a medication. While sometimes a short half-life is beneficial for patients, other times a long half-life is advantageous for a patient.

HALF-LIFE OF THROMBOLYTIC DRUGS

Alteplase has an extremely brief half-life of approximately five minutes. There is an advantage and a disadvantage to the short half-life in alteplase. An advantage is that alteplase is a powerful clot busting medication that lyses only a specific clot and is eliminated from the body in a very brief time. A disadvantage of alteplase's short half-life is the increased possibility of reocclusion (Greer 2007). In contrast, streptokinase has a much longer half-life than that of alteplase. However, the longer half-life of streptokinase also has disadvantages, since streptokinase has antigenic properties and poses the risk of other allergic reactions. Additionally, it is usually unsafe to administer streptokinase a second time within 6 months due to its highly antigenic property (Rivera-Bou and Brown 2010).

CONCLUSION

The Food and Drug Administration approved alteplase as a treatment for acute ischemic stroke on June 8, 1996. Astonishingly, to this very day, alteplase is the sole approved medication for patients suffering from ischemic stroke. Additionally, it has bumped stroke from the third leading cause of death to the fourth leading cause of death in the United States

(Center for Disease Control 2010). The Gusto-I and Gusto-III trials indicate that alteplase is a more practical and powerful drug than streptokinase in patients suffering from myocardial infarction with ST segmentation. In fact, according to the American Heart Association, the ideal time to administer thrombolytic drugs is within 90 minutes after the heart attack. However, if administered up to 12 hours after the onset of symptoms, the chances of surviving and recovering are still enhanced (Health Guide 2010).

Pulmonary embolism is not an easily detected condition, but deep vein thrombosis, an equally serious condition, is detectable. The use of alteplase in acute massive pulmonary embolism has shown more efficient and steadfast results than that of anticoagulation therapy. Due to an extremely brief half-life, possibly resulting in reocclusion, alteplase has come under fire. However, with the use of recombinant technology, more generations of alteplase are being synthesized and produced to enhance the already proficient drug.

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Is Thrombolytic Therapy Safe When Used to Treat Elderly Patients?

Daniel Yaeger

Abstract

Recently, research has been conducted to determine if thrombolytic therapy works to dissolve clots and if it is a safe treatment option. The specific purpose of this study is to determine if thrombolytic therapy is safe for use in the elderly. This study was conducted by reviewing the relevant literature that has been published from the time that research began to test the usefulness of thrombolytic therapy. Numerous journals were examined to ensure impartiality and emerge with an unbiased conclusion. The journals were discovered using PubMed article finder, using Google as a search engine, and by scrutinizing relevant references found within articles citing previous studies. It was discovered during the course of the study that thrombolytic therapy has been proven to be a useful treatment. The main danger of the therapy comes not from the drug itself but from the possibility of causing an intracranial hemorrhage due to reperfusion to the ischemic tissue. Thrombolytic therapy should not be withheld from the elderly based solely upon age; rather, each patient's full history should be considered, and the decision should be based on the patient's blood pressure, glucose level, age, and history of recent surgery or trauma. The issue of microbleeds has been examined, and there is no conclusive evidence to suggest that they cause or do not cause intracranial hemorrhage in patients treated with thrombolytic agents.

Discussion

There are two types of strokes: embolic and hemorrhagic. Hemorrhagic strokes are caused by ruptured blood vessels in the brain. Embolic strokes are caused by a blood clot that forms in the brain and prevents blood from reaching the neurons or from a blood clot that forms in the extremities and subsequently travels to the brain with the same deleterious effect. The axiom doctors refer to when treating embolus strokes is, "Time lost is brain lost." The longer the brain fails to receive fresh, oxygenated blood the more damage there is likely to be. In a typical middle cerebral artery ischemic stroke, two million nerve cells are lost each minute reperfusion has not been achieved (Saver et al. 2010). One of the newest, most innovative, and controversial treatments for both strokes and heart attacks is thrombolytic therapy. This therapy involves using drugs that break down clots to restore blood flow. These drugs have been proven to be effective in treating both strokes and heart attacks. However, there is an inherent risk in using thrombolytic therapy. Drugs commonly used for clot lysis are not specific to brain clots; they break apart all clots formed anywhere around the body. The irony of thrombolytic therapy is that, while attempting to curtail damage caused by a stroke, it may end up causing an intracranial hemorrhage.

In general, any therapeutic approach must conform to the sequence of events of a stroke: after interruption of the blood supply, while some tissue probably suffers irreparable damage within minutes, a variable amount remains deactivated but in a viable state for several hours. Restoration of the blood supply, therefore, may save the ischemic tissue and improve the patient's overall outcome. Although research suggests that the blocked artery will open by itself sometime between twenty-four hours and a week, information on this is scanty at best; many factors including composition, age, and location (e.g. middle cerebral vs. superior cerebellar) contribute to overall recovery. There is clear evidence, however, that spontaneous lysis of a thrombus does occur. Current research is aimed at studying if the spontaneous process can be accelerated in time to restore the useful brain function without unacceptable risk (Wardlaw and Warlow 1992).

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Research on thrombolytic therapy has increased in response to strokes being the third most common cause of death in the developed world. In addition, strokes leave many people disabled and dependent on family and social or health services. Stroke has defied the considerable efforts of medical science to find an effective treatment (Wardlaw and Warlow 1992). Considerable interest in the use of thrombolytics also stems from the fact that they have been proven to work in patients suffering acute myocardial infarctions. The general thought is that if thrombolytic agents have been proven useful in heart attacks, they should also be a viable alternative to stroke treatment (Hommel et al. 1996).

To fully appreciate the benefits and risks of thrombolytic therapy, it is necessary to first understand the biochemistry and molecular biology of thrombolytic drugs. The assumed

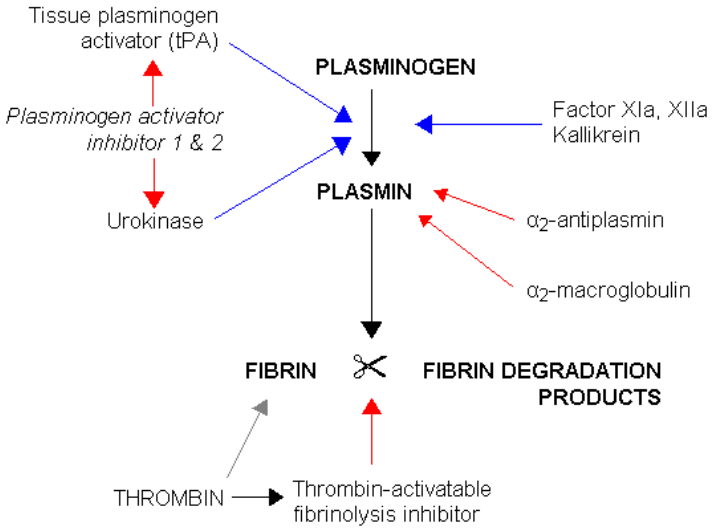

Figure 1: Schematic representation of the fibrinolytic system
Source: Kunamneni et al. 2007

culprit of strokes is fibrinogen; the goal of the clot-busting drug is to dissolve the fibrin clot, a process known as fibrinolysis. Plasminogen is an inactive proteolytic enzyme, which upon activation becomes plasmin. Plasmin is able to hydrolyze many types of coagulation proteins, most notably fibrinogen. Plasminogen is activated by plasminogen activators released from endothelial cells, normally. Plasminogen activators include streptokinase (SK), urokinase (UK), and tissue plasminogen activator (TPA). In the case of an acute heart attack or stroke, these activators can be given to the patient. In cellular biology, where one finds activators, one also finds inhibitors. All the different plasminogen activators are inhibited by plasminogen activator inhibitor-1 (PAI-1). Plasmin, which breaks down fibrin to fibrin degradation product (FDP), is inhibited by numerous inhibitors including alpha-2-antiplasmin (Figure 1) (Kunamneni et al. 2007).

A thorough understanding of the biochemical mechanisms of thrombolytic drugs is important in understanding how they work. If the activators are being used as a cure for heart attacks and strokes, then perhaps, in the event of an intracranial hemorrhage, use can be made

of the inhibitors to prevent fibrinogen breakdown and induce clotting. Perhaps these inhibitors can even be used to assist in treating hemophiliacs[1].

If clot-busting drugs carry such a high risk of intracranial hemorrhage, why would doctors use these drugs as opposed to another safer method? The answer is that no other medications seem to work. Antiplatelet agents such as aspirin and anticoagulant agents such as heparin reduce platelet aggregation and thrombin formation; however, they have no effect on fibrinogen and blood viscosity. In fact, in two different studies, aspirin and heparin were given to patients having acute strokes, and they both showed very little effect. Thrombolytic agents, on the other hand, fill all three functions in one therapy: they lower the viscosity of the blood by fibrinogen reduction, potentially allowing blood flow back into the blocked region of the brain; they act as anticoagulants, and they may act as antiplatelet agents (Lowe 1998).

Antiplatelet agents, anticoagulant agents, and thrombolytic agents pose nearly the same risk of interfering with hemostatic plug formation (the forming of a clot to maintain hemostasis). It can be inferred that the risks of intracranial hemorrhage are not necessarily reduced using alternative drugs. Indeed, based on trial evidence, alternative drugs do lower the chances of recovery. Aspirin was tested as a preventive drug against strokes, and it was noted during the 4.6-year long trial that, not only did aspirin not prevent stroke (in men with a mean age of 57), it also increased the risk of intracranial hemorrhage. Although some studies found aspirin to be helpful to middle aged women predispositioned to certain types of stroke, women who took it in excess (> 15 tablets/week) had a higher rate of hemorrhage (Cornett et al. 2008. In a later study, patients treated with thrombolytic therapy also received both a platelet aggregation inhibitor (aspirin) and an anticoagulant (heparin), and neither the heparin nor the aspirin were associated with intracranial hemorrhage. It can be suggested that the seeming disassociation between aspirin, heparin, and intracranial hemorrhage was because the aspirin and heparin were given several hours after the thrombolytics were administered. In fact, if signs of intracranial hemorrhage became apparent, the heparin and aspirin would not have been given (Simoons and Maggioni 1993).

This information would seem to suggest that there is a connection between fibrinogen levels, blood viscosity, and instance of stroke. A study proving the connection appears in the New England Journal of Medicine as early as 1984. The study shows that fibrinogen is as important a predictor of stroke as high blood pressure; consequently, patients with both high blood pressure and high fibrinogen levels have the highest risk of stroke (reviewed in Lowe 1998). Another study, appearing in 1996, shows that fibrinogen levels predict atrial fibrillation and congestive heart failure, which are both risk factors for strokes. This study, though, shows that high fibrinogen is a secondary cause of stroke, not a direct cause. A study comparing incidence of stroke and fibrinogen levels in Americans vs. Japanese shows that Japanese have a lower level of fibrinogen and, correspondingly, a lower incidence of stroke (reviewed in Lowe 1998). In another early study, showing a more direct relationship, the fibrate drug clofibrate (used to lower cholesterol levels in blood) lowered the plasma fibrinogen and blood viscosity, resulting in a significant increase in the cerebral blood flow (reviewed in Lowe 1998).

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[1] In the event of an intracranial bleed it would seem to be too late to try using deactivators; however, this knowledge could present a possible entry point into looking for a treatment.

Once scientists realized the potential for thrombolytic therapy, they had to ask themselves: Is this treatment option really going to work? And, even if this potential treatment does work, is it safe? In 1992, research was conducted to assess whether or not thrombolytic therapy is effective. The results of 10 different trials were combined and examined, and it was noted that there was a reduction in the risk of death and deterioration to stroke patients treated with thrombolytic therapy. This provides some evidence that thrombolysis is beneficial in acute ischemic stroke. The study concludes that although this was not enough evidence to recommend widespread usage of the thrombolytic drugs, there was enough improvement in patient outcome to encourage larger randomized trials (Wardlaw and Warlow 1992).

The main problem with this retrospective study is that the administration of thrombolytic drugs, which should have begun within 24 hours from the time of symptom onset, ranges anywhere from 24 hours up to five days later (the median being 72 hours). Additionally, the severity of the stroke was not taken into account.

Hommel et al. (1996) record the results of a trial done to determine if thrombolytic therapy is indeed safe. The trial included 154 patients in a placebo group and 156 patients in a streptokinase group. Six months after treatment, 124 patients from the streptokinase group and 126 patients from the placebo group had either died or were severely disabled. At ten days after treatment, more patients who received the thrombolytics had died than placebo patients. Researchers noted that most of the deaths came about because of cerebral hemorrhage. Hommel et al. conclude, "Treatment with streptokinase resulted in an increase in mortality. The routine use of streptokinase cannot be recommended in acute ischemic stroke." However, they did note at the end of the article that those patients who had been treated with the thrombolytic agent and survived the treatment without subsequent disability had significant improvement in their level of function. Fewer patients in the streptokinase group were disabled as compared to the placebo group. Hommel et al. cautiously speculate that one possible explanation of this can be that thrombolytic agents do actually work to improve function.

It is important to note that when trial participants were treated with these drugs, the doctors did not know about the various contraindications. The criteria for exclusion during this trial were previous hemorrhagic stroke, recent surgery or trauma, pregnancy, or other illness known to compromise the prognosis. Nowadays researchers know more about the contraindications, e.g., hypertension, diabetes, gender, use of oral anticoagulants, and, possibly, age. Furthermore, only 25% of the patients treated with the thrombolytic agents received the therapy within 3.75 hours of symptom onset. The median delay from symptom onset was 4.6 hours. Presently, the drug companies are trying to have the FDA approve the drugs to be given after 4.5 hours; less than 25% of patients in this trial received the drug in that time slot.

In May 2009, the American Heart Association guidelines for the administration of recombinant tissue-Plasminogen Activator (rt-PA) following acute stroke were revised to expand the window of treatment from 3 hours to 4.5 hours to provide more patients with an opportunity to receive benefit from this therapy. Eligibility criteria for treatment in the 3 to 4.5 hours after acute stroke are similar to those for treatment at earlier time periods; however, there are additional exclusion criteria: patients older than 80 years, patients taking oral anticoagulants, and patients with a history of stroke and diabetes. Patients falling into any of these categories are excluded (Saver et al. 2010).

In 2004, a research group pooled together all the data of six randomized placebo-controlled trials of intravenous rt-PA to study whether or not thrombolytics are helpful after 3 hours of symptom onset. They retrospectively analyzed 2775 patients from more than 18 countries, thus ensuring a large sample population and randomization in the test subjects. The problem was that every research group that did a trial used their own eligibilty criteria, and they grouped the patients differently based on time of treatment. The group did, however, feel it safe to confirm on the basis of the collective data that rapid treatment is associated with better outcomes at 3 months. The group also stated that the possible benefit of rt-PA treatment may extend beyond 3 hours, but it definitely does not extend to 6 hours, because, as time goes on with the stroke untreated, progressive disappearance of the ischemic penumbra (ischemic but still viable cerebral tissue) occurs. Accordingly, patients who have potentially viable ischemic brain tissue at later time might have substantial treatment benefits (Hacke et al. 2004).

During the course of the research, the team noticed a striking phenomena. Patients with more severe strokes arrived at the hospital earlier than those with less severe strokes, thereby receiving rt-PA earlier. The mortality and disability rates are high for those patients in early treatment intervals due to the severity of the stroke, but, at the same time, the effect of the rt-PA is greatest in those treated early despite the greater stroke severity (Hacke et al. 2004) . This proves the effectiveness of rt-PA when given early.

One of the big concerns that doctors have is the presence of microbleeds. A microbleed is a tiny focal collection of blood breakdown products adjacent to histologically abnormal small vessels, resulting from blood leakage through the fragile vessel wall (Figure 2). The actual size of a microbleed is likely to be less than a millimeter. It is suggested that microbleeds result from small vessel damage. Recent research suggests that there may be a genetic component as well (Werring 2007).

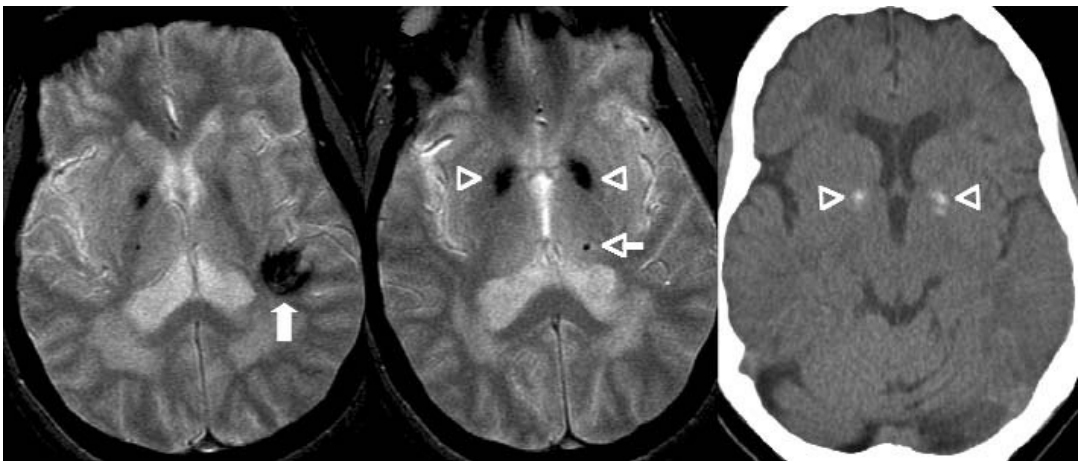

Figure 2: Cerebral microbleeds: A microbleed can be seen in the middle picture located near the right thalamus and marked with an arrow. Source: Werring 2007

One of researchers' concerns regarding microbleeds is whether there is a connection between cerebral microbleeds and treatment with a thrombolytic agent during an ischemic stroke. The basis for this well placed assumption is that cerebral microbleeds are found in 54% to 71% of all primary intracerebral hemorrhage occurrences (Werring 2007). Ho Sung Kim et al. (2006) retrospectively evaluated 279 patients and selected 65 patients (37 men, 28 women, mean age being 67 years old) who fit their extremely selective criteria. The criteria

included: symptom onset within 6 hours of receiving the drug, no previous history of intracranial hemorrhage, thrombolytic treatment immediately after the initial MRI, and another MRI 1-3 days after the thrombolytic treatment. They found that, after being treated with thrombolytic agents, 25 patients of those 65 had microbleeds. Of the 40 patients who did not have microbleeds, 9 had signs of hemorrhage. Of the 25 patients that did have microbleeds, 8 had signs of hemorrhage. From this research it seems clear that there is no correlation between presence of microbleed and subsequent intracranial hemorrhage as a result of treatment with a thrombolytic agent.

A limitation of this experiment is that the test group used is very small. They began with 279 patients which is already small, and the test numbers were further reduced due to the very selective criteria. Further research with more test subjects involved is needed to reach a firm conclusion. The author also mentions that the maximum number of microbleeds any patient had was 10, and, in future studies, he would like to see what happens to patients with more than 10 microbleeds.

Another study comparing location of microbleed and symptomatic hematoma (collection of blood outside blood vessels) concluded that there is a correlation between the two; however, they were not sure how to interpret the correlation (Roob et al. 2000).They note that there was some tendency toward a regional association between microbleed location and the site of a symptomatic hematoma, but they could not discern the specific patterns of microbleed distribution. Kim's study, which mentions that presence of a microbleed is not a contraindication to thrombolysis, seems to contradict Roob's study, which notes a correlation between the two.

Also interesting is that in Roob et al.'s study, which was also done retrospectively, the population consisted of 109 patients who had an intracranial hemorrhage (as opposed to 65), which is still very small for a study, and 54% of those patients had microbleeds. However, the patients in this study had up to 90 lesions, which is a great deal more than anyone else in Kim et al.'s study, but the average number of microbleeds was still 14, which is not that much more than Kim et al.'s study. In Roob et al.'s study, the researchers grouped the individual with 90 microbleeds in the same group as the patient with one microbleed. What would be interesting is if there was research conducted which would compare number of microbleeds to incidence of intercranial hemorrhage, because there is limited data to suggest that microbleed number may actually be a predictor of future bleeding risk after intracranial hemorrhage. In fact, microbleeds are associated with larger volume of intracranial hemorrhage (Werring 2007).

One of the primary concerns of both doctors and researchers is: is thrombolytic therapy safe for use on the elderly? The problem that researchers had is that most of the information about hemorrhage as a result of thrombolytic therapy comes from clinical trials, and elderly individuals are either scarcely selected due to the highly selective criteria of the trials, or not included in the trial at all. The reason for this is that elderly patients often have a poor prognosis for recovery, high mortality rate, high health care costs, and in the case of a heart attack the doctor can always treat the patient with angioplasty. Therefore, information about how the elderly would respond to thrombolytic agents is scarce (Brass et al. 2000). Pundik et al. (2008) set out to determine three things: the rate of intracranial hemorrhage associated with thrombolytic therapy for elderly patients suffering an acute heart attack, whether there are any independent predictive factors for intracranial hemorrhage, and a way of estimating the risk of an intracranial hemorrhage.

Stroke is more common in the elderly, and the morbidity and mortality after a stroke are higher with increasing age. The incidence of stroke doubles with each consecutive decade above the age of 55, and the mortality for patients over 85 years old is tripled compared with a younger group. In addition, the rates of a favorable outcome after a stroke are significantly lower in older patients. Because stroke rates are so high in the elderly, researchers and clinicians need to find a viable treatment, and thrombolytic therapy seems to be the most promising. The problem is that, unlike higher admission blood pressure, treatment delay, and hyperglycemia, which have all been proven to be risk factors of intracranial hemorrhage, advanced age is not a clear indicator (Pundik et al. 2008).

A research team analyzed the charts of 218,663 patients who were eligible and treated 31,732 of them with thrombolytic therapy. The mean patient age was 73 years old. Out of 31,732 patients, 455 (1.43%) of the patients ended up with intracranial hemorrhage, and 72% of the patients that had intracranial hemorrhage died within 6 months compared to 18% of the patients who did not receive the therapy and died within 6 months (Brass et al. 2000).

The goal then was to figure out what some of the risk factors for intracranial bleeding are, because if some of the risk factors can be identified, the individual patient's risk of hemorrhaging can be assessed. Studies previous to the 1993 study by Simoons and Maggioni suggested that age, hypertension, overdose of drugs based on body weight, being female, previous central nervous disease, and the use of oral anticoagulants are all associated with increased risk of hemorrhage. It has been suggested that the reason the elderly are at greater risk is not because of age per se; it is actually because elderly patients tend to have more contraindications to thrombolytic therapy. They found that patients over the age of 75 were more likely to have a history of hypertension, diabetes, congestive heart failure, previous bypass, and stroke among others (DeGeare and Grines 2000). Poor outcomes after ischemic stroke in the elderly could also be caused by other factors that are associated with aging. It has been shown in animal and clinical studies that effects of ischemia and reperfusion on brains are hastened in aged organisms (Pundik et al. 2008).

Researchers theorized that the reason elderly patients with congestive heart failure do not respond well to thrombolytics is because poor cardiac pump function results in decreased perfusion, making it difficult for the thrombolytic agents to penetrate the occlusive thrombus. This theory is hard to accept because, if the thrombolytic agent is not able to penetrate the thrombus because the heart is not pumping strong enough, it stands to reason that the thrombolytic agent would not be able to cause a hemorrhage either. If the elderly patients would simply not respond to the drug at all then they would have a valid point, but it does not seem to follow that the thrombolytic agent cannot get to the thrombus but can get to the brain.

As stated above, one of the primary predictors of intracranial hemorrhage is hypertension. However, research suggests that the bleeding risk depends not only on the state of the intracranial blood vessels but also predominantly on the blood pressure at the time of the thrombolytic therapy. This is based on an observation that patients with intracranial stroke often have higher blood pressure than those patients experiencing other types of strokes. Consequently, before beginning any course of thrombolytic treatment, the patient's blood pressure should be assessed and, if necessary, corrected (Simoons and Maggioni 1993).

In 2002, Tanne et al. reported that intravenous rt-PA is an effective therapy for acute ischemic stroke, but they wanted to identify baseline factors that are associated with thrombolysis related intracranial hemorrhage. During their retrospective investigation of 1,205 patients treated with rt-PA within 3 hours of symptom onset, of which 72 patients (6%)

developed intracranial hemorrhage, they determined that the main contraindications were diabetes melitus, past cardiac disease, increasing stroke severity, advancing age, use of an antiplatelet agent before treatment, and elevated pretreatment blood pressure. They determined that advanced age is a risk factor because they noted a particularly low rate of intracranial hemorrhage in patients under 60 years old (Tanne et al. 2002).

In 2004, research was done by Simon et al. to assess whether or not thrombolytic agents are safe for the elderly. As part of an ongoing monitoring process, a database was created that records all the details of patients treated for strokes with rt-PA since 1996. The database gives researchers access to many important details such as demographic data, blood pressure and glucose level at time of admission, severity of the stroke, and age of the patient. Of the hundreds of patients that received rt-PA during those years, 62 patients were over 80 years of age. Six out of those 62 patients experienced a severe intracranial hemorrhage, only slightly higher than the 6.4% of patients under 80 years of age who experienced severe intracranial hemorrhage. Thus, while it is true that patients over the age of 80 suffer from higher mortality and morbidity rates than younger patients, the elderly should not be denied the chance to benefit from thrombolytic therapy based on age alone (Simon et al. 2004).

In 2006, a systematic review performed by Engelter, Bonati, and Lyrer analyzed data taken from many websites such as PubMed, MEDLINE, and Science Citation Index and found many instances where elderly stroke victims were treated with thrombolytic agents. They found that stroke patients over 80 years old that receive rt-PA do have a substantially higher mortality rate than patients under 80. Additionally, older patients, even if they do recover, will not have as favorable a recovery (based on various neurological tests such as the Rankin scale). However, they also found that the risk of Intracranial hemorrhage was similar in both groups. They believe that age is a predictor of how well an elderly patient will recover after a stroke , but, because the likelihood of intracranial hemorrhage did not favor one age group, they conclude that rt-PA is a viable treatment option for the elderly, because the potential bleeding risks are unlikely to outweigh the potential benefit of treatment.

In 2008, researchers collected data from the Brain Attack Database at University Hospitals in Cleveland, Ohio. The database contained all cases of acute stroke dating back to 1993. The researchers had 488 cases where the patients suffered an acute stroke and were subsequently treated with a thrombolytic agent. Of the 488 patients, 404 were under age 80 (mean age 62 years old with a range of 20-79) and 78 patients where above age 80 (mean age 83 with a range of 80-99). The rate of intracranial hemorrhage in the elderly was 12.82%, but that figure was then adjusted. The reason is that most of the elderly patients who subsequently hemorrhaged did so as a result of treatment with intra-arterial therapy (not intra-venual therapy). The rate of hemorrhage in the younger group was 10%. Based on this research evidence, thrombolytic therapy should not be ruled out as a treament for an elderly patient simply because of advanced age, although method of treatment should be considered (Pundik et al. 2008).

There are some interesting trends in the demographic characteristics that deserve mentioning. In the younger group (age under 80), 43% of the patients treated for stroke were women, and in the older group, 55% of the patients were women. Pundik et al. (2008) suggest that the reason for this is because there is a prevalence of acute ischemic stroke in older women. Also interesting to note is that 61% of the younger patients exhibited hypertension compared to 80% that exhibited hypertension in the elderly group. Another significant trend is that in the younger group, 22% of the patients had a history of smoking compared to 8% of the older group that has a history of smoking.

The problem with their conclusion is that they suggest that thrombolytic therapy is safe for patients above 80 with no maximum age. Perhaps it would be helpful if in the future, the research team would, instead of using ranges of 20-79 and 80-99, break the ranges down into 5 year intervals. By ranging the patients in groups of 80-85, 85-90, and so on, the researchers can perhaps establish a maximum age for thrombolytic therapy.

Doctors believe in thrombolytic therapy as a treatment for acute stroke, and they want this therapy to work. The problem is that a great danger is associated with the therapy. What if doctors could lower the risk of intracranial hemorrhage? Researchers compared how patients treated in a CCSU – a Critical Care Stroke Unit recovered as compared to those patients treated in an ASU – Acute Stroke Unit. They found that patients treated in Acute Stroke Units received more treatments with rt-PA, had a shorter length of stay in the hospital, and had a lower 90-day mortality and disability rate. The researchers suggest that the reason for the better outcome is because in the Acute Stroke Unit, patients receive care quicker, and they are monitored very closely for fever, hypertension, hypotension, cardiac arythmias, and glucose levels. It is suggested that the continued monitoring and specialized nursing of the Acute Stroke Unit permitted the early detection, control, and treatment of factors related to hemorrhage (Roquer et al. 2008).

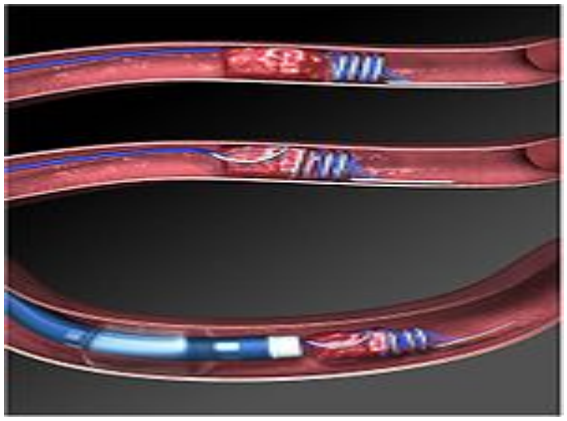

Figure 3: Mechanical Thrombectomy. Source: wikipedia.com

Perhaps the biggest problem with t-PA is that the FDA states that after 3 hours it is not safe for a physician to administer this potentially life saving or life ending drug. Is there an alternative treatment to t-PA for after 3 hours? In 2005, research was conducted to see if a blood vessel occlussion could be removed by mechanical means. This would be accomplished by inserting a catheter into the femoral artery, directing it into the cerebral circulation, and deploying a corkscrew-like device to ensnare the clot which is then withdrawn from the body. (Patients who were candidates for treatment with t-PA were excluded from this trial.) The Mechanical Embolus Removal trial was attempted as long as 8 hours after the onset of symptoms. The overall mortality was 44% of the patients, which is very high although not unexpected, as 8 hours is a long time after symptom onset. However, in 48% of the patients, recanalization was achieved, which means that the treatment has potential but just needs some fine tuning to be made safe and more effective (Cornett et al. 2008).

Another treatment method that is being looked into is angioplasty and stenting. The theory is that if angioplasty has been proven effective in preventing heart attacks, it should also be effective in preventing strokes. Early trials suggest that this could be a viable treatment option for patients who can not receive thrombolytics, but more research is needed (Saver et al. 2010).

Conclusion

Thrombolytic agents restore cerebral blood flow in some patients with acute ischemic stroke and may lead to improvement or resolution of neurologic deficits. Unfortunately, thrombolytics can also cause symptomatic intracranial hemorrhage. Therefore, if a patient is a

candidate for thrombolytic therapy, inclusion and exclusion criteria must be reviewed thoroughly. The exclusion criteria focus largely on identifying the risk of hemorrhagic complications associated with thrombolytic use (Saver et al. 2010). Based on research done within the last 5-10 years, it would seem that if a patient comes into a hospital having a stroke (assuming it is a stroke severe enough to warrant treatment with a thrombolytic agent) and the patient is in otherwise good health, i.e., no prior history of heart disease, not a diabetic, not hypertensive, and no history of bleeding disorders, and it is within 3 hours of symptom onset (perhaps 4.5 hours of symptom onset), then it would seem to be safe to administer the drug. If any of these contraindications are present the doctor needs to weigh the possible risk of hemorrhage vs the severity of the stroke. Interestingly enough, based on the recent research, clinicians should be more concerned about treating an obese 40 year old with a history of high blood pressure than an 85 year old who is in otherwise perfect health.

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