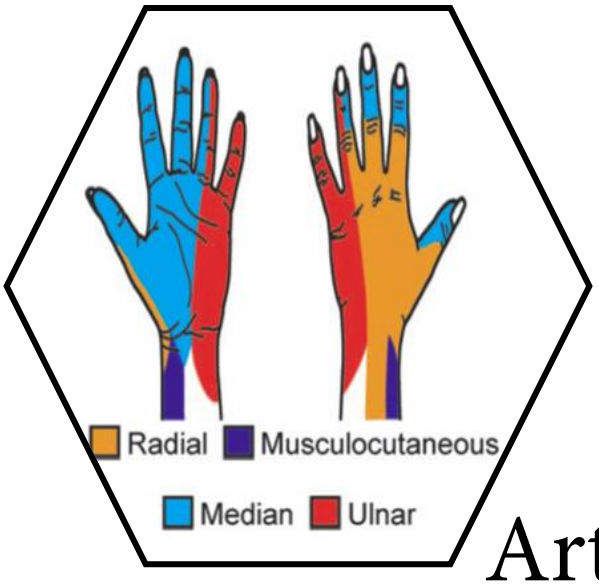

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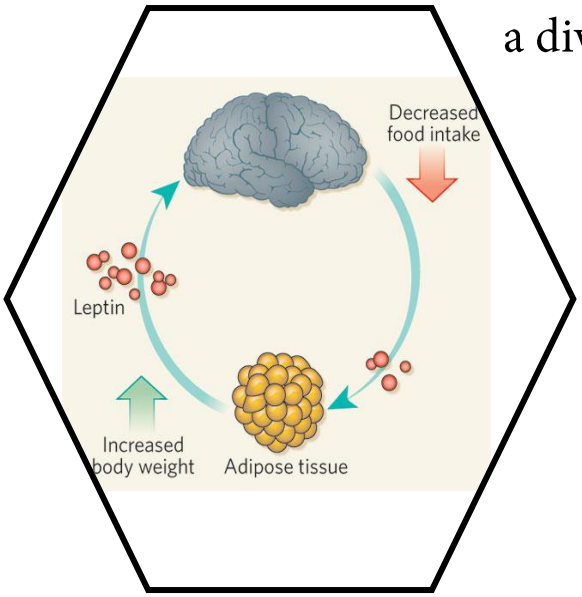

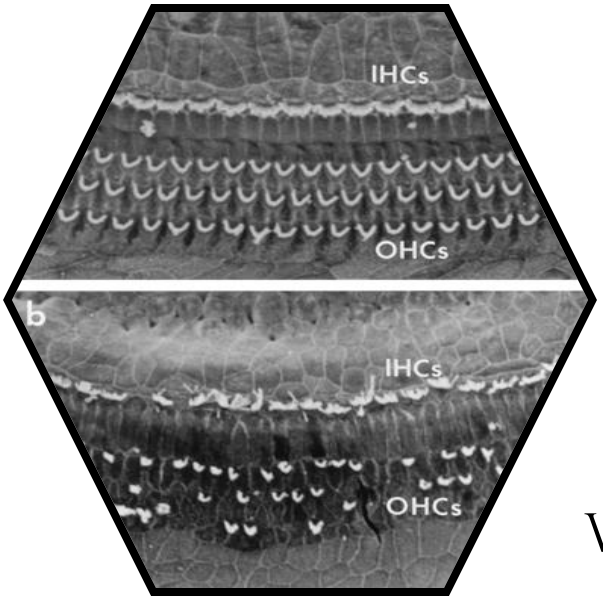

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The Lander College of Arts and Sciences at Touro in Flatbush

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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MELATONIN AND ITS EFFECT ON LEARNING AND MEMORY

Nechama Leah Bauman (Cahn)

ABSTRACT

Melatonin is a neurohormone produced by the pineal gland and secreted into the body in a circadian rhythm. Melatonin is known to be involved in many vital body functions, including sleep, reproduction, and immune response. Exogenous melatonin, sold as over the counter natural supplements in drugstores, is commonly taken by many people to help cure various ailments. Melatonin also plays a role in the hippocampus. This paper investigates the effects of melatonin on long-term potentiation in the hippocampus. Long-term potentiation, described as a long-lasting strengthening of synapses between nerve cells, is thought to be responsible for long-term memory retention. It is found that melatonin has a negative effect on long-term potentiation, inhibiting its magnitude. As long-term potentiation is related to some forms of learning and memory, melatonin inhibits learning and memory too. The practice of taking melatonin supplements causes one's long-term potentiation to be inhibited to a greater degree than it would be under normal conditions and can significantly impact one's learning and memory. In conclusion, although more studies need to be conducted, one should be wary and display caution before using melatonin supplements with any regularity.

INTRODUCTION

Melatonin, N-acetyl-5-methoxytryptamine, is a neurohormone synthesized mainly by the pineal gland. It is synthesized in a series of steps, starting with the conversion of tryptophan to serotonin. Serotonin is then converted to melatonin (Cardinali and Pevet 1998) (Figure1). Melatonin is metabolized mainly in the liver. The major metabolite of melatonin is 6-sulfatoxymelatonin (Macchi and Bruce 2004).

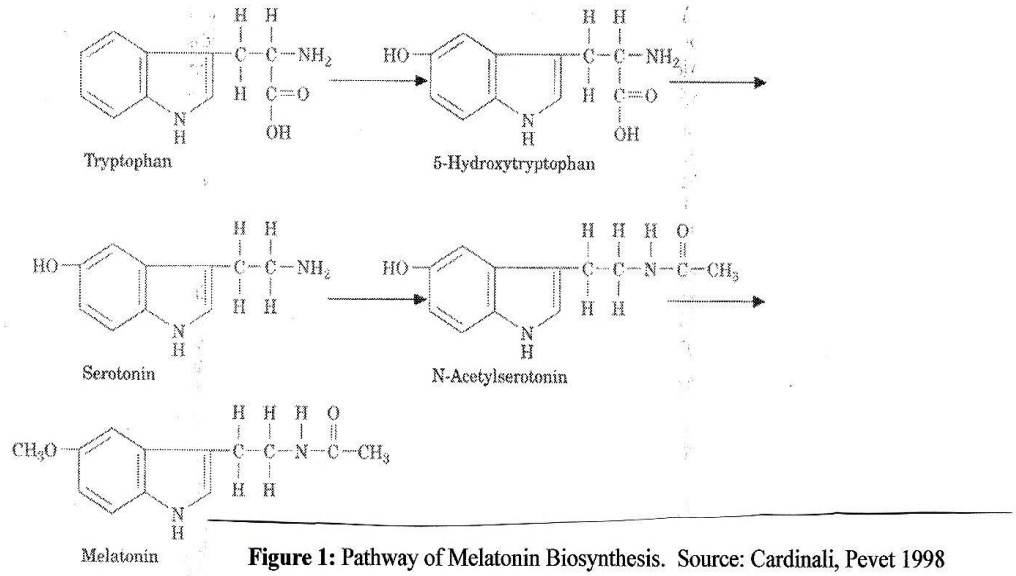

Figure 1: Pathway of Melatonin Biosynthesis. Source: Cardinali, Pevet 1998

The suprachiasmatic nucleus controls melatonin levels in the body, which follows a circadian rhythm of high melatonin levels by night and low melatonin levels by day.

This pattern is controlled by an endogenous, independently run pacemaker in the suprachiasmatic nucleus. Light and darkness do not cause the circadian rhythm of melatonin but do have the ability to change its timing. Light inhibits melatonin production, while darkness stimulates it. Photic information from the retina is sent to the suprachiasmatic nucleous and from there to the pineal gland in the form of norepinephrine. When norepinephrine enters the pineal gland, melatonin is synthesized. When light hits the retina, the retinal photoreceptor cells become hyperpolarized, inhibiting norepinephrine release, thereby inhibiting melatonin production. When it is dark outside, the retinal photoreceptors do release norepinephrine, thereby stimulating melatonin production. (Brzezinski 1997).

Melatonin is circulated to the rest of the body by passive diffusion into the bloodstream. It acts by binding to receptor sites. The receptors are part of the guanosine triphosphate-binding proteins and are G-protein coupled receptors (Brzezinski 1997). There are two subtypes of melatonin receptors: MT1 and MT2. Both subtypes are found in many areas of the body, including the cerebellum, retinal rods, ganglion cells, lymphocytes, and blood platelets. Since melatonin is very easily diffused, it has a systemic effect even without receptors at the basic cellular level, altering cytoskeletal and mitotic functions by binding to calmodulin, and acting as a free-radical scavenger (Macchi and Bruce 2004).

Endogenous melatonin is involved in many of the processes of the body, including sleep, reproduction, and the immune system. Exogenous melatonin, sold as over-the-counter tablets in drugstores and health food stores, is used by many people to cure various disorders.

Melatonin plays a major role in sleep. The circadian rhythm by which melatonin is synthesized is connected to sleep, and melatonin is also secreted in higher amounts at night, when people typically sleep (Brzezinski 1997). The effect of melatonin on sleep may also be related to changes in body temperature at night, with the nighttime decrease in body temperature, which is connected to the onset of sleep, increasing the evening secretion of endogenous melatonin. In fact, the highest point of melatonin production at night corresponds with the lowest point of body temperature (Macchi and Bruce 2004). Exogenous melatonin is often taken to correct sleeping problems. Flying over different time zones (jet lag) and working the night shift can disturb one's circadian rhythm, and many people take melatonin supplements to try and cure this. Also, people with insomnia, who have trouble falling asleep or staying asleep, often take melatonin supplements (Brzezinski 1997).

Melatonin also plays a role in the reproductive system. In animals that are seasonal breeders, the seasonal cycle, which is controlled by melatonin, regulates reproductive activity (Macchi and Bruce 2004). In humans, too, melatonin is involved in reproduction. Melatonin inhibits the reproductive process (Brzezinski 1997). Accordingly, melatonin supplements are sometimes taken by men and women to try and influence their reproductive systems. Additionally, there may be a relationship between endogenous melatonin levels and puberty (Macchi and Bruce 2004).

Melatonin also plays a role in the immune system, increasing the immune response. This is thought to happen by high levels of melatonin stimulating T-helper cells and other parts of the immune system (Macchi and Bruce 2004). Melatonin is also a strong antioxidant. It is a free-radical scavenger, scavenging against toxic hydroxyl

radicals as well as other oxygen-centered radicals. This protects the macromolecules of the body, especially DNA (Brzezinski 1997). Melatonin is also thought to have oncostatic properties, slowing down the development of tumors, and is taken by some as a treatment for cancer (Macchi and Bruce 2004).

Melatonin may also have an influence on the cardiovascular system. It may also have a connection to some psychiatric and neurological disorders (Macchi and Bruce 2004). Some people take melatonin to prevent or to reduce the effects of coronary disease, Alzheimer's disease, and Parkinson's disease (El-Sherif et al. 2002).

Melatonin is also thought to play a role in the processes of the brain. Melatonin receptors are present in the hippocampus, indicating that melatonin plays some role in that area (Wang et al. 2005). This research paper will look at the role of melatonin in the hippocampus and, specifically, at its effect on the process of long-term potentiation. It will also focus on the question of whether taking melatonin supplements, which inundate one's body with greater levels of melatonin than are naturally synthesized, has a harmful effect on the long-term potentiation in the hippocampus.

METHODS

The information in this paper was obtained by critical analysis of scientific research articles. The articles used have to do with studies conducted on the topics of melatonin, long-term potentiation, and the connection between the two. The articles were found in the Touro College library databases. ScienceDirect was the database most frequently used.

DISCUSSION

The hippocampus is an essential part of the brain, located in the medial temporal lobe of the brain. The hippocampus is made up of several structures: the hippocampus proper, the dentate gyrus, and the subiculum. There are three main excitatory pathways in the hippocampus: the perforant pathway, the mossy fiber pathway, and the Schaeffer collaterals. The hippocampus is part of the limbic system and is involved in the formation of long-term memory (Rison and Stanton 1995).

Learning and memory are stored in the brain as changes in the synapses between neurons (Medina and Izquierdo 1995). If two cells are active at the same time, the synapse between these cells is strengthened (Bliss and Collingridge 1993). In 1973, long-term potentiation (LTP), a method in which learning and memory are stored in the hippocampus, was discovered by Tim Bliss and Terje Lomo. They found that short bursts of high-frequency stimulation to excitatory pathways in the hippocampus caused an increase in synaptic excitability that was long lasting, lasting even months long (Rison and Stanton 1995). Later on, it was discovered that LTP also causes a change in the ionic current, causing the ionic current to be different than that in regular synaptic transmission (Morris 2003).

Long-term potentiation is induced by a specific mechanism. First, a high-frequency tetanus is given to the neurons. This causes the postsynaptic membrane to become strongly depolarized by AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid) receptors located on the dendritic spine. The depolarization removes the magnesium (Mg) barrier of the NMDA (N-methyl-D-aspartate) receptors, also located on the dendritic spine. This allows sodium (Na), potassium (K), and calcium (Ca) to flow through (See Figure 2). Calcium concentrations rise in the dendritic spine, triggering calcium dependent processes necessary in order for LTP to occur. The calcium dependent

processes cause changes in the synapse that increase synaptic strength, achieving long-term potentiation (Rison and Stanton 1995).

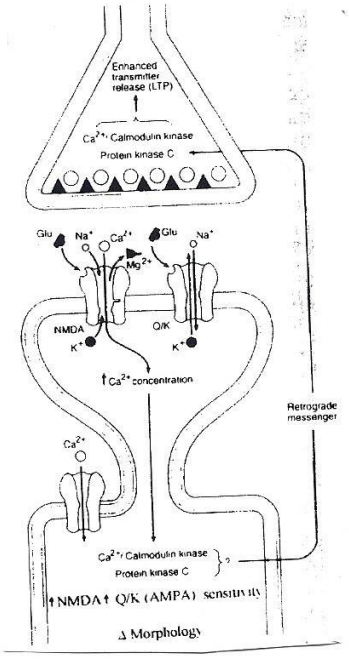

Figure 2: Model for the induction of LTP. Source: Rison, Stanton 1995

In order for the synapses to be strengthened in LTP, there must be correlated activity, meaning that the presynaptic and postsynaptic neurons must be active simultaneously. NMDA receptors make this happen by opening their channels only when stimulated by both neurons. Only after receiving glutamate from the presynaptic neuron as well as the removal of the magnesium block by depolarization of the postsynaptic neuron do NMDA receptors open their channels and enable the rest of the LTP pathway to occur (Tsien et al. 1996).

Long-term potentiation is connected to memory and learning. Synaptic plasticity, meaning change in synaptic strength, is caused by LTP and seems to be the way in which learning and memory are stored in the brain. Studies show that synaptic weights changed after learning, showing that learning is connected to LTP. Also, when the mechanisms involved in synaptic plasticity were changed, the rate of learning was also changed. Even after the learning was completed, changing of the synaptic weights affected the experimental animals' ability to remember what they learned. Interfering with LTP is seen to also interfere with learning and memory, showing that LTP is very involved with these tasks (Morris 2003).

In order to experiment with long-term potentiation, it must be easily stimulated and measured. During experimentation, long-term potentiation is usually induced by giving a tetanus, a stimulus, to a hippocampal slice. The tetanus must be sufficiently strong, usually at least 100 Hz, in order to invoke LTP (Bliss and Collingridge 1993). LTP is measured by recording the field excitatory postsynaptic potential (fEPSP) in the hippocampal slice after the tetanus. Experimenting with changes in the strength of synaptic connection allows one to learn about what causes these changes and how it might be linked to learning and memory (Wang et al. 2005).

Many studies have been performed investigating the effect of melatonin in the hippocampus. Their results showed that melatonin affects LTP by changing the synaptic transmission between neurons (Wang et al. 2005).

One such study, performed by Louisa M. Wang, proved that melatonin inhibits LTP in neurons. Wang proved this in an experiment using hippocampal slices of mice. First, LTP was induced with high-frequency stimulation, and the results were recorded for 60 minutes. Melatonin was then applied to the hippocampal slices, and LTP was induced again. This time, the field excitatory postsynaptic potential (fEPSP) slopes were much lower, showing that the melatonin had reduced the magnitude of LTP (Wang et al. 2005) (See Figure 3).

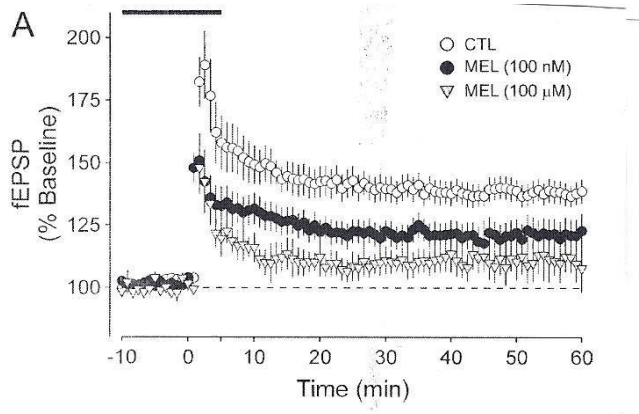

Figure 3: Inhibition of LTP by application of melatonin to hippocampal slices.
Shown is a comparison of the melatonin slices vs. the control slices. The melatonin slices have lower fEPSP, indicating an inhibition of LTP.
Soure: Wang, et. al. 2005

Yoshiyuki Takahashi also experimented with melatonin, investigating its role in LTP. Using hippocampal slices from rat brains, he tested the effect of melatonin on LTP in the CA1 region of the hippocampus. Compared to the control group, melatonin considerably lowered the expression of LTP (Takahashi and Okada 2011).

These two studies illustrate that one role of melatonin in the hippocampus is to inhibit LTP. In both experiments, after the addition of melatonin, LTP was significantly lower than the control groups.

After learning that melatonin inhibits long-term potentiation, the next step is to figure out how it is accomplished. What mechanism is in place that connects melatonin an indoleamine neurohormone, to LTP, a process of memory?

Of the two known melatonin receptor subtypes, MT1 and MT2, the inhibitory effect of melatonin seems to occur through the MT2 receptor. Wang illustrates this with his receptor-specific experiments. Luzindole, a nonselective melatonin receptor antagonist, blocked melatonin's inhibitory effect on LTP. This was expected, for if melatonin is not able to bind to receptors then it cannot act. However, 4-P-PDOT, a MT2-selective antagonist, also blocked melatonin's inhibitory effect on LTP. Here, melatonin was able to attach only to the MT1 receptors and not the MT2 receptors, and it did not act on LTP. This shows that melatonin inhibits LTP through the MT2 receptors (Wang et al. 2005).

Wang additionally proved this idea through experiments on genetically modified mice. In mice deficient in both MT1 and MT2 receptors, melatonin exhibited no inhibitory effects because it had no receptors on which to attach. In mice deficient only in

MT2 receptors, again melatonin had no effect. However, in mice deficient only in MT1 receptors, melatonin did inhibit LTP. Thus, it is the MT2 receptors which allow melatonin's effect on LTP, and as long as the MT2 receptors are present, melatonin works its effect in the hippocampus (Wang et al. 2005).

Dawn R. Collins suggested that perhaps the mechanism for melatonin's inhibition of LTP is based on N-methyl-D-aspartate (NMDA) receptors. Melatonin is similar in structure to some NMDA receptor antagonists, and if melatonin blocks NMDA receptors, then LTP would be inhibited. However, in experimentation, melatonin was found to have no effect on NMDA receptor-mediated responses, thus not inhibiting LTP through a mechanism involving the blockade on NMDA receptors (Collins and Davies 1997).

Wang hypothesized that the mechanism for LTP inhibition by melatonin involves the inhibition of the Adenylyl cyclase- protein kinase A pathway (AC- PKA pathway), which is involved in LTP. As MT2 receptors are negatively coupled to AC and PKA activity, and melatonin is mediated through MT2 receptors, it seems possible that melatonin's mechanism of action is through the AC-PKA pathway. If it is true that melatonin inhibits LTP through the inhibition of the AC-PKA pathway, then PKA inhibitors should likewise inhibit LTP the same way that melatonin does. Therefore, Wang tested H89, a PKA inhibitor, in its ability to inhibit the induction of LTP. H89 did inhibit LTP, to the same extent as melatonin did. This experiment, as well as further experiments testing the hypothesis, shows that melatonin works to block LTP induction by a mechanism involving the inhibition of the AC-PKA pathway (Wang et al. 2005).

However, the mechanism for melatonin action in the hippocampus is not straightforward. Takahashi demonstrated that melatonin blocked the induction of LTP with a mechanism involving the inhibition of the nitric oxide (NO) signaling pathway. The nitric oxide cascade is a precursor to LTP. In order for LTP to occur, a high-frequency stimulation must be given, leading to postsynaptic calcium concentrations. The calcium activates the production of nitric oxide. Nitric oxide leads to cGMP synthesis, protein kinase G activation, and finally to LTP induction. Thus, by melatonin inhibiting the nitric oxide signaling pathway, it leads to inhibition of LTP. One method Takahashi used to prove this experimentally was the application of L-NAME, a nitric oxide synthase inhibitor, to hippocampal slices. L-NAME inhibited LTP, just as melatonin did. Because melatonin inhibits LTP by inhibiting nitric oxide pathway, both melatonin and nitric oxide inhibitor should have the same end result of LTP inhibition. Each of them should achieve the same LTP inhibition, and putting both melatonin and nitric oxide inhibitor should not increase the level of LTP inhibition, because they both act on the same nitric oxide pathway. Takahashi tried this and got the hypothesized results, supporting the idea that melatonin inhibits LTP by inhibiting the nitric oxide cascade (Takahashi and Okada 2011).

Both the AC-PKA pathway and the nitric oxide pathway are mechanisms involved in melatonin inhibition of LTP in the hippocampus. There is thought to be an interaction between the two pathways (Takahashi and Okada 2011).

As previously mentioned, long-term potentiation is involved in learning and memory. Everything discussed above about melatonin inhibiting LTP means that in some way, melatonin is inhibiting the brain's ability to learn and store memory. With the endogenous melatonin produced naturally by the pineal gland, this inhibition is part of the body's natural cycle. Just as melatonin is produced in a circadian rhythm, LTP is also

found to have a circadian rhythm. The magnitude of LTP in the hippocampus is larger during the day, when less melatonin is produced, and smaller in the night when melatonin production rises (Takahashi and Okada 2011). Studies conducted by Arun V. Raghavan confirm the change in LTP strength between day and night. Raghavan found that hippocampal slices taken from hamsters during daytime showed a much greater measure of LTP than in those taken from hamsters during the nighttime (Raghavan et al. 1999). Since LTP is involved with learning and memory, learning and memory also must show a circadian rhythm (Takahashi and Okada 2011).

However, melatonin's effect on LTP is concentration dependent. Higher concentrations of melatonin have been found to inhibit LTP to a greater extent than lower concentrations (Wang, et. al. 2005). This information is crucial when considering the effects of melatonin on learning and memory. The circadian rhythm of learning and memory, from the circadian rhythm of endogenous melatonin, is a normal part of our body functioning. However, the intake of exogenous melatonin supplements places higher doses of melatonin into our body then usual, which causes LTP to be inhibited to a greater degree then normal. It is possible that melatonin supplements can seriously inhibit LTP, negatively affecting one's learning and memory to a significant degree. In fact, Xiu-Jing Cao conducted studies on the long-term effect of low dose melatonin on long-term potentiation and its subsequent effects on learning and memory, focusing especially on spatial learning. Spatial learning is awareness about one's surroundings and orientation in space. The study concluded that exogenous melatonin causes lasting harm to learning and memory (Cao et al. 2009).

Cao experimented with rats. The rats were given melatonin for sixty days and were then tested to evaluate their ability for spatial learning and to measure their LTP levels. Spatial learning was tested using the Morris Water Maze test. In this test, the rats were placed in a pool of water. In order to escape the water, the rats had to find the platform hidden in the water. The rats' spatial memory was tested by their ability to remember the platform's position by using spatial cues. When using more spatial memory, the platform was found faster. In this experiment, both the control group and the melatonin group showed decreasing reaction time, finding the platform faster as the experiment progressed. However, the group with melatonin still took significantly longer in the maze than the group without melatonin. The exogenous melatonin weakened the rats' spatial memory (Cao et al. 2009).

The long-term potentiation of the rats' hippocampi was then tested. As expected, the results showed that LTP had been inhibited in the melatonin-exposed rats, when compared to the control group. The fEPSP slope of the melatonin group was significantly less than that of the control group (Cao et al. 2009) (See Figure 3 above).

This study shows that melatonin inhibits LTP, impairing spatial memory and learning, because this type of learning and memory is related to LTP (Cao et al. 2009).

The melatonin in this study was given to the rats in low doses of 3 mg/kg for a relatively long period of 60 days. This is the same way that many people take melatonin supplements. Countless people take a low dose melatonin pill daily. However, the Cao study shows proof that this daily melatonin supplement can be harmful. It actually lowers LTP and affects spatial learning and memory. As Cao concluded, "melatonin should not be used as… [a] dietary supplement," for it harms learning and memory (Cao et al. 2009).

An experiment conducted by Ruben Soto-Moyano found that melatonin's inhibition of long-term potentiation damages visuo-spatial memory. Visuo-spatial skills involve one's visual perception of spatial relationships. Soto-Moyano tested visuo-spatial memory using the 8-arm radial Olton maze. This maze consists of a central point with eight arms extending out from it. The rat is required to run up and down the arms to find the food placed at the end of one of the arms. This maze tests visuo-spatial working memory (Soto-Moyano et al. 2005). The experiment included rats treated with melatonin and a control group of rats not treated with melatonin. In the maze, the rats treated with melatonin made more errors and took more time to solve the task than the control group. This shows that melatonin weakened the visuo-spatial working memory of rats. The control group performed better as time went on, meaning that they used long-term memory to help remember the maze. The rats with melatonin had their long-term memory damaged by the melatonin, proven by the higher number of errors even over many days of testing (Soto-Moyano et al. 2005).

This experiment by Soto-Moyano demonstrates that the LTP inhibition caused by exogenous melatonin harms visuo-spatial memory. Taking melatonin supplements may inhibit one's visuo-spatial learning and memory. Tasks that involve visuo-spatial processing, such as estimating distance and depth, may be damaged by melatonin supplements.

CONCLUSION

As seen in the above studies, melatonin inhibits long-term potentiation, consequently inhibiting learning and memory, especially spatial memory and visuo-spatial skills. With the ingestion of melatonin supplements, melatonin enters the body in amounts greater than usual. These higher levels of melatonin cause a greater inhibition of LTP and significant inhibition of learning and memory.

Additional studies must be conducted to learn more about melatonin's effect on the hippocampus. None of the findings discussed above are fully conclusive, and more research is needed on order to clarify the guidelines for the safe use of melatonin. However, it can be concluded from the studies discussed above that there is a definite relationship between melatonin, LTP inhibition, reduced spatial memory, and learning.

Melatonin is sold over the counter in the form of natural supplements in drugstores throughout the United States. These melatonin pills are often intended to help with various ailments, including jet lag, insomnia, and reproduction. However, people should be advised that taking melatonin pills long term or on a regular basis could possibly have negative side effects, impairing one's learning and memory capability. Melatonin supplements, although unregulated and promoted as natural, should not be taken unless medically advised, and even then, only with extreme caution.

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IS LAUGHTER THE BEST MEDICINE?
AN EVALUATION OF THE PHYSIOLOGICAL EFFECTS OF LAUGHTER

Annette Dalezman

ABSTRACT

Laughter directly affects one's physiology. Laughter causes various muscle contractions which, in turn, affect body systems. Specifically, the cardiovascular, immune, and respiratory systems are impacted by laughter. Stress levels and pain tolerance thresholds are also directly impacted by laughter. Research has been done on the effects of laughter in patients with cancer, dementia, and atopic dermatitis. Based on the review of multiple experiments, a direct correlation between laughter and multiple body systems and diseases seems to exist.

INTRODUCTION

Laughter is an audible expression of happiness and content. It is the physiological response to humor and other similar stimuli. Laughter is contagious and has the ability to create a positive atmosphere that can lift people's spirits. Scientific evidence indicates that laughter is more than just a pleasant act that people like to engage in. In fact, laughter is thought to directly affect the physiology and a number of systems of the body, possibly even having healing abilities. Research indicates that the cardiovascular, immune, and respiratory systems may benefit from laughter. Stress and pain tolerance levels also seem to be directly affected by laughter. Patients suffering from prevalent diseases such as cancer, dementia, and atopic dermatitis may benefit from laughter therapy as well.

That laughter is beneficial to one's health is not a new concept. In ancient Greece, hospitals were built next to amphitheaters, for they believed in the healing effects of laughter. Similarly, William Shakespeare believed in the health benefits of laughter. In a play he produced, *The Taming of the Shrew*, he writes, "And frame your mind to mirth and merriment, which bars a thousand harms and lengthens life" (Zillmann et al. 1993). It was only as recent as 1989, though, that the Journal of the American Medical Association acknowledged that laughter therapy has healing effects on chronic diseases and immediate symptom-relieving effects (Ljungdahl 1989).

NORMAN COUSINS

One of the pioneers of laughter therapy who had first-hand experience with the effects of laughter is Norman Cousins. Cousins is known as the man who "laughed his way out of a crippling disease" (Cousins 1976). In 1964, Cousins exhibited severe joint pain and fever. He was diagnosed as having ankylosing spondylitis (AS), a progressive rheumatoid disease involving inflammation of the spine (Martin 2004). He hypothesized that since laughter is a eustress, or positive from of stress, perhaps it would have the opposite effects that stress has. Based on that, he hired a nurse to read to him humorous stories and watched Marx Brother movies (Sahakian and Frishman 2007). These helped relieve his pain, allowing him to fall asleep. He claimed that 10 minutes of hearty laughter can provide two hours of pain-free sleep (Martin 2001). Against all odds, he was out of the hospital within a few weeks and lived to be 75 years old. He was the inspiration and driving force for scientists to investigate and research the healing effects of laughter (Sahakian and Frishman 2007). Nevertheless, despite the evidence indicated by this incident, it is possible to attribute Cousins' recovery to the high doses of vitamin C that were administered simultaneously with the laughter therapy (Martin 2001).

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EFFECTS ON PHYSIOLOGY

What happens to the body while laughing? What are the actual physiological effects of laughter on the body? Laughter, being a physical act, causes motion of several groups of muscles (more than 300 individual muscles altogether), the most visible being motion of the facial muscles (Figure 1), specifically near the oral region (Mora-Ripoll 2010). When smiling, the zygomaticus major, orbicularis oris, and orbicularis oculi contract. When laughing, these muscles contract along with simultaneous contraction of the facial, pharyngeal, and respiratory muscles (Takeda et al. 2010). Laughter also causes contraction of powerful muscles of the diaphragm. The audible sound of laughter is due to the repetitive vocal sounds produced by the actions of the chambers of the pharynx, nasal cavities, and mouth (Mora-Ripoll 2010), accompanied by changes in respiratory patterns (Sahakian and Frishman 2007). These movements directly affect the cardiovascular, respiratory, immune, muscular, and neuroendocrine systems, both short term and long term (Fry 1994). Additionally, there may be both healing and preventive effects against various diseases.

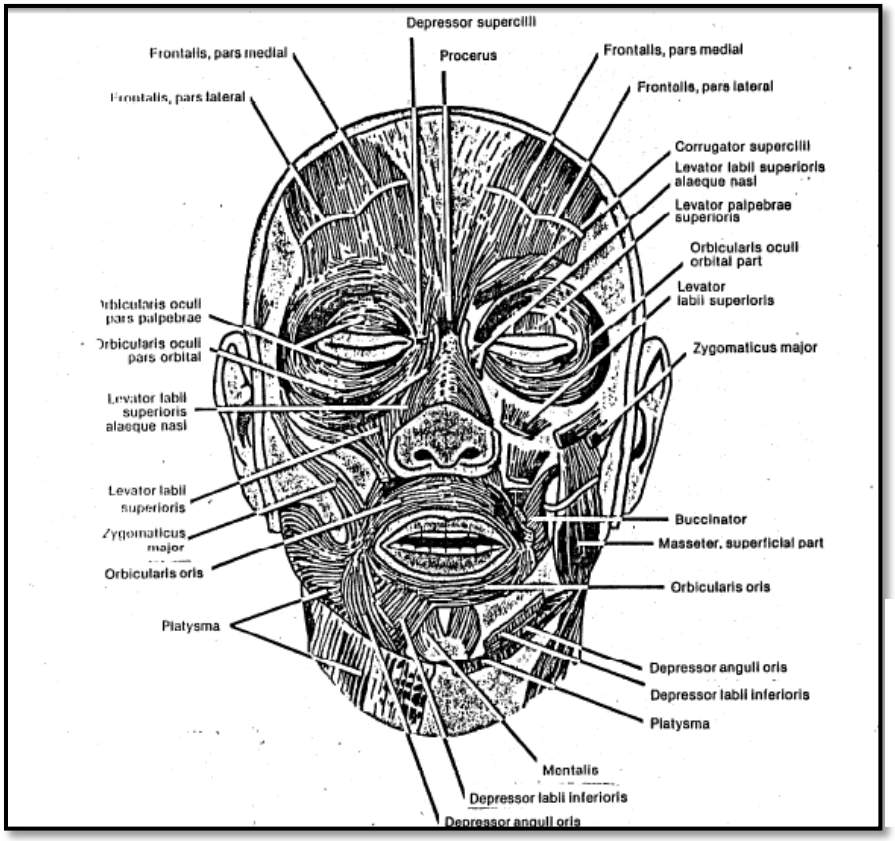


Figure 1: Muscles of the face and neck affected by laughter and smiling. Source: Ruch and Ekman 2001

Laughter can have negative physiological effects too, albeit few. Intense laughter can cause spasmodic contraction of skeletal muscles throughout the body, triggering profound physiological effects (Sahakian and Frishman 2007). There have been cases reported in which intense laughter led to a gelastic (laughter-induced) syncope. Fainting due to intense laughter is a rarity, however, and can be prevented if the laughter is controlled (Braga et al. 2005). Asthma can be exacerbated by strong laughter as well (Sahakian and Frishman 2007).

LAUGHTER TYPES

There are various forms of laughter. Different kinds of laughter have different effects on one's physiology. Therapeutic laughter is mainly derived from spontaneous laughter. Spontaneous laughter, triggered by external stimuli and positive emotions, causes contractions of muscles around the eye socket. Self-induced laughter, triggered by oneself at will, is another form of therapeutic laughter. Stimulated laughter, resulting from the physical action of certain external factors, such as tickling to one who is ticklish, is another form of therapeutic laughter, although to a lesser extent (Mora-Ripoll 2010). These laughter types are not necessarily a result of humor.

EFFECTS ON THE CARDIOVASCULAR SYSTEM

One of laughter's greatest impacts is on the cardiovascular system. The contraction of skeletal muscles during laughter increases venous return, thus reducing causes of venous stasis (Sahakian and Frishman 2007). The movements of laughter also help exercise the heart, thus increasing oxygenation of the blood (Martin 2001) and, consequently, also increasing stroke volume and thus cardiac output (Mora-Ripoll 2010). Additionally, laughter may prevent cardiac related diseases as it directly affects both systolic and diastolic arterial blood pressures (Fry and Savin 1988; Sugawara et al. 2010). Heart rate is also directly related to laughter (Sugawara et al. 2010).

An experiment was done to study and assess the direct effects of laughter on circulation (Miller et al. 2006; Sahakian and Frishman 2007). A group of 20 men and women were assigned to watch one of two 30-minute films to induce either stress or laughter. They returned two days later to watch the 30-minute segment not previously seen. Over a 100 vascular flow measurements were taken before and after the video segments. The findings showed that 95% of the volunteers demonstrated an increase in flow-mediated vasodilation during the laughter phase, and 74% showed a decrease during the mental stress phase (Figure 2) (Miller et al. 2006). These results clearly indicate a correlation between laughter and increased cardiovascular function.

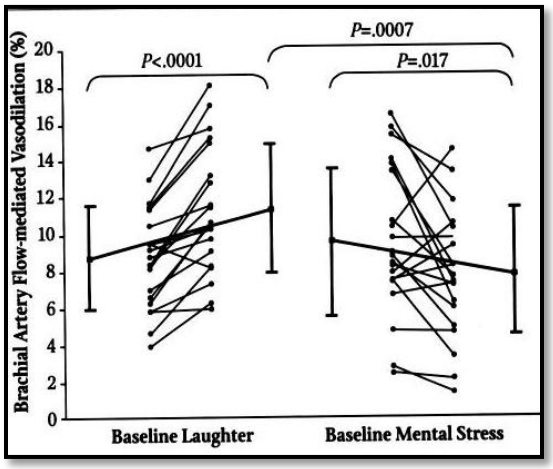
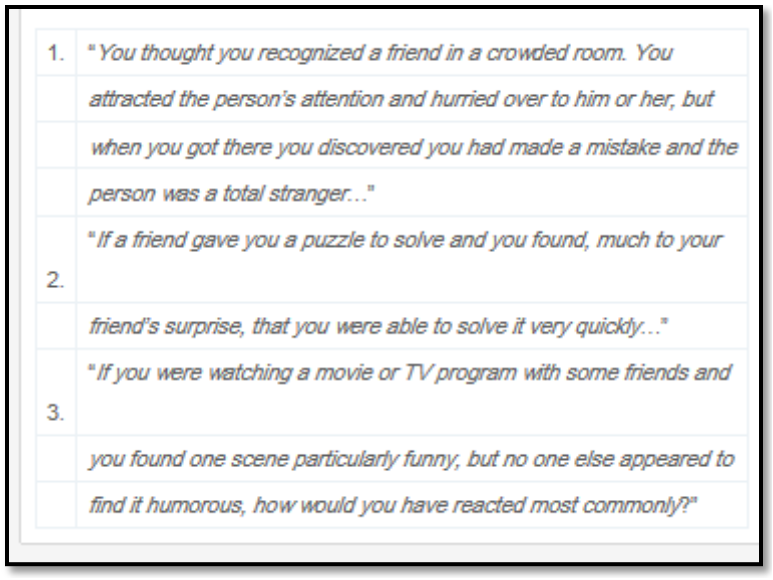


Figure 2: Measurements of flow mediated vasodilation during laughter and mental stress. Source: Miller et al. 2006

Coronary heart disease is known as the leading cause of death in the United States (Clark et al. 2001). Doctors at the University of Maryland have conducted research to determine the association between laughter and this prevalent disease. Questionnaires were given to 300 men and women, half of whom had coronary heart disease. The questions presented every day scenarios that can be found either humorous or annoying (Figure 3). The results showed that people with coronary heart disease laughed 40% less than those that were healthy, indicating an inverse association between laughter and coronary heart disease (Clark et al. 2001). This research is not very convincing as it was done on people that already have a disease. Perhaps it was the disease that was responsible for them laughing less rather than the lack of laughter being responsible for the disease.



1.	"You thought you recognized a friend in a crowded room. You attracted the person's attention and hurried over to him or her, but when you got there you discovered you had made a mistake and the person was a total stranger…"
2.	"If a friend gave you a puzzle to solve and you found, much to your friend's surprise, that you were able to solve it very quickly…"
3.	"If you were watching a movie or TV program with some friends and you found one scene particularly funny, but no one else appeared to find it humorous, how would you have reacted most commonly?"

Figure 3: Samples from the Situational Humor Response Questionnaire. Source: Clark et al. 2001

A study was done suggesting that laughter can positively impact people who suffered myocardial infarctions (MI). Two groups of patients exhibiting myocardial infarctions were followed during rehabilitation. The experimental group was allowed to watch self-selected humorous videos for 30 minutes each day. Results showed that the group viewing the humor tapes had less arrhythmias, lower plasma and urinary cholamines, required less beta blockers and nitroglycerine, and had less recurrence of myocardial infarctions (Balick and Lee 2003; Berk et al. 2001). These results show that recovery for MI patients can be positively impacted by laughter and humor. Similarly, research has shown that mirthful laughter led to a lower incidence of myocardial infarction in high-risk diabetic patients (Mora-Ripoll 2010).

Research conducted to determine the effects of laughter on various hormones points to cortisol, a stress hormone that can raise blood pressure and thus directly related to the cardiovascular system, as being greatly affected by laughter (Mora-Ripoll 2010). An experiment was done by Dr. Lee Berk, a well-known researcher in the field of laughter therapy, that took several blood samples from a group of volunteers before and after they watched a humor movie. The blood samples were examined to compare cortisol levels (among other hormone levels) before and after the laughter. The results indicated that laughter decreases cortisol levels (Sahakian and Frishman 2007). This would indicate that laughter can lower blood pressure, thereby decreasing one's chances of contracting chronic hypertension and heart failure.

Effects On the Immune System

Research has proven that people who laugh more respond better to disease. Laughter boosts immunity by increasing production and activity of interferon-gamma, natural killer cells, activated T cells, and B cells (Ziegler 1995). Laughter also affects immunoglobulin A, immunoglobulin G, and immunoglobulin M levels (Mora-Ripoll 2010).

Interferon-gamma, also known as immune interferon, is the only member of the type II class of interferons. It is a protein that is released by host cells in reaction to tumor cells, viruses, bacteria, and parasites. Interferon-gamma is secreted specifically by helper T cells, cytotoxic T cells, and natural killer cells. Interferon-gamma has antiviral, anti-tumor, and immunoregulatory properties. A study on the effects of laughter on interferon-gamma demonstrated that not only does mirthful laughter lead to an increase of interferon-gamma levels, but the heightened levels lasted into the next day as well (Ziegler 1995).

Natural killer cells are integral for the innate immune system. Natural killer cells provide rapid responses to virally infected cells and to tumor cells. They play an integral role in fighting cancer and in dealing with viral illnesses (Bennett et al. 2003). Multiple studies have been done to assess the effects of laughter on natural killer cell activity. A group of 33 women were randomly assigned to watch a humor video or a distraction video. The subjects' laughter was measured using the standard human response scale. Results indicated a correlation between laughter and natural killer cell activity (Figure 4). Lee Berk conducted a similar experiment with male subjects and yielded similar results (Berk et al. 2001).

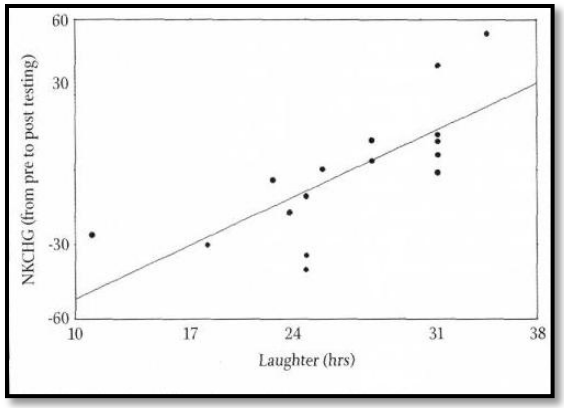


Figure 4: Effect of laughter on natural killer activity. Source: Bennett et al.

Immunoglobulin A, immunoglobulin G, and immunoglobulin M are affected by laughter as well. Immunoglobulin levels seem to increase as the amount of laughter increases (Martin 2001). Immunoglobulin A is an antibody that plays a great role in mucosal immunity (Bennett et al. 2003). Secretory immunoglobulin A is a component of the immune system, found in saliva. It is involved in defense against upper respiratory infections. An experiment done showed a significant increase in secretory immunoglobulin A due to laughter (Martin 2001). There are skeptics who question the use of immunoglobulin A to measure one's immunity, because of variations in individual salivary flow rate (Bennett et al. 2003). Therefore, natural killer cells seem to be a better method of assessing one's immune function.

EFFECTS ON THE RESPIRATORY SYSTEM

The respiratory system is another system greatly affected by laughter. The physical act of laughing assists with breathing by helping eliminate air and clear respiratory secretions during the process (Lebowitz et al. 2011). Additionally, vocalization of laughter leads to higher positive airway pressures and activation of additional muscle groups (Ruch and Ekman 2001). In one study, individuals with a sense of humor seemed to experience fewer respiratory diseases (Bennett et al. 2003).

Specifically, research has been done on the effects of laughter on patients with chronic obstructive pulmonary disease (COPD). Chronic obstructive pulmonary disease is a progressive disease characterized by chronic obstruction of airflow, hyperinflation of the lungs, and persistent ventilator impairment (Lebowitz et al. 2011). Data suggest that smiling and laughing may result in temporary reduction of hyperinflation of the lungs in individuals with chronic obstructive pulmonary disease (Brutsche et al. 2008).

An experiment was done on 19 patients suffering from chronic obstructive pulmonary disease and 10 healthy individuals. A humorous clown was used as the means to trigger laughter. Plethysmography, a test used to measure changes in volume in different parts of the body, was done before and 24 hours after the intervention. The subjects' laughter and smiling were recorded on video and analyzed, and their real-time breathing was assessed. The results indicated that smiling and moderate laughter were able to reduce hyperinflation in patients with severe chronic obstructive pulmonary disease. Intense laughter, on the other hand, was shown to potentially lead to hyperinflation, because higher intensities of laughter demand increased ventilation and oxygen consumption (Figure 5) (Brutsche et al. 2008). A similar experiment that

used a humor video as the means to trigger laughter showed analogous results (Lebowitz et al. 2011). Thus, maintaining a balance of laughter strength would seem to be most beneficial for the respiratory system.

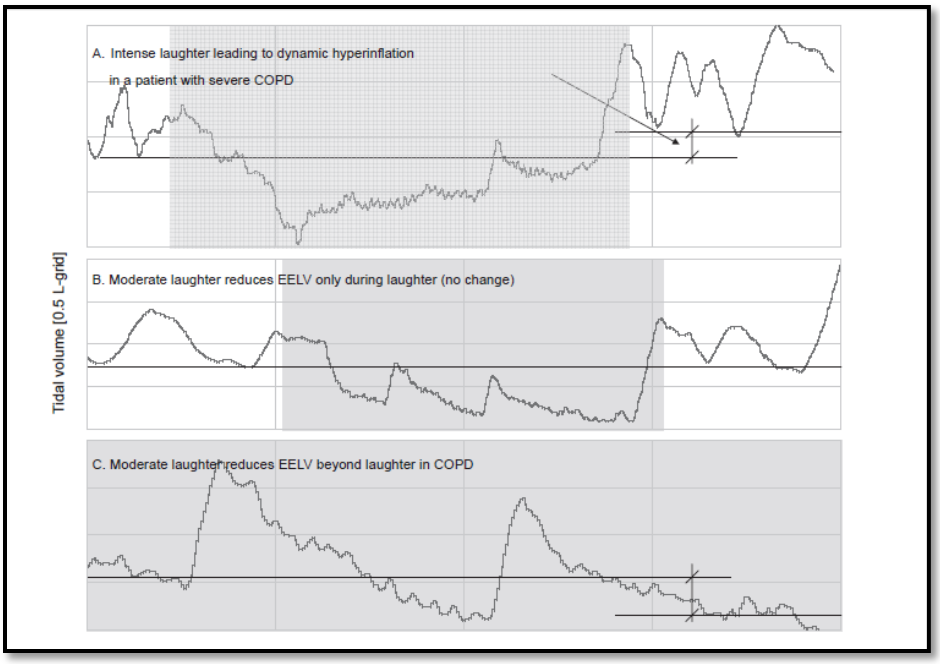


Figure 5: Spirogram from the real time monitoring of the breathing pattern showing the varying effect of laughter on end-expiratory lung volume (EELV) in patients with COPD. Source: Brutsche et al. 2008

EFFECTS ON STRESS

Stress can have negative physiological effects on the cardiovascular system and can lead to suppression of the immune system as well (Martin 2001). Therefore, if laughter can lower stress levels, it may prevent various problems related to the cardiovascular and immune systems. The Association for Applied and Therapeutic Humor has proposed that humor and laughter may stimulate an appreciation for the absurdities in life, thereby relieving stress while simultaneously promoting healing (Sahakian and Frishman 2007). Stress levels seem to be directly associated with laughter. Humor and laughter may, thus, be a possible complimentary therapy to reduce stress levels. Laughter seems to affect both the physiological as well as the psychological components of stress (Martin 2001). Similarly, laughter can reduce anxiety levels (White and Winzelberg 1992).

Stress levels can be lowered by the hormone cortisol. Since laughter helps to decrease cortisol levels, laughter can be used to reduce stress levels as well (Bennet et al. 2003). An experiment was done to determine the connection between laughter and stress. Volunteers were brought to laughter by watching a humorous video of their choice (Sahakian and Frishman 2007). The experiment results indicated that increased mirthful laughter correlated with decreased stress scores (Figure 6). Additionally, those who laughed more reported a lower level of stress post-laughter (White and Winzelberg 1992). A similar experiment was done on dental patients. The study showed that patients who joked and laughed before dental procedures reported less stress during and after the procedures (Bennet et al. 2003).

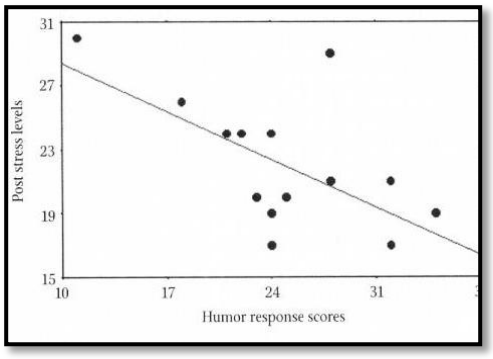


Figure 6: The relationship between laughter and post stress levels. Source: Bennett et al. 2003

At Stony Brook University, another study was conducted to assess the relationship between laughter and stress. Groups of people were shown a stressful movie containing images of a gory industrial accident. The subjects were told to describe what they saw in both a serious manner and in a manner that they found humorous. Their stress levels were measured before and after their descriptions. Stress levels were determined by monitoring heart rate, skin conductivity, and skin temperature. The results were identical for both those subjects who were humorous by nature and those subjects not humorous by nature but with just an appreciation for humor. In both subject types, when describing the video humorously, the subjects had lower measurements on all three accounts. This indicated that through the humor and laughter, they alleviated the stress caused due to viewing the gory accident. When describing the video in a serious manner, results indicated that their stress levels increased (Ziegler 1995). This is likely due to the fact that stress is affected by the actual laughter and not by the source of laughter.

EFFECTS ON PAIN TOLERANCE

Norman Cousins' life experience and research suggest potential analgesic effects of laughter (Martin 2001). Laughter seems to reduce pain and increase one's pain tolerance levels. Perhaps it is by stimulation of the production of endogenous opioids, such as beta endorphin, that pain levels are reduced. Laughter also seems to lower pain thresholds, thereby proving as effective as relaxation (Christie and Moore 2004). Much experimentation has been done to prove and to further understand these theories.

A study was done to determine the effects of various degrees of laughter and humor on pain tolerance. A humor video was shown to 56 female subjects who were randomly assigned to one of three groups. Depending on what group they were part of, they were told to react to the humor film a certain way. Those assigned to the cheerfulness group were expected to get into a cheerful mood without laughing, those assigned to the exhilaration group were told to smile and laugh extensively, and those assigned to the humor production group were expected to not only laugh but to produce humorous commentary as well. As part of the study, the cold pressor test was used to measure pain tolerance levels. The cold pressor test works by submerging a subject's hand in ice-cold water and determining how long the pain can be tolerated (Zweyer et al. 2004). In this study, the cold pressor test measured the pain tolerance levels of the three groups before, immediately after, and 20 minutes after the humor film. In all three groups, the cold pressor test

results indicated that pain tolerance levels increased due to laughter and remained high even 20 minutes later (Figure 7). The subjects' enjoyment levels were assessed using the facial action coding system. This is an objective coding technique that measures facial movement (due to laughter) and its intensity and duration. Subjects also answered a questionnaire that indicated their enjoyment level of the film (Zweyer et al. 2004). The results showed that even cheerfulness alone can lead to an increase in pain tolerance levels, although actual laughter is likely to achieve more significant results. In order to assess the effects of pain tolerance levels on men, a similar experiment using male subjects should be done.

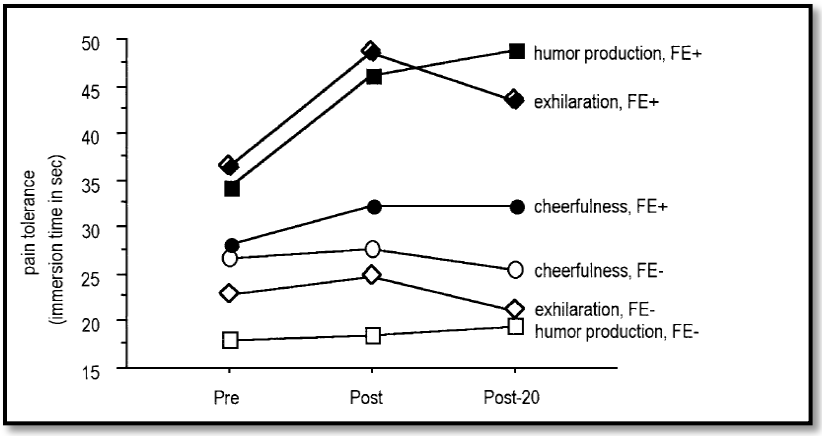


Figure 7: Changes in pain tolerance for the three groups. (FE - = few enjoyment displays, FE+ = many enjoyment displays). Source: Zweyer et al. 2004

A similar experiment, using 40 college students, was done on both male and female subjects. Students assigned to the laughter group listened to a 20-minute comedy audiotape, students assigned to the relaxation group listened to a 20-minute progressive muscle relaxation audiotape, students assigned to the dull narrative group listened to a 20-minute audiotape on ethics, and another group did not receive any listening material. Discomfort thresholds were measured using a blood-pressure cuff. The cuff was inflated until the subject could not tolerate the pain, and the maximum pressure was recorded. Pain thresholds for the laughter group were significantly higher after the 20-minute movie, further suggesting a correlation between laughter and pain tolerance levels (Martin 2001).

EFFECTS ON ATOPIC DERMATITIS

Atopic dermatitis is a chronic inflammatory skin disorder that involves scaly and itchy rashes (Kimata 2001). In patients with atopic dermatitis, plasma nerve growth factors (NGF) and neurotrophin-3 (NT-3) levels are elevated (Kimata 2004). Patients with atopic dermatitis also exhibit allergic skin wheal responses in which their skin flares up, forming red blotchy rashes. Recent studies indicate that laughter may help to reduce these symptoms (Kimata 2001; Kimata 2004). Consequently, laughter may also reduce the number of incidents of nighttime awakenings among children with atopic dermatitis. Additionally, laughter may reduce allergen-specific Immunoglobulin-E production. (Mora-Ripoll 2010).

An experiment was done to observe the effects of laughter on patients with this disease, specifically observing the wheal responses. The experiment studied 26 patients with atopic dermatitis, all of them allergic to dust mites. Skin prick tests, using commercial allergen extract, were performed before and after viewing an 87-minute humor video. Wheal size was measured 15 minutes after each of the skin prick tests. The results indicated that wheal size induced by house dust mite allergens were significantly reduced after the humor intervention (Figure 8). Wheal response size was also measured before and after the patients watched an 87-minute informative video, and no change in wheal size was observed (Kimata 2001). The same experiment was performed using cedar pollen and cat dander, and again the results indicated a

significant reduction of the wheal size for allergies in atopic dermatitis patients. The wheal caused by cedar pollen was reduced from 8 mm to 2 mm, and the wheal caused by cat dander was reduced from 7mm to 2mm (Kimata 2001).

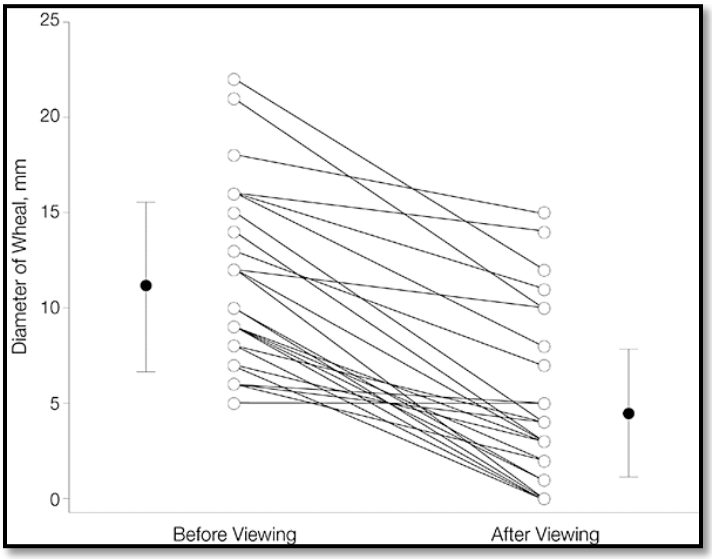


Figure 8: Effect of laughter on wheal responses induced by house dust mite allergen. Source: Kimata 2001

Another experiment was done to determine the connection between laughter and allergic responses in patients with atopic dermatitis. In addition to assessing the effect of laughter on the skin wheal size, changes in plasma nerve growth factors (NGF) and neurotrophin-3 (NT-3) levels were observed as well (Kimata 2004). The results indicated that wheal size was reduced significantly due to the laughter and that laughter caused the NGF and NT-3 levels to be reduced as well (Table 1).

Variable	Control		Laughter	
	Before viewing	After viewing	Before viewing	After viewing
Plasma (pg/ml)				
NGF	2159 ± 154	2193 ± 145	2267 ± 156	1831 ± 83**
NT-3	1784 ± 72	1859 ± 81	1859 ± 80	1201 ± 47**
Wheal (mm)				
HDM	8.3 ± .3	8.5 ± .3	8.5 ± .3	6.7 ± .2**
JCP	9.6 ± .3	9.4 ± .3	9.6 ± .3	7.3 ± .3**
Cat dander	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Histamine	7.3 ± .2	7.2 ± .2	7.5 ± .3	7.2 ± .2
Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0

(NGF= Nerve Growth Factors; NT- 3= Neurotrophin-3; HDM- House Dust Mites; JCP- Japanese Cedar Pollen; **Significant reduction compared with before viewing)

Table 1: Effect of laughter on plasma neurotrophins and wheal responses for various allergies. Source. Kimata 2004

Although the effect of laughter on skin wheal reactions was observed only on patients with atopic dermatitis, it is possible that these effects may occur in patients with other allergy

diseases as well. In order for this to be confirmed, further experimentation on those patients would have to be done. Even though there is no indication that laughter cures atopic dermatitis, laughter seems to greatly enhance the quality of life for patients with atopic dermatitis.

LAUGHTER THERAPY MAY AFFECT PATIENTS WITH CANCER

Cancer is a prevalent and deadly disease. Although no experimentation was done on the effects of laughter on cancer, cancer patients have reported greatly benefitting from the use of humor intervention (Christie and Moore 2004). Humor and laughter was cited as the second most common form of intervention (after prayer) for patients with cancer (Bennett et al. 2003).

A study conducted on a group of women suffering from breast cancer demonstrated humor and laughter as powerful tools in coping with a breast cancer diagnosis. The women felt a strong need to laugh to survive low moments, find humor with others through support groups, and use humor to help them relax (Johnson 2002). Although this study is suggestive that humor may enhance the quality of life and treatment period for cancer patients, experimentation would have to be conducted in order to assess the healing effects of laughter on cancer patients.

Although there is no clear evidence that indicates a relationship between laughter and cancer patients, the relationship between natural killer cells and laughter may lead to further speculation of this possibility. Natural killer cells play an integral role in fighting cancer, as they attack and fight off tumor cells. Since natural killer cells seem to be directly affected by laughter (Bennett et al. 2003), perhaps it is indicative that cancer can be affected as well. In order to fully assess these ideas, though, further research would need to be done.

LAUGHTER THERAPY MAY AFFECT PATIENTS WITH DEMENTIA

Dementia is a progressive disease in which patients lose many of their cognitive abilities (Takeda et al. 2010). Similar to cancer patients, humor and laughter therapy may be helpful and therapeutic for dementia patients as well. Laughter may be able to help these patients release tension and help to alleviate their pain (Zillmann et al. 1993). However, due to dementia being a disease in which patients lose many of their cognitive abilities, the forms of humor intervention deemed appropriate may be limited. Therefore, it is worth studying the different forms of laughter generation. Laughter can be evoked by a release in tension, it can be associated with pleasant feeling, and it can be used as a form of social communication. These categories can be further subdivided as delineated in Table 2.

As a patient's dementia progresses, the ability to process information is lost. Therefore, laughing as a communication tool is lost already in the early stages (Table 3). Laughing due to release of tension, however, is preserved throughout the disease. Thus, dementia patients placed in a relaxed, tension-free environment are more likely to laugh, and, if used appropriately, humor can be an effective form of therapy (Takeda et al. 2010). In order to further assess how the physiology of dementia patients is affected by laughter and how it may affect the disease, further research and experimentation would have to be done.

CONCLUSION

Laughter has profound effects on human physiology; it is an internal jogging mechanism that stimulates the physiological systems. Various kinds of laughter have effects on the cardiovascular, immune, and respiratory systems, among others. Stress levels and pain tolerance thresholds seem to be affected as well. There seems to be use for laughter therapy to help ease and possibly even heal the discomfort of patients with various diseases, including coronary heart disease, chronic obstructive pulmonary disease (COPD), atopic dermatitis, cancer, and dementia. Based on experimentation, it seems that laughter rather than humor is what has an impact on the physiology. Laughter seems to have effects both during and after laughing. Laughter, however,

may also have negative effects, specifically intense laughter. Therefore, one must be careful if using laughter therapy.

Laughter therapy is easy to use and is a relatively cheap and safe form of therapy. Although there is much speculation to the validity of this seemingly easy course of treatment, the evidence is very suggestive and the negative effects are few. However, in order to incorporate laughter therapy into a medical setting and in order to determine if laughter can be an all-around healing agent, more investigation should be done to fully assess all the possible risks and benefits. Further research should also be done to determine the effective duration of laughter and to assess how long these effects can last.

A) Laughter evoked by a release of tension- laughing in order to relax	
A1. Release from strong tension	Spontaneous laughter as a result of a release of strenuous tension
A2. Release from weak tension	Spontaneous laughter as a result of a release of lesser tension
B) Laughter associated with pleasant feelings	
B1. Fulfillment of instinctive needs	i.e.- a baby smiles after feeding
B2. Fulfillment of expectations	i.e.- one has a pleasant feeling after an accomplishment
B3. Feelings of superiority	Scornful laughter or a cold smile
B4. Feelings of disharmony	i.e.- one laughs after a harmless mishap
C) Laughter used for social communication	
C1. Cooperative	i.e.- smiling when one shakes a hand
C2. Defensive	i.e. -when one smiles/laughs to conceal their inner feelings (of hurt)
C3. Aggressive	Scornful laughter - laughing at someone else
C4. Devaluating	Smiling to devalue something. i.e.- smiling after a train door slams in one's face

Table 2: Various forms of laughter that were tested on dementia patients. Source: Takeda et al. 2010

Preservation in dementia		
Type of laughter/smile	**Early Stages**	**Late Stages**
A1. Release from strong tension	+	+
A2. Release from weak tension	+	+
B1. Fulfillment of instinctive needs	+	+
B2. Fulfillment of expectations	+	-
B3. Feelings of superiority	+	-
B4. Feelings of disharmony	+/-	-
C1. Cooperative	-	-
C2. Defensive	-	-
C3. Aggressive	-	-
C4. Devaluating	-	-

Table 3: Relationship between laughter/smile and the progression of dementia. (+ = humor that is preserved in dementia patients; - = humor that is not preserved in dementia patients). Source: Tikada et al. 2010

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NOISE-INDUCED HEARING LOSS AS A GROWING THREAT TO SOCIETY: AN EXPLORATION OF THE DAMAGING EFFECTS OF IPODS AND CONCERTS ON AUDITORY FUNCTION

Rachela Greenman

ABSTRACT

The purpose of this paper is to determine the exact dangers of leisure music to society, as peoples' hearing can be negatively impacted by excessive exposure to music, in terms of both duration and sound (dB) level. Two types of studies are analyzed. One study analyzes the effects of concert and disco style music on musicians and party guests, primarily through experiments which test pure-tone audiometry, distortion product otoacoustic emissions (DPOAE), and general sound levels of people and places before, during, and after exposure. Another study analyzes the effects of personal listening devices (PLDs) on the population, mainly through studies, questionnaires, hearing tests, and experiments. The results of many studies show that, due to the popularity of personal listening devices, people are listening to more music, more often, in more places, and at higher dB levels than ever before. The results determine that whereas both PLDs and concerts are harmful to society, PLDs pose a greater threat to users' auditory functions.

INTRODUCTION

In America today, over 27 million people suffer from moderate levels of hearing loss, and the numbers are steadily increasing (Daniel 2007; Matusitz and McCormick 2010). About half of these people lose a portion of their hearing due to exposure to loud noise for just a short period of time (Daniel 2007). Many are school-age children and young adults who voluntarily expose themselves to increasingly loud music over time (Matusitz and McCormick 2010). Adults have begun to lose their hearing at earlier ages than ever before, and more young children and teenagers have begun to suffer from hearing loss than in years past. The National Institute for Occupational Safety and Health (NIOSH) has found that 30 million Americans are exposed daily to unsafe levels of noise, and that one out of every eight children shows symptoms of hearing loss (Daniel 2007).

How loud is loud noise? The National Institute for Occupational Safety and Health states that exposure to sound levels above 85 dB over the course of an eight-hour work day is considered hazardous to one's hearing because it can cause damage to hair cells or actually cause temporary hearing loss. Although it is well known that prolonged exposure to loud noise will likely cause damage to one's hearing, studies have shown that even brief exposure to noise levels above 85 dB can potentially damage the hearing (Daniel 2007; Matusitz and McCormick 2010). In this paper, sound volume and length of exposure are highlighted because they are the two exposure factors over which individuals have the most control.

The most common form of hearing loss is noise-induced hearing loss (NIHL). What exactly is noise-induced hearing loss, and how does it occur? In the cochlea, a section of the inner ear, there is a region called the Organ of Corti which contains inner and outer hair cells embedded in a sensory epithelial layer of tissue. The Organ of Corti is situated on top of the basilar membrane which is stiff and wide in some areas and the opposite in others. Sound waves vibrate the basilar membrane; low frequencies vibrate the apical section, and high frequencies vibrate the basal section. The inner hair cells of the cochlea normally transmit the vibrations from the ear to the brain via transduction, which causes an impulse to go from the eighth cranial nerve in the inner ear to the cerebral cortex. The outer hair cells (OHCs) "boost the stimulus by

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electromechanical feedback… it increases both the amplitude and frequency selectivity of basilar-membrane vibrations for low-level sounds" (Mahendrasingam et al. 2010). For this reason, the outer-hair-cell region is also known as the cochlear amplifier and, as such, is extremely important in the discussion of hearing loss. When a person is exposed to extremely loud noise, the hair cells that detect high-frequency noise are damaged (Daniel 2007).

There are two different ways that noise can damage hair cells of the inner ear. The first is when the ear is exposed to noise louder than 140 dB for a short amount of time (for example, an explosion or a bang). The force of the sound's energy can cause such a strong vibration that the Organ of Corti can completely detach itself from the basilar membrane, causing immediate permanent hearing loss. The second way in which the Organ of Corti can be damaged by sound is through high intensity noise (85 dB and above) that the ear is exposed to over a longer period of time. Through prolonged exposure, the stereocilia of outer hair cells can gradually be destroyed, and the mechanical functions of the hair cells can be damaged. This second kind of exposure can, eventually, also lead to inner hair cell damage, causing permanent hearing loss as well (Zhao et al. 2010) (See Figure 1). Noise-induced hearing loss that damages hair cells in the cochlea is irreversible, and even a hearing aid cannot restore that aspect of hearing, because the damaged or destroyed hair cells can no longer transmit sound signals from the inner ear to the brain (Daniel 2007). In addition, a secondary result of sensory cell destruction is the destruction of spiral ganglion cells. Spiral ganglion cells play a key role in the best treatment for hearing loss that is available right now, namely, cochlear implants. Cochlear implants operate by stimulating the spiral ganglion cells of the cochlea. Thus, with the loss of spiral ganglion cells, noise-induced hearing loss can leave lasting and permanent damage to the ear (Watanabe et al. 2010).

Studies suggest that with longer exposure to loud noise, more hair cells are damaged. An incredible observation from scientists has shown that 30-50 % of all hair cells can be destroyed before any degree of hearing loss is even discovered (Daniel 2007; Matusitz and McCormick 2010). For this reason alone, prevention is the most important way to avoid hearing loss.

Since there are noise limit regulations for the workplace, the most common source of NIHL is not due to unsafe work environments but, rather, to recreational sources of noise. There are currently no clear guidelines for the noise levels deemed safe in recreational environments. The main sources of recreational loud noise are amplified music from discos and portable music players. These can cause either temporary or permanent threshold shifts (TTS/ PTS), depending on the intensity of the music and on the duration of exposure to the music. A temporary threshold shift generally lasts for up to two days and then goes away due to "regeneration mechanisms of the inner ear" (Muller et al. 2010). A permanent threshold shift occurs over a longer period of time and

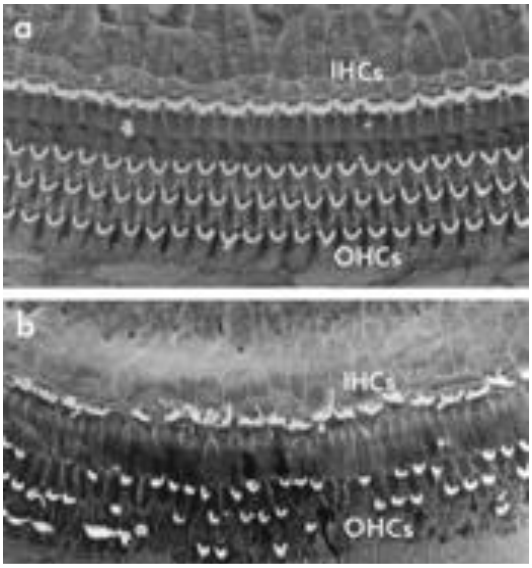

Figure 1: Scanning electron micrographs of the (a) normal and (b) damaged cochlear sensory epithelium. In the normal cochlea, the stereocilia of a single row of inner hair cells and three rows of outer hair cells are present in an orderly array. In the damaged cochlea, hair cells are missing, and stereocilia are abnormal, leading to hearing loss. Source: Ryan 2000

is gradual because it is caused by irreversible outer hair cell damage in the cochlea (Muller et al. 2010). Ringing ears and temporary hearing loss are warnings that if unhealthy noise exposure continues, NIHL may occur (Loftis 2007).

Listening to personal listening devices such as iPods and mp3 players has become one of the most common forms of leisure that exposes people to extremely loud music. Recent studies have shown that the most popular PLDs have the capacity to reach a sound output level of over 100 dB. The sound level is further increased if the recording is at a louder than average dB level to begin with. Those who listen to their PLDs at maximum capacity for just 3-12 minutes exceed safety limits and put themselves at risk for permanent noise-induced hearing loss (McNeill et al. 2010).

This paper considers the impact of excessive duration and volume (dB) of the music that one listens to, in order to determine which source of leisure music—live music at social events or PLDs—is most harmful to society. The desired result is to educate readers when to take extra precautionary measures to protect their hearing.

DANGERS OF SOCIAL EVENTS TO THE EAR

One category of excessive leisurely noise that poses a large threat to peoples' hearing is social events. This category includes weddings, concerts, and discos. There is a twofold problem that arises regarding social events. The first problem is that the noise levels are generally controlled by musicians and DJs who themselves are often victims of NIHL. In fact, many musicians who perform in rock or jazz bands have been shown to display either one or a number of the most commonly tested for hearing disorders among musicians, namely, tinnitus, hyperacusis, distortion, diplacusis, and temporary threshold shift (Kahari et al. 2003). Due to their difficulty hearing, musicians tend to play the music very loud, exposing their audiences to unsafe sound levels above 85 dB, putting them at risk of NIHL even with short exposure (Santos et al. 2007). Indeed, as has been documented, "Recently there has been rising concern about the prevalence of NIHL caused by excessively amplified sound activities in leisure settings" (El Dib et al. 2008). Studies focusing on musicians at nightclubs and rock bands found that, in many cases, the non-musicians such as bar tenders, waitresses, DJs, and doormen were all exposed to sound levels above 85 dB (Santos et al. 2007).

The second problem tied to social events is that today's generation has grown accustomed to louder volumes of music, and, as such, the younger members of the audience at social events demand louder sound levels from the bands. As a result, the bands and DJs play music at higher dB levels to please their audiences, leading to increased exposure to louder music for more members of the population. Thus, more people are at risk for developing NIHL by attending loud concerts and other social events that feature loud music (Levey et al. 2011).

DANGERS OF PERSONAL LISTENING DEVICES TO THE EAR

A second category of excessive leisurely noise that poses a serious threat to peoples' hearing is personal listening devices. This category includes iPods and mp3s. Many members of the younger population are listening to PLDs at higher volumes, for longer periods of time, on headphones that send noise directly through the outer ear canal to the eardrum (Levey et al. 2011). Even noise that is at a safe dB level in a free field may not be safe when listened to through in-the-ear headphones because the volume increases in the ear canal due to resonance (Henry and Foots 2012). Increased volume in the ear canal poses greater danger to one's hearing because the noise causes stronger vibrations of the eardrum, leading to excessive movement of the outer hair cells, inner hair cells, and stereocilia. The excessive movement can, in turn, damage them.

Teenagers specifically tend to listen to their PLDs at higher intensities than other members of the population. Many do not realize the extent to which they are endangering their hearing (Levey et al. 2011). In fact, according to one study, since PLDs today can reach a maximum volume of well over 100 dB, close to the sound intensity of a chain saw, the noise emitted by a PLD today is powerful enough to damage the listener's hearing in just five minutes (Matusitz and McCormick 2010). A study done in 2009 found that adolescents who frequently listen to mp3 players are four times more likely to raise the volume and listen to louder music than infrequent listeners (Kahari et al. 2011). As such, the growing danger of noise-induced hearing loss due to PLDs is indeed a true threat to the hearing of today's society as PLD use becomes more and more frequent. In addition, many PLD users tend to listen to their music in settings where there is outside noise interference, such as on the street, subway, and bus. There are reports suggesting that when outside noise increases to 72 dB, the average PLD user raises the device sound level from 69 dB to 85 dB on average. While 85 dB is still not too extreme, due to the current popularity of the Apple iPod, which is conveniently small and contains a large amount of memory and long battery life, people are taking more risks with respect to their hearing by exposing themselves to prolonged periods of 85 dB sounds. This suggests a greater risk of NIHL occurrence in today's population (Levey et al. 2011). PLDs can be particularly dangerous for yet another reason. Being that they are primarily used for private activities in which only the listener is aware of and in control of the sound level, there is little room for third parties, such as teachers and parents, to gauge the amount of noise exposure to which children are exposing themselves (Matusitz and McCormick 2010).

TYPES OF HEARING LOSS

There are several types of hearing loss. This paper, however, focuses on sensorineural hearing loss (SNHL) in particular. Sensorineural hearing loss occurs when the inner ear or auditory nerve is not functioning properly and sound is not being processed as it should be. One of the main causes of sensorineural hearing loss is excessively loud noise. Sensorineural hearing loss that is caused by loud noise is called noise-induced hearing loss.

Other auditory defects that generally come along with NIHL are hyperacusis, diplacusis, tinnitus, and distortion. Hyperacusis is described as hypersensitivity to general sound, which is normally an innoxious stimulus. It occurs when sound that is normally considered of average level suddenly becomes too loud for a person to handle. Diplacusis occurs when one has problems with pitch perception. Tinnitus occurs when one hears buzzing or ringing in the ears, particularly when there is no outside stimulus producing any noise. Distortion occurs when one hears harmonies, frequencies, or other tones as fuzzy, out of tune, unclear, and not in the correct form that the sound was produced (Kahari et al. 2003).

METHODS USED TO MEASURE HEARING LOSS

Hearing loss can be measured in a variety of ways. Different methods target specific sections of the ear, discerning between the effects of differing sounds on various parts of the ear. In the studies analyzed below, the procedures most generally used were pure-tone audiometry, distortion product otoacoustic emissions (DPOAE) testing, and questionnaires. Pure-tone audiometry measures overall hearing loss by determining the lowest tone that is still audible to a person, otherwise known as the pure-tone threshold. Distortion product otoacoustic emissions, which is a type of otoacoustic emissions (OAE) testing, detects the status of the cochlea, measuring the extent of damage to outer hair cells. Because outer hair cells play a large role in auditory functions, when they are damaged, hearing loss occurs. Otoacoustic emissions testing, therefore, is most helpful in measuring NIHL because the outer hair cells are the most vulnerable

part of the ear when it comes to loud noise exposure. Otoacoustic emissions are thought to emerge from the ear due to transduction of healthy outer hair cells. Thus, the less otoacoustic emissions that emerge after a given stimulus, the greater the indication that outer hair cell damage has occurred (Muller et al. 2010; Bhagat and Davis 2008).

PURE-TONE THRESHOLD AND OTOACOUSTIC EMISSION MEASUREMENTS AT SOCIAL EVENTS

An experiment was conducted to study the effects of three hours of loud disco music on the audience's hearing, particularly on their pure-tone threshold and on their distortion product otoacoustic emissions. A pure-tone threshold is the lowest audible sound that one can hear, and a distortion product otoacoustic emission is emitted from the ear in response to two tones of one frequency and one sound pressure that are presented into the outer ear canal. Pure-tone threshold testing, as previously explained, measures overall hearing loss, and distortion product otoacoustic emission testing measures the amount of functioning outer hair cells in the cochlea.

In this particular experiment, 15 subjects with a mean age of 25, who were all healthy and had normal hearing levels, were exposed to disco style music of about 104 dB for three hours. Pure-tone threshold and distortion product otoacoustic emission measurements were taken both before and after exposure to the disco music. The stimuli for both measurements were administered and recorded via ear probes and a digital signal processing card. Pure-tone noises were calibrated using in-the-ear simulators. For pure-tone threshold measurements, the stimuli ranged in frequency from 3469 Hz to 4500 Hz, steadily increasing in increments of 47 Hz. This range was used because human hearing is the most sensitive to noises around 4000 Hz. Beginning with 40 dB level noise, the subject was exposed to a pure tone and instructed to press down on a PC mouse until the sound could no longer be heard. The dB level was decreased in increments of 2 dB at a time until an average was reached at which the subject ceased to press down on the PC mouse. The dB level at this point was determined to be the subject's pure-tone threshold. For distortion product otoacoustic emission measurements, the frequency range of the stimuli was similar to the range used in the pure-tone threshold measurements, and the distortion product otoacoustic emissions were detected using ear probe microphones.

Measurements were taken immediately before, immediately after, and one day after exposure to loud music. Pure-tone thresholds shifted dramatically immediately after disco exposure by approximately 8-14 dB. Individually, there was a wide range of deterioration from 0 to 26 dB. After one day, many subjects significantly recovered; however, they did not recover completely. There was an overall deterioration of about 4 dB (max= 9 dB) among all participants in the experiment. All of the participants in the study displayed diminished distortion product otoacoustic emission levels, which recovered somewhat after the first day post-exposure. However, none of the participants recovered completely (Muller et al. 2010) (See Figure 2).

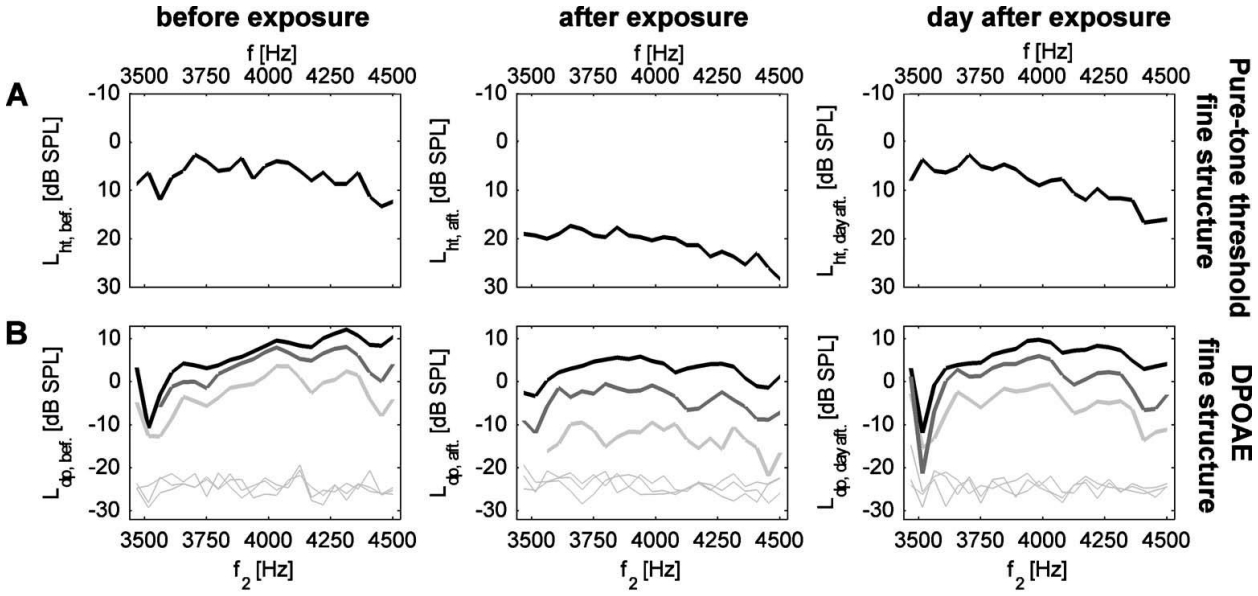

Figure 2: (A) Pure-tone threshold fine structure data taken before (left panel), immediately after (middle panel), and the day after (right panel) discotheque attendance. (B) DPOAE fine structure data for L=40, 30, and 20 dB (from top to bottom). Light gray lines show the particular noise floor; the noise floor is the level of background noise generated by the measuring tools. Source: Muller et al. 2010

Overall, three hours of exposure to music averaging 102 dB caused a clear deterioration in pure-tone thresholds and in distortion product otoacoustic emission levels of the participants. In addition, the decreased distortion product otoacoustic emission levels indicated an increase in distortion product otoacoustic emission threshold, showing that the outer hair cells (i.e. the cochlear amplifier) suffered a loss of sensitivity, particularly at low tone levels. If after only three hours of exposure to 102 dB, outer hair cells and overall hearing capability were clearly impacted, one can infer how much more so would hearing and outer hair cell function be affected by repeated exposure to these high noise levels (Muller et al. 2010).

Another experiment was conducted in Sweden to ascertain the extent of NIHL that rock and jazz musicians suffer from due to their constant environment. This experiment was of particular importance because at social events, such as weddings and discos, the musicians and DJs are generally in charge of the music sound level. If the musicians suffer from NIHL, they may turn up the volume on the amplifiers to their instruments, and this would put all of the party guests at great risk for developing temporary threshold shift or even permanent threshold shift and NIHL due to the excessively loud music.

The participants in this experiment were all musicians who were over the age of 25, had at least five years of experience working in the music industry (i.e. they were exposed to the sound from their instruments directly, not through headphones), and had no preexisting hearing disorders other than possible NIHL due to their daily occupational music exposure. In total, 139 musicians participated. First, pure-tone audiometry was conducted using a calibrated audiometer and headphones, in a soundproof booth. Participants were tested at least eight hours after their last exposure to music in order to avoid temporary threshold shift that would interfere with the

data. Next, all participants filled out a questionnaire to determine the presence of any other hearing disorders such as hyperacusis, diplacusis, tinnitus, and distortion. Based on the results of the questionnaire, the musicians were divided into groups of those with no hearing disorders, those with one hearing disorder, and those with two or more hearing disorders.

According to the information collected from the questionnaires, most of the musicians played for four days a week and five hours a day, all in amplified sound environments where music levels exceeded safety limits. The pure-tone audiograms showed that, overall, the female musicians had lower (i.e. better) hearing thresholds than the males. Interestingly, all the musicians who had five different hearing disorders (five being the most) played the drums, with some also playing the saxophone, both of which are very loud instruments. Some had even worn customized protective earplugs during performances. The results of the testing showed that 74% of the 139 musicians displayed affected hearing due to their excessive music exposure, a frightening statistic. However, it is also interesting to note that the sound levels measured in the listeners' positions were only above safety levels 50% of the time (Kahari et al. 2003). That being said, the listeners are apparently at a slightly lower risk than the musicians to develop NIHL

A cross-sectional study similar to the previous experiment was conducted on 177 Brazilian participants in 2007 to determine the prevalence of high-frequency hearing loss due to noise overexposure (i.e. NIHL) among sound technicians as compared to the rest of the members of the population. Non- sound technicians who were exposed regularly to high sound levels due to their occupations were excluded from the study. Of the participants, 50% were sound technicians and 50% were non-sound technicians. All participants filled out questionnaires regarding their specific exposure to music. They had their ear canals inspected and had hearing tests administered via audiometers. The results of the study showed that 50% of the sound technicians had high-frequency hearing loss and only 10.5% of the non-sound technicians displayed signs of high-frequency hearing loss (El Dib et al. 2008)

In the above three studies, the effects of excessively loud music exposure on the hearing of both musician and listener were analyzed and tested. The conclusions gathered from the studies will be discussed below

PURE-TONE THRESHOLD AND OTOACOUSTIC EMISSIONS MEASUREMENTS AND QUESTIONNAIRES FROM PLD USERS

In 2007, a study was conducted to determine the effects of 30 minutes of exposure to PLD noise that is at the maximum safe exposure level of 85 dB. The participants were 20 young adults who had very good hearing thresholds to begin with. Pure-tone threshold and distortion product otoacoustic emission (DPOAE) measurements were taken before, during, and after the music exposure in sound treated booths. Pure-tone threshold measurements were taken using a calibrated audiometer and headphones. Distortion product otoacoustic emission measurements were taken by emitting primary tones to the ear canal using a probe that contained mini loudspeakers and a mini microphone. The participant was exposed to 30 minutes of straight rock music via an ear bud headphone that was inserted into the ear canal. Immediately after the music exposure, DPOAE and pure-tone threshold measurements were taken to assess immediate recovery abilities of the ear. In addition, to test for long-term hearing loss as a result of the music exposure, DPOAE and pure-tone threshold measurements were taken again 48 hours after the noise exposure.

The results of the above study are interesting. The pure-tone threshold measurements showed no long-term significant threshold shifts as a result of the 30-minute rock music

exposure of 85 dB. However, there were significant reductions in DPOAE half-octave band levels, specifically in the higher frequency range, for a short period of time. Although the DPOAE levels returned to pre-exposure levels within 48 hours of the music exposure, the results indicate that even at the maximum safe level of music volume, certain parts of the ear can be temporarily affected. The results of this study indicate that DPOAE changes can be possible early warning signs that if exposure continues, real ear damage may occur (Bhagat and Davis 2008).

A field study conducted in Sweden gathered data regarding the listening preferences and habits of PLD users, the prevalence of hearing disorders among the population, and sound exposure differences between males and females. Over the course of a single day, data was collected in the main hall of a central station. Passersby who were listening to PLDs were invited to fill out a questionnaire and then to measure their preferred listening volume. There were 41 participants deemed fit to have their results be part of the study. The questionnaire covered three main topics. The first questions discussed the participant's PLD usage history, addressing the type of PLD and headphones used and the earliest age of usage. The second group of questions covered the participant's general usage habits, addressing length of time, environments, maximum volume, and type of music that the participant generally listened to. This section also questioned whether the participant listened to music when falling asleep at night. The third category of questions discussed the hearing health of the participant, inquiring whether there was tinnitus, distortion, hearing fatigue, ultra sensitivity to noise, and whether the participant had basically good hearing or not.

The sound pressure levels of the PLDs were measured with a KEMAR manikin equipped with a coupler and microphone. The microphone was connected to a preamplifier and to a 3560B Frontend (a system for noise and vibration analysis) that was used together with PULSE software to measure and analyze all of the incoming data. Each participant was asked to pick a preferred song and volume, and after 30 seconds of the music playing, a minute-long sample time was measured. The components observed were mean listening level (L_{Aeq}^{60s}), maximum level, and peak level from 20 Hz-10 kHz. In this field study, both genders had begun to use PLDs in their early 20s. More than half of the participants reported listening to their PLDs every day; they were dubbed frequent listeners. The majority of participants reported listening to their devices in loud environments, namely trains and buses (see Figure 3). In addition, more than half of the participants listened to their PLDs at sound levels 75-100% of the device's maximum volume. In this study, only half of the participants showed no signs of affected hearing (Kahari et al. 2011).

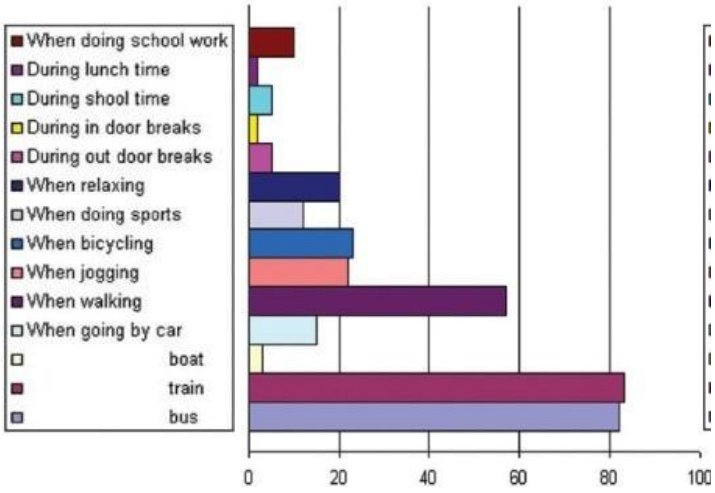

Figure 3: Bar graph plots number of participants per category versus type of environment in which PLD is listened to. The majority of PLD users listen to their devices in loud environments, namely trains and buses. Source: Kahari et al. 2011

ADDITIONAL STUDIES REGARDING PLD USAGE AND DANGERS

In 2006, the American Speech-Language-Hearing Association conducted a survey of 1,000 adults and 301 high school teens in the United States to review the prevalence of hearing difficulties in different parts of the population. The survey showed that a larger percentage of teens than adults displayed three symptoms of noise-induced hearing loss: (1) Television volume was turned up by 28% of teens vs. 26% of adults. (2) During regular conversation, 29% of teens said "what" or "huh" vs. 21% of adults. (3) Tinnitus was experienced by 17% of teens vs. 12% of adults (Levey et al. 2011).

In an unrelated study conducted on 189 college students (average age being 22 years old) using PLDs and walking right out of the subway station onto a CUNY campus, the goal was to determine the percentage of college students at risk for noise-induced hearing loss as a result of their PLDs. The earphones of all participants were measured, and questionnaires were filled out regarding their backgrounds and details surrounding their PLD usage (i.e. recent volume adjustments, type of PLD, frequency of usage, etc). The earphone volumes were measured via a mannequin fitted with a sound level meter that had the microphone situated in the silicon ear canal of the mannequin and was connected to an ER-7C Probe Microphone System via a probe tube that was connected to a computer which recorded the incoming data. The data gathered from the study showed that over 50% of the PLD users were at risk of NIHL due to unhealthy PLD listening habits; too many of the students preferred listening levels above the safe limit (Levey et al. 2011).

Another study, examining 28 undergraduate students, included a questionnaire, hearing health assessment, and measurements of the students' PLD sound levels. Males were discovered to listen to music at an average of 8 dB higher than females (McNeill et al. 2010).

Yet another study tested the hypothesis that PLD users raise the volume on their devices when in a loud environment. Noise measurements were made using two Etymotic ER-7C probe tube microphones placed in the participants' ear canals at a safe distance from the eardrum and connected to a PC able to record the data. The hypothesis was proven correct, as the majority of listeners on average raised their dB levels by 10 dB when in noisy environments. In addition, this study also found that male PLD users tended to listen to louder music than females (Henry and Foots 2012).

DISCUSSION: SOCIAL EVENTS VS. PLDS

DANGERS OF MUSIC AT SOCIAL EVENTS

The first issue raised in this paper regarding social events, claiming that those in control of music volume are themselves likely to have NIHL, inclining them to expose their audiences to excessive volumes, has substantial evidence backing it. In one Swedish experiment described above, which analyzed musicians, 74% of the 139 musicians displayed affected hearing due to their excessive music exposure (Kahari et al. 2003). In the Brazilian study described above, 50% of the sound technicians examined had high-frequency hearing loss (El Dib et al. 2008). Additional studies have shown that most musicians who perform in rock or jazz bands display one or more symptoms of hearing loss (Kahari et al. 2003).

The second issue raised regarding social events, claiming that music volumes are often excessively loud, above 85 dB, placing the audience at great risk of developing NIHL, also has substantial backing. Sound level measurements that were carried out at a number of random musical performances, using meters attached to listeners' and musicians' collars near the ears, showed that all of the musicians were exposed to volumes that exceeded the country's safety limits. Even worse, at rock and jazz concerts, the sound levels that the listeners were exposed to

ranged from 90 to 150 dB, definitely unsafe according to the universal safety limit of 85 dB (Kahari et al. 2011). In addition, the abovementioned study which examined the effect of three hours of excessively loud music on hearing showed that three hours of 102 dB level music can cause outer hair cell deterioration and general hearing loss (Muller et al. 2010). Clearly, NIHL has the potential to occur as a result of loud concerts.

Minimizing the urgency of this data, however, is El Dib's aforementioned study which, although finding that 50% of the sound technicians had high-frequency hearing loss, found just 10.5% of the non-sound technicians to be significantly affected by the effects of loud music. Another point to consider is that females have been shown to possess better hearing thresholds than males (Kahari et al. 2011).

DANGERS OF MUSIC FROM PLDS

There were a number of issues raised in this paper regarding PLDs. Firstly, since many members of the younger population are listening to PLDs at higher volumes and for longer periods of time, they are putting themselves at risk for developing NIHL (Levey et al. 2011). Secondly, many PLD users tend to listen to their music in settings where there is outside noise interference, such as on the street, subway, or bus. In such settings, where there is a high level of noise interference, the average PLD user raises the listening level to excessively loud and, therefore, unsafe volumes. Thirdly, even noise that is at a safe dB level in a free field may not be safe when listened to through in-the-ear headphones, because the volume increases in the ear canal due to resonance (Henry and Foots 2012).

These issues have validity, as in one study, over 50% of PLD users were shown to be at risk for developing NIHL due to unhealthy PLD listening habits (Levey et al. 2011). Another study found the majority of PLD users listening to their devices in loud environments and at more than 75% of the maximum volume (Kahari et al. 2011). In another study, the majority of PLD users were shown to raise their PLD volume by 10 dB when in noisy environments (Henry and Foots 2012). As for the third issue, ear buds in particular have been shown to be extremely harmful to one's hearing because they are placed in the outer ear canal and project noise towards and within very close proximity to the eardrum. The noise emitted from the ear bud is more concentrated and louder in the ear canal and, as such, can cause greater damage to the ear than free field noise that is heard at concerts and parties. To make matters worse, because most ear buds do not block outside noise, and many PLD users listen to already loud music in loud environments, they tend to turn up the music even louder on their PLDs, leading to greater resonance of noise within the ear canal, which increases the sound intensity by about 10 dB (Henry and Foots 2012).

POSSIBLE IMPROVEMENTS FOR SOCIAL EVENTS AND PLDS

A possible improvement to address excessive sound levels at social events is to set legal limits on the volumes at which bands and DJs are allowed to play music during events. A possible improvement to prevent excessive sound levels in PLDs is to lower their maximum volume capabilities. Devices geared towards the adult population can have two sound-level limits, one at baseline level that limits the user to safe volumes and a second, higher volume that can only be activated consciously (e.g. a password). Devices geared toward children and teens, who tend to use PLDs more recklessly than adults, should have just one maximum volume that is well within the safe range of noise exposure (Kahari et al. 2011). Dr. Brian Fligor, director of diagnostic audiology at Boston Children's Hospital, recommends limiting PLD volume to 50% of its maximum sound level for one who listens all day and to 80% if listening for just 90 minutes (Matusitz and McCormick 2010).

Another possible improvement to prevent NIHL might be to raise awareness of the positive effect that intake of antioxidant-rich foods has on auditory function. Antioxidants help lower radioactive oxygen species (ROS) levels in the body. Within the ear, ROS levels in cochlear tissue are raised due to exposure to loud noise. Raised levels of ROS can cause NIHL. As such, the increased uptake up antioxidant-rich foods would be a possible improvement to help prevent NIHL (Hirose et al. 2008).

CONCLUSION

Whereas social events do pose some auditory health risks to the general audience, the music is in a free field and is not exposed to the general public for nearly as long or as often as PLD sounds are. PLDs are used more often and in more private settings, allowing little regulation by others. Thus, although both can reach equal noise levels, PLDs pose a greater danger than social events in regard to developing NIHL, because the devices are more frequently listened to than social events are attended.

At concerts and discos, although studies show the musicians at great risk of developing NIHL, the audience does not have as great a risk. With PLD use, however, all users, both male and female, are at risk of developing NIHL due to unsafe listening habits (Levey et al. 2011), although males have been shown to be more susceptible to hearing loss than females, and the left ear (in both males and females) has been shown to be more sensitive than the right ear (Zhao et al. 2010). In reality, although both social events and PLDs pose threats to society's hearing, one can be worse than the other depending on the frequency of exposure and the dB levels that each individual listens to. Therefore, everyone should take proper precautions to protect their ears in all settings, regardless of the source of the music.

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DELAYED VERSUS EARLY UMBILICAL CORD CLAMPING

Bracha Yaffa Sachs

ABSTRACT

Immediate cord clamping is a part of the active management of the third stage of labor. Active management is standard birth protocol because it significantly reduces the risk of maternal postpartum hemorrhaging. However, since recent evidence advocates delayed cord clamping, various medical practitioners and health organizations would like to incorporate delayed cord clamping in place of immediate cord clamping as a part of standard birth protocol. Proposed benefits include a serious decline in the prevalence of anemia, especially, in countries where anemia is endemic, as well as a decrease in the risk of intraventricular hemorrhage and late onset sepsis. Although these advantages are significant and very important, there are concerns associated with increased risks such as neonatal jaundice, polycythemia, and maternal postpartum hemorrhage.

In order to come to a conclusion, researchers and professionals must calculate the risks versus the benefits of delayed cord clamping based on numerous experiments and randomized controlled trials. Based on the latest research data in the postnatal health arena, delayed cord clamping is a beneficial and risk free technique to manage the umbilical cord for the first few minutes in healthy neonates.

INTRODUCTION

The vein and two arteries within the umbilical cord provide the necessary nutrition for the fetus and dispose of its wastes. The umbilical vein brings oxygenated blood to the fetus, and the two umbilical arteries carry deoxygenated blood away from the fetus (Tortora and Grabowski 2003a). Without the umbilical cord connection, a fetus cannot survive in its mother's uterus. Immediately after an infant is born, the decline of the infant's intrathoracic pressure will draw blood from the umbilical cord into the lung. Therefore, as long as the umbilical cord remains unclamped, blood passes through to the infant at 19 ml/kg of the infant's birth weight, increasing the neonatal blood volume (Weeks 2007) (See Figure 1). Upon birth, active management is generally implemented. Active management is standard birth protocol, intended to significantly reduce the risk of maternal postpartum hemorrhaging. Included in active management are controlled cord traction, immediate clamping and drainage of the umbilical cord, and the administration of uterotonic agents. The standard practice is to tie and cut the umbilical cord within the first 15 seconds of birth, a process known as immediate or early cord clamping (ICC or ECC, respectively) (Eichenbaum-Pikser and Zasloff 2009). However, a growing body of evidence indicates that it would be beneficial to delay this clamping, a procedure known as delayed cord clamping (DCC). Is the evidence displaying potential benefits of delayed cord clamping substantial enough that mothers should delay the clamping of the cord to maximize the benefits that their children receive?

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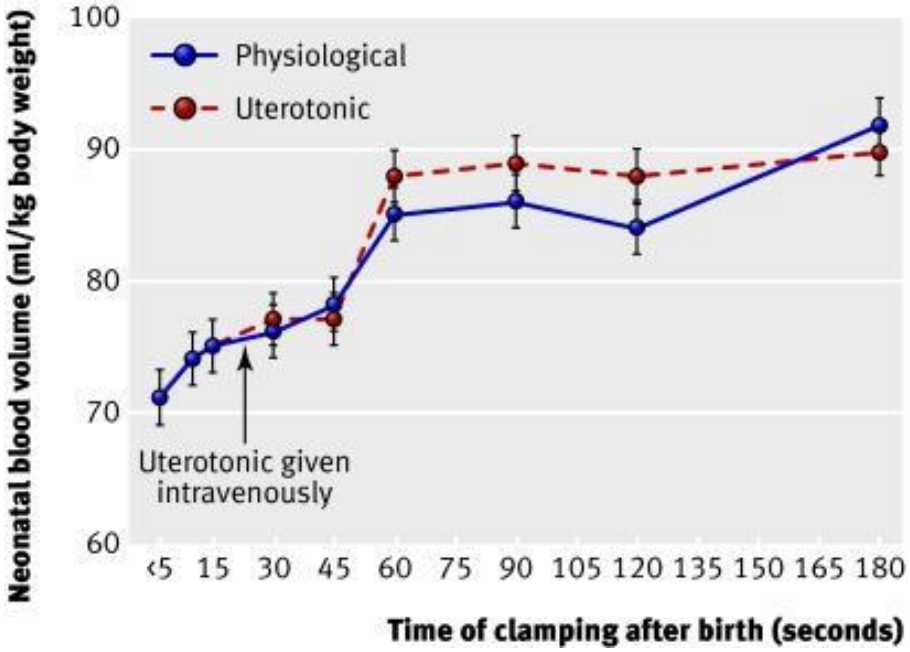

Figure 1: Changes in the neonatal blood volume with increasing delay of cord clamping, with and without the use of a uterotonic. Source: Weeks 2007

TIMING OF CORD CLAMPING AND INFANT POSITIONING

While the timing of immediate cord clamping in full term, preterm, and cesarean section born infants, ranges from clamping of the cord immediately after the baby is delivered up until 30 seconds post birth, the timing is not as defined in delayed cord clamping. Table 1 displays a range of times referred to in the literature as DCC.

Author, Year	Study Population	Cord Management	Statistically Significant Results	Recommendations
Strauss et al. 2008	Partially blind randomized controlled trial, < 36 wks EGA; early, n = 60; delayed, n = 45	Early = within 15 sec; delayed = 1 min	Circulating RBC vol/mass increased and Hct values were higher after delayed clamping	1 min delay in infants 30–36 wks EGA who do not need resuscitation
Hutton and Hassan 2007	15 randomized controlled trials, full-term infants	Early = immediately after birth; delayed = minimum of 2 min	Improved hematologic status over 2–6 mos with delayed clamping	Minimum of 2-min delay
McDonald and Middleton 2008	11 randomized controlled trials, full-term infants	Early = within 60 sec; delayed = > 1 min after birth or when cord pulsation ceased	No difference in rates of PPH, increase in neonatal Hgb/Hct; increase in jaundice	A more "liberal" approach to delaying clamping in healthy term infants

Jahazi et al. 2008	Healthy, full-term, vaginally born neonates; delayed, n = 34; early, n = 30	Early = 30 sec; delayed = 3 min	No increase in Hct noted; significantly increased ENBV	Potential benefit should be considered by providers
Utlee et al. 2007	randomized controlled trial, 34–37 wks EGA; early, n = 19; late, n = 18	Early = < 30 sec; late = > 180 sec	Delayed groups had higher Hgb levels at 1 hr postpartum and 10 wks old; no difference in the ferritin levels at 10 wks	Immediate cord clamping should be discouraged
Van Rheenen et al. 2007	Delayed, n = 46; control, n = 45	Awaited cessation of pulse, mean clamping time 305 sec (control mean clamping time, 15 sec)	Increase in PCV, increased Hgb at 4 mos	3-min delay
Cernadas et al. 2006	Early, n = 90; delayed 1, n = 90; delayed 2, n = 92	Early = within 15 sec; delayed 1 = 1-min delay; delayed 2 = 3-min delay	Hct at 6 hrs highest in delayed cord clamping, lowest in early; increase in anemia at 6 hrs and 24–48 hrs in early	Delay of at least 1 min
Van Rheenen and Brabin 2006	Four randomized controlled trials	Immediate = within 20 sec; delayed = 30 sec to 2 min	Decreased anemia up to 4 mos, higher iron levels up to 6 mos	Delay of at least 3 min
Rabe and Diaz-Rossello 2004	Seven randomized controlled trials	Early = within 30 sec; delayed = 30–120 sec	Decreased IVH, fewer blood transfusions	Delay of 30–120 sec

EGA= estimated gestational age; ENBV= estimated neonatal blood volume; Hct= hematocrit; Hgb= hemoglobin; IVH= intraventricular hemorrhage; PCV= packed cell volume; PPH= postpartum hemorrhage; RBC= red blood cell; RCT= randomized controlled trial.

Table 1: Variation in the definition of delayed cord clamping. Source: Eichenbaum- Pikser and Zasloff 2009

In DCC of a full term, healthy neonate, most doctors will wait until the cord ceases pulsating before clamping it (Kent 2010). Pulse cessation can either be defined as the complete

absence of a pulse, or as the presence of only a weak pulse. Van Rheenen et al. (2007) found that, on average, cord pulsation ceased completely 305 seconds after birth. Some doctors and midwives, however, consider DCC as clamping of the cord at any point after 30 seconds from birth (Eichenbaum- Pikser and Zasloff 2009). In DCC of preterm infants, the variance tends to be smaller, with trials reporting a clamping time of 30 seconds to one minute (Mercer et al. 2006; Strauss et al. 2008).

To maximize the volume of transfused blood, the neonate should be held about 10 to 15 inches below the site of delivery, and no unnecessary pressure should be placed on the cord (Mercer et al. 2006).

Benefits of Delayed Cord Clamping

Improved Hematocrit Levels, Decreased Iron Deficiency, and Prevalence of Anemia

Iron deficiency and iron deficiency anemia are endemic in underdeveloped countries and poor populations. Since the brain requires iron for neuron myelination, dendritic growth, neurotransmission, and in neural and glial energy metabolism, iron deficiency in infants is linked to neurodevelopmental delays (Lewis 2011). Some of the detrimental effects that happen as a result of iron deficiency are irreversible, even after iron treatment. Delaying umbilical cord clamping after birth increases the volume of blood transfusion and results in a decrease in infant iron deficiency (Mercer and Erickson-Owens 2006).

A large, randomized study conducted in Mexico City found that infants in the DCC group had less iron deficiency and iron deficiency anemia than infants in the ICC group. Additionally, infants in the DCC group had a higher mean corpuscular volume, higher levels of body and stored iron, higher levels of ferritin, and a lower ratio of transferrin receptor to ferritin than those in the ICC group (Chaparro et al. 2006) (Figures 2 and 3).

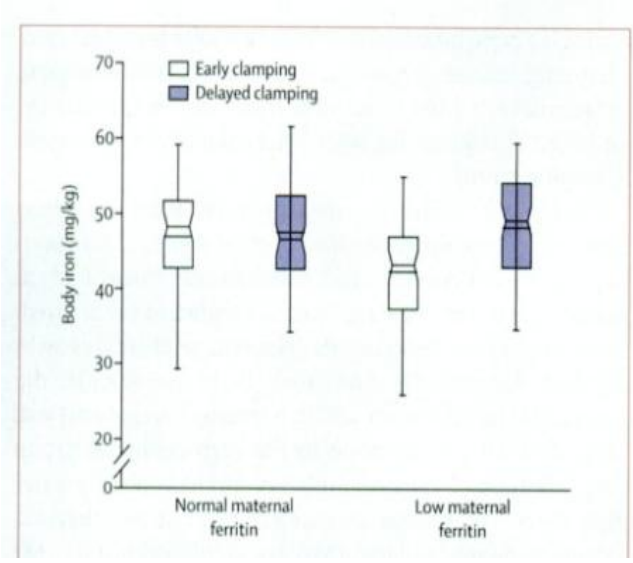

Figure 2: Box-and-whisker plot of two-way interaction effect of treatment group and maternal ferritin on infant body iron (mg/kg) at 6 months of age. Boxes represent the inter-quartile range (25th to 75th percentile), and whiskers indicate the 5th and 95th percentiles for the unadjusted data. The notch in each box represents Cl about the median, represented by horizontal line at the middle of the notch. Additional horizontal line represents the mean of each subgroup. Treatment difference (early clamping vs. delayed clamping, adjusted for maternal ferritin and employment) in body iron in infants born to mothers with normal ferritin concentrations was -0.8 mg/kg (95% Cl- 5.0 to 3.4 mg/kg). Treatment difference (adjusted) in body iron in infants born to mothers with low ferritin concentrations was -6.5 mg/kg (-10.2 to -2.8 mg/kg). Low maternal ferritin is <12 μg/L; normal maternal ferritin is ≥ 12 μg/L. p=0.008 for interaction term. Source: Chaparro et al. 2006

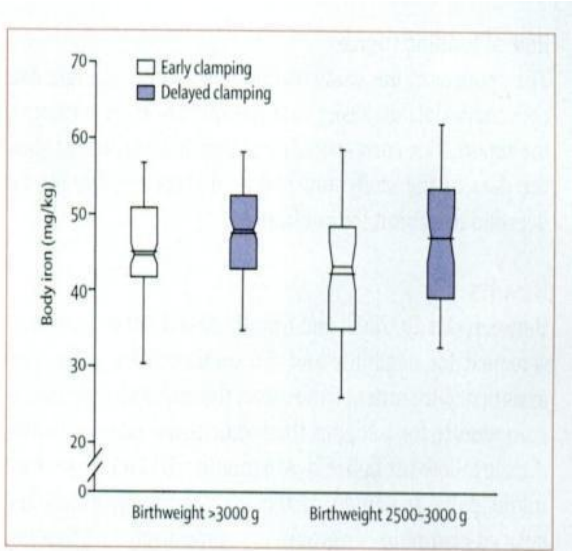

Figure 3: Two-way interaction effect of treatment group and infant birth weight on infant body iron (mg/kg) at 6 months of age. Treatment difference (early clamping vs. delayed clamping, adjusted for maternal ferritin and employment) in body iron in infants born with birth weight more than 3000 g was -3.5 mg/kg (95% Cl- 5.9 to 0.9 mg/kg). Treatment difference (adjusted) in body iron in infants with birth weight between 2500 g and 3000 g was 7.1 mg/kg (-11.9 to -2.4 mg/kg). p=0.04 for interaction term. Source: Chaparro et al. 2006

DCC can also reduce anemia in preterm infants. A review analysis of ten randomized trials, with a total of 454 premature infants within 37 weeks gestational age, found that infants with DCC had significantly higher hematocrit levels and less transfusions for anemia than those with ICC (Rabe et al. 2008) (See Table 2). However, the groups in these studies were relatively small, containing only between 19 to 86 participants.

Study	ECC n/N	LCC n/N	Relative risk (fixed-effects model)
Outcome: transfused for anaemia			
Kinmond et al. 1993	7/13	1/13	7.00 (1.00, 49.16)
McDonnell et al. 1997	6/23	4/23	1.50 (0.49, 4.62)
Rabe et al. 2000	16/20	9/19	1.69 (1.00, 2.85)
Total	56	55	2.01 (1.24, 3.27)
Total events: 29 early, 14 delayed			
Test for heterogeneity: x^2=2.26, df=2 (p=0.32), I^2=11.5%. Test for overall effect: Z=2.81 (p=0.005).			

Study	N	ECC	N	LCC	WMD (fixed)
Outcome: transfused for anaemia					
Kinmond et al. 1993	13	1.91 ± 1.62	13	0.46 ± 0.66	1.45 (0.50, 2.40)
Rabe et al. 2000	20	2.40 ± 1.95	19	1.20 ± 1.60	1.20 (0.08, 2.32)
Oh et al. 2002	17	4.00 ± 4.00	16	3.60 ± 3.80	0.40 (-2.26, 3.06)
Mercer et al. 2006	36	2.47 ± 3.70	36	1.94 ± 3.10	0.53 (-1.05, 2.11)
Total	86		84		1.16 (0.52, 1.80)
Total events: none recorded					
Test for heterogeneity: x^2=1.29, df=3 (p=0.73), I^2=0%. Test for overall effect: Z=3.55 (p=0.0004).					

Table 2: Figures in parentheses are 95% CI. ECC= Early cord clamping; LCC- late cord clamping; WMD= weighted mean difference; df= degrees of freedom. Source: Rabe et al. 2008

A slightly larger trial, consisting of 105 preterm infants, also found that infants in the DCC group had higher hematocrit levels, red blood cell volume, and iron levels. Infants in this study had a gestational age of 36 weeks or less (Strauss et al. 2008).

DCC can also benefit healthy, full term infants born in developed countries. A study of 400 mother-infant pairs in Sweden found higher ferritin concentration and total body iron in the DCC group at four months. The DCC group also had lower numbers of anemic infants at two days from birth (Andersson et al. 2011).

According to one research group, the higher birth weight of 96 grams on average in infants with delayed cord clamping denotes a higher placental transfusion in the DCC group. Additionally, in two-day-old infants, the placenta retained less blood and raised levels of hemoglobin and hematocrit (Andersson et al. 2011). However, perhaps the increased birth weight is a result of natural factors. Also, although a possible explanation accounting for the lower hemoglobin concentration in the DCC is proposed, there is no scientific reason given. On one hand, the trial proves the effectiveness of increasing iron stores and decreasing iron deficiency at four months in infants born in countries that do not have an endemic iron deficiency anemia. Yet, the research confirms the conclusion only in full term healthy infants, and not in premature births or in births from complicated pregnancies in the same setting.

DECREASED RISK OF INTRAVENTRICULAR HEMORRHAGE

Premature infants are at a higher risk for intraventricular hemorrhage (IVH) than full term infants, with infants born within 28 weeks gestational age having the greatest risk (Kling 2010).

A study by Mercer et al. (2006) found that infants with ICC had a higher risk of IVH than those with DCC. In the ICC group, IVH affected some 42 % of males and 29 % of females, whereas IVH affected only 9 % and 23 % respectively, in the DCC group. A recent follow-up study found similar results, with infants in the DCC group showing a 50 % reduction in IVH (Mercer et al. 2010). This study also tested for neurodevelopmental outcomes of DCC and showed that as a result of lower rates of intraventricular hemorrhage, the infants had better motor performance at seven months. Although the positive motor performance is dominant in male infants (Mercer et al. 2010), a reason for this particular outcome is unknown. The improvement of the neurodevelopmental status in male infants resulting from lower rates of intraventricular hemorrhage may be due to the fact that intraventricular hemorrhage has shown to be more widespread in the males with ICC.

Table 3 below reviews a number of studies reflecting a lower rate of IVH in infants with DCC.

Study	ECC n/N	LCC n/N	Relative risk (fixed-effects model)
Outcome: intraventricular haemorrhage			
Hofmeyr et al. 1993	11/46	8/40	1.20 (0.53, 2.68)
Hofmeyr et al. 1988	10/13	8/23	2.21 (1.17, 4.17)
McDonnell et al. 1997	1/16	0/15	2.82 (0.12, 64.39)
Rabe et al. 2000	3/20	1/19	2.85 (0.32, 25.07)
Oh et al. 2002	4/17	2/16	1.88 (0.40, 8.90)
Mercer et al. 2003	5/16	3/16	1.67 (.48, 5.83)
Mercer et al. 2006	13/36	5/36	2.60 (1.03, 6.54)
Total	164	165	1.90 (1.27, 3.84)
Total events: 47 early, 27 delayed			
Test for heterogeneity: x^2=2.17, df=6 (p=0.90), I^2=0%. Test for overall effect: Z=3.13 (p=0.002).			

Table 3: ECC= early cord clamping, LCC= late cord clamping, df= degrees of freedom. Source: Rabe et al. 2008

Several studies propose possible mechanisms for this decrease in occurrences of intraventricular hemorrhage. Dr. Backes suggests that the results are due to an increase in progenitor cell percentages within 48 hours of birth. However, this increase is not significant anymore by the time the infants are 30 days old (Kling 2010). Alternatively, Rabe et al. (2008) suggest that this decrease may be a reflection of improved cardiovascular stability that results from the delayed clamping. However, none of these explanations have been confirmed.

Strauss et al. (2008) found no significant decrease in intraventricular hemorrhage incidence in DCC v. ICC infants. However, the Society of Obstetricians and Gynecologists of Canada says that DCC in prematurely born infants reduces occurrences of intraventricular hemorrhage (Gordon 2010). Strauss et al. know that the outcome of their trial does not agree with previous outcomes and have suggested that the neonates in their trial were at least 30 weeks gestational age whereas those in other trials were generally under 30 weeks. However, the Society of Obstetricians and Gynecologists of Canada says that DCC significantly reduces intraventricular hemorrhage incidence in infants born at less than 37 weeks gestational age (Gordon 2010).

DECREASED RISK OF LATE ONSET SEPSIS

Decreases in the incidence of late onset sepsis (LOS) have been recently discovered as another potentially important outcome of DCC. Late onset sepsis is the probable cause behind infection and inflammation of infants born prematurely (Rabe et al. 2008).

In one study, while 22% of infants in the ICC group tested positive for LOS, only 3% of the DCC group tested positive. As an interesting aside, a higher percentage of late onset sepsis occurred in the more prematurely born infants. Of the eight infants from the ICC group and one from the DCC group diagnosed with LOS, six of them were born 24 to 27 weeks gestational age, and the other three were born 28 to 31 weeks gestational age. Interestingly, infants with sepsis had lower initial hematocrit levels, even when controlled for gestational age (Mercer et al. 2006).

In a more recent study, the results were almost identical to the previously mentioned study, with 21% of infants in the immediate cord clamping group and 3% of infants in the delayed cord clamping group testing positive for late onset sepsis (Mercer et al. 2010).

As seen with reduced incidence of intraventricular hemorrhage, gender comparisons indicate that with DCC, male infants have a greater reduction in late onset sepsis than female infants. In one experiment, six males and two females in the ICC group acquired LOS, while in the DCC group the number of infants with late onset sepsis was zero and one respectively (Mercer et al. 2006). Another trial records the same trend but does not give detailed data (Mercer et al. 2010). Although a clinically plausible explanation for this trend has not yet been proposed, it may be because late onset sepsis, like IVH, is more prevalent in male infants.

Babies born via a caesarean section would also greatly benefit from delayed cord clamping; only their precarious state generally requires earlier intervention. Nevertheless, a delay of at least one minute should be attempted (Weeks 2007). The same applies to full term or preterm babies requiring special care and support prior to umbilical cord pulse cessation (Eichenbaum- Pikser and Zasloff 2009).

Risks of Delayed Cord Clamping

Increased Risk of Neonatal Jaundice, Resulting in an Increased Phototherapy Treatment

Delayed cord clamping is often assumed to be liable for an increase in the incidence of neonatal jaundice (Eichenbaum-Pikser and Zaloff 2009). In Strauss et al. (2008) randomized trial, a significantly higher percentage of infants in the early cord clamping group required phototherapy to treat neonatal jaundice. However, there were no differences in the serum bilirubin levels, the age which the phototherapy treatment began, and the length or intensity of the treatment between the two groups. Another experiment indeed found higher bilirubin levels in DCC infants, but the levels generally did not exceed the phototherapy threshold, and the infants did not require exchange transfusion. The number of incidences where treatment was required did not differ significantly between the two groups (van Rheenen and Brabin 2006). In Chapparo et al. (2006) study of normal weight, full term Mexican infants, there was no difference between the two groups in the number of infants diagnosed with neonatal jaundice. In a study of healthy Swedish infants, only one infant in the DCC group required phototherapy for jaundice, as opposed to two infants in the ICC group. According to the Cochrane review (a collection of databases in medicine and other healthcare specialties), the fear of increased risk in neonatal jaundice relies on unpublished data (Andersson et al. 2011), and there is no evidence validating a relationship between DCC and neonatal jaundice (Strauss et al. 2008).

Increased Risk of Polycythemia

Polycythemia is diagnosed in neonates if they display venous blood hematocrit levels greater than 65% (Tortora and Grabowski 2003b). However, because the red blood cell volume from a peripheral vein is usually higher than other veins, some doctors may use 70% venous blood hematocrit as their cutoff mark (Chaparro et al. 2006). Concerns have been expressed that polycythemia might be a potential adverse effect of DCC (Andersson et al. 2011). In fact, according to Basile and Southgate (2004), delayed cord clamping is said to be the most common cause of polycythemia in full term healthy neonates since it results in higher levels of hematocrit.

However, studies on full term, healthy infants do not support this concern and conclude that polycythemia is not a risk of delayed cord clamping. In the study of Mexican infants by Chapparo et al. (2006), none of the infants presented with hematocrit levels greater than 70%. Although there were two infants in the DCC group whose hematocrit levels were slightly above

65%, they were not diagnosed with polycythemia. In the abovementioned Swedish study as well, none of the infants were diagnosed with polycythemia (Andersson et al. 2011).

Similar results were obtained from trials done on preterm and very low birth weight infants. In Strauss et al. (2008) study of preterm infants, although each time the hematocrit levels were assessed, the levels displayed in infants from the DCC group were significantly higher than those of the ICC group, they were always below 65% (Table 4). Like the full term healthy infants, none of the preterm infants were diagnosed with polycythemia.

	Day 0-1		**Day 7**		**Day 14**		**Day 21**		**Day 28**	
Clamping time	**Hct**	**PLT**	**Hct**	**PLT**	**Hct**	**PLT**	**Hct**	**PLT**	**Hct**	**PLT**
Immediate (n = 55)	53 ± 1.1	248 ± 17	47 ± 0.9	292 ± 28	41 ± 0.07	375 ± 33	36 ± 0.7	456 ± 36	31 ± 0.6	434 ± 37
Delayed (n = 41)	56 ± 1.3	241 ± 54	52 ± 1.0	334 ± 45	46 ± 0.8	365 ± 60	41 ± 0.9	401 ± 56	35 ± 0.8	469 ± 103
	$p = 0.188$		$p = 0.005$		$p < 0.0001$		$p < 0.0001$		$p < 0.0001$	

Table 4: Hct= Hematocrit, PLT=platelet. Source: Strauss et al. 2008

Some experiments, however, do show DCC infants exceeding hematocrit thresholds. In a trial analysis conducted by Van Rheenen and Brabin (2004, quoted in Mercer et al. 2007), three infants in the DCC group were diagnosed with polycythemia. A similiar analysis conducted by Hutton and Hassan (2007, quoted in Eichenbaum-Pikser and Zaloff 2009) displays some infants with polycythemia at 7, 24, and 48 hours after birth, with a greater risk for DCC infants. However, in both cases, all of the diagnosed infants had asymptomatic polycythemia. Additionally, in a double blind randomized controlled trial by Jahazi et al. (2008, quoted in Eichenbaum-Pikser and Zaloff 2009), infants in both groups presented with polycythemia at two hours after birth, with no significant differences between the two groups.

INCREASE RISK OF MATERNAL POSTPARTUM HEMORRHAGE

Active management, versus expectant management, of the third stage of labor has been proven to decrease maternal postpartum hemorrhaging (PPH) (Miller et al. 2004). Included in active management of the third stage of labor are controlled cord traction, immediate clamping and drainage of the umbilical cord, and the administration of one or two uterotonic agents, e.g. oxytocin (Chelmow 2008). Expectant management is the delivery of the placenta via maternal effort, waiting for cessation of the cord pulse to clamp it, and refraining from administering uterotonic agents (Rogers et al. 1998). Chaparro et al. (2006) report that there is no evidence proving that ICC alone decreases the risks of maternal hemorrhage. They suggest that perhaps it is the combination of all the components of active management, including ICC, which decreases the risk of postpartum hemorrhage. Their study, however, suffered from an inability to properly measure maternal blood loss and can, therefore, be considered inconclusive.

Another study on the effects of delayed cord clamping on maternal postpartum hemorrhage showed no increase in hemorrhage with DCC. In this study, women were given either a simplified package of active management from which ICC was omitted, or a full package

of active management including ICC. The women who received the simplified package showed no substantial increase in maternal postpartum hemorrhage (Garcia 2012a). Dr. David Hutchon says that ICC is an unproven intervention for decreasing postpartum hemorrhaging (Gordon 2010). Andrew Weeks (2007) assumes that ICC was not intentionally added to the active management, since there is no evidence of it playing any role in reducing postpartum hemorrhage by itself or together with the other components of active management. Rather, Weeks assumes, the sole reason for the inclusion of ICC is most likely due to its having been included into standard birth protocol. The World Health Organization recommends the administration of oxytocin as well as controlled cord traction and suggests delaying the clamping and cutting of the cord until a healthcare worker is prepared to apply cord traction (Garcia 2012b).

CONCLUSION

Much research has been done concerning delayed cord clamping in the full term, preterm, and cesarean section neonates. In all the experiments, while none of the outcomes confirm the possible risks of DCC, substantial evidence verifies its benefits. Therefore, not only should mothers be educated about the concept of DCC, but DCC should also be integrated into standard birth protocol in healthy infants.

With regard to infants born with major health issues, further research is still required to determine whether or not the DCC method is safe enough to be incorporated in standard birth protocol.

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BIOLOGICAL ENGINEERING: ADVANCES AND METHODS

Joel Schwartz

ABSTRACT

The last few decades have seen a tremendous growth in the field of bioengineering. As the need for further treatment and innovation for tissue repair, partial to full organ replication, and gene therapy continues to increase, the field of bioengineering will be tasked with curing and preventing disease and traumatic injuries. The two primary fields currently being focused on in the lab are the way cells interact and communicate to build tissues, and the nature and materials utilized in scaffolding to allow differentiation and migration when cells are seeded. Within those two fields are subsets of different methods, materials that vary greatly. Some stem cells offer certain benefits, yet lack viability due to a host of obstacles, such as ethical questions about their procurement, to their technical obstacles, such as materials utilized for best profusion in a scaffold. It appears that proper and adequate funding for research into finding solutions will be pivotal in having the next medical breakthrough in science. It may very well be referred as one of the greatest advancements in modern history and forever change the face of science should this technology become successful and accessible. Indeed, recent successes in patients would be a strong indicator that this technology and innovation is not too distant in the future.

INTRODUCTION

Currently, in the US alone, there are over 100,000 candidates awaiting organs on the national organ waiting list. On average, a person on that waiting list dies every 90 minutes (UNOS 2012). In addition, as the amount of recipients is increasing, the amount of donors is decreasing. This is due to factors such as ethical debates and customs regarding organ donation, and the fact that organs may only be harvested coincident with brainstem death, which necessitates hospitals utilize additional resources to keep patients on life support (Briggs et al.1997). While technology and advanced methods have fine-tuned the science of organ transplants, it does not address the need for more organs. In addition, tissues such as cartilage, muscle, and even neural tissue for regeneration in vivo can have a tremendous impact on overall life quality in a patient. In response to this need, the last 30 years have been a whirlwind of activity for researchers to try and replicate and build new tissues and organs, thus creating the field of biological engineering. An early example of tissue engineering was developed by Dr. J. Burke of Massachusetts General Hospital. He created a synthetic neodermal skin utilizing chrondroitin 6-sulface in tandem with collagen, to cover burn patients while their skin was in the process of regenerating.

Bioengineering is multifaceted and has many working and intrinsic parts. It requires an in depth and interdisciplinary understanding of the biological cell such as its embryonic origin, cycle, replication, metabolism, energy requirements and proliferation stages, as well as a keen understanding of structural engineering, materials unique in biological adsorption or absorption, chemical compounds and nanotechnology to pair them together in a hybrid organ.

At the fundamental level, it begins with the cell, specifically using an embryonic stem cell or mature adult progenitor cell (MAPC) to direct and grow. However, having cells proliferate is not enough, as a clump of differentiated cells is all that would be present, hence the need for scaffolding as well. Scaffolding is tasked with providing a three dimensional structure for the cells to grow on and to deliver growth factors to nurture the cells. Study and research analyzing embryonic and adult stem cells and its methods of use, as well as the requirements scaffolding need to become viable, and assess and evaluate which of the many scaffolding

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materials/methods currently being tested, hold the most promise for full organ replication in the near future.

1. Stem Cell Role in Regenerative Engineering

Stem cells were discovered to have the ability to replicate and divide into any type of cell in the body (plasticity). This opened up the possibility for regenerating diseased tissue or failing organs by reseeding those areas with stem cells to differentiate and proliferate to become the new cells in those areas. When combined with the proper method of feeder lines and guided cell growth, the development of the cell can be accomplished. There are several different types of stem cells, each with its own unique set of benefits and characteristics. Some exhibit obstacles as well, and are in the process of being studied.

Human Embryonic Stem Cells

Stem cells isolated from the embryo are achieved in chronological proximity to egg fertilization. These cells are derived from the inner mass of the blastocyst cell after four to six days of gestation, at which point they number approximately one hundred fifty cells, comprised of none differentiated and totipotent cells. They will continue on to form the more than two hundred tissue types in the human body. Derivation is executed through several means. The primary and most successful method utilized, is by removing the outer trophectoderm via immunosurgery thus exposing the inner mass cell (ICM) for disaggregation and plating on a feeder cell layer for further culture. Cultivation of stem cells is maintained by providing the proper nutrients such as proteins and growth factors, which can be efficiently done if cells are in a suspension medium, specific to the cell's needs. At day six, these cells will enter the blastocyst stage and have differentiated into trophectoderm and an inner cell mass that includes the progenitors of all the cells in the body. Thus the embryonic stem cell has tremendous potential for differentiation into any type of cell needed, and is unique in its capacity to propagate without losing pluripotency, thus may readily differentiate into various cell types of the three embryonic germ layers. An additional advantage of human embryonic stem cells is the relative ease with which they can be genetically engineered by a broad range of techniques such as transfection, electroporation, and viral infection (Power and Rasko 2011). Human embryonic stem cells have the additional advantage of rapid mitosis as well, and can divide at an exponential rate. However, unless the stem cell is banked from the recipients own stem cells, rejection is still possible and quite likely. Additionally, there are many ethical debates about whether utilizing embryonic stem cells. Primarily, the concern seems to be that according to one school of thought, life begins at inception, so extracting cells from a fertilized egg is akin to destroying life (Briggs et al. 1997). Additionally, due to its extreme proliferative nature, they often can become cancerous and form teratomas. Indeed, there have been documented cases of tumors containing fully formed teeth or retinas.

On the other hand, there are many advantages to utilizing embryonic stem cells. Primarily, these cells exhibit the ability to unlimited or prolonged self-renewal and the potential to produce all the differentiated cell types necessary for any specific tissue. Their embryonic state allows for exponential growth that can propagate, assuring robust growth. Due to their ability to differentiate into any type of cell, all tissue types can be generated. Additionally, the extreme plasticity of the cell makes it an ideal candidate for programming the cell DNA to express the specific differentiation desired. However, although human embryonic stem cells are not yet differentiated, they are already coded in their DNA for the proteins and markers they will exhibit. This can lead to host rejection should an immune response be initiated. Creating a cell bank with all the different types of HLA (human leukocyte antigen) to act as a reserve for

matching the specific needs of recipients would certainly address the issue of host rejection and would drastically decrease the amount of time a recipient would need to wait for the quorum of cells needed for transplant (Cabrera et al. 2006).

MATURE ADULT PROGENITOR CELLS

Mature progenitor cells have proven most successful in the clinical aspect. This is due to its already partially differentiated state, which makes the manipulation and direction of differentiation more viable. Mature adult progenitor cells do not illicit host rejection, when it is the recipient's very own cell. However, it does have certain limitations. The main obstacle with progenitor cells, is that they are already differentiated, thus making them only viable for their specific cell of origin. Additionally, sometimes the area where the progenitor cells may be from, are in a necrotic state and viable cells for extraction is no longer possible. When that is the case, receiving progenitor cells from a donor will result in the same problems of host rejection due to different phenotype. (Shokier et al. 2010). Additionally, the use of stem cells eliminates the need for lifelong immunosuppressant drugs as the stem cells utilized for regeneration is the recipient's own cells. Additionally it has been found that when these cells are encapsulated with Fibrin, they secrete more collagen. (Lin et al. 2010)

Mature adult progenitor cells are primarily selected from bone marrow derived mesenchymal stem cells (BMSCS) which are capable of differentiating into a number of different cell types of mesoderm lineages, including adipocytes, osteocytes, chondrocytes, and other mesodermal cells (Fillmore et al. 2005). They are generally cultured by using colony forming unit fibroblasts. Raw or ficoll-purified bone marrow is placed onto the plates. Mesenchymal stem cells can exhibit signs of adherence to the tissue culture plastic within 1-2 days. Hematopoietic cells do not adhere. Also, flow cytometers are used to filter for STRO-1 markers. Lin and Bo experimented with mature adult progenitor cells to see how well they differentiate into hepatocyte like cells. They used a 21 gauge needle inserted into the bone which was then flushed with Dulbecco's modified Eagles medium (DMEM). The cells were then centrifuged with a Ficoll step gradient of 1.077g/ml at 1500 rpm. Mononuclear cells were then collected and re-suspended in Dulbecco's modified Eagles medium with 10% fetal bovine serum (Fillmore et al. 2005). This process was done for both chondrocytes and osteoblasts. For chondrocytes, enough extracellular matrix was formed to adequately plug areas of tissue damage in knee joints. For osteoblasts, mineral deposits were observed to show that the osteoblasts were indeed calcifying the extracellular matrix and creating bone. This has had strong clinical success and will hopefully be available as bone replacement in the near future. It is also important to note that mature adult progenitor cells are currently the only type of stem cell approved by the FDA for medical application. This is mostly due to the fact that as mature adult progenitor cells or only multipotent, they do not exhibit the exponential growth of embryonic or cord derived stem cells, thus not posing a risk of cancer or teratoma formation. However, the main obstacle of mature adult progenitor cells are they are very limited in clinical applications due to their very stable properties. It has been speculated that with dedifferentiation, this obstacle might be overcome. Through several unique pathways, namely the NOGGIN pathway, it is possible to reprogram an already differentiated adult cell back to its basic non-differentiated state, thus having the same capabilities as embryonic stem cells, but not have the rapid mitotic characteristic that can become cancerous. (Cai et al. 2007). Dedifferentiation is not a farfetched concept as it is seen in many amphibians, animals and even humans. Amphibians, have exhibited the ability to regenerate damaged organs to fully functional states (Davenport 2005). This makes

a strong argument for dedifferentiation to be funded and researched extensively, as it might hold the key for repairing damaged organs in humans as well.

CORD-DERIVED MESENCHYMAL STEM CELLS

Data supports the indication that cord derived mesenchymal stem cells are a unique combination of the positive qualities of both adult progenitor and human embryonic stem cells, with none of the disadvantages either of those cells exhibit. They are extremely proliferative due to its embryonic nature (not quite as proliferative as human embryonic stem cells), yet have developed enough to exhibit signs of mature progenitor cells. This allows them to be programmed to differentiate into specific types of cells. Additionally, due to their chronological proximity to their phenotypic state, it is quite possible that certain cells are original stem cells much like those found in the human embryo. Drawbacks include possible rejection by host because they already have markers. Much like the idea of a human leukocyte antigen bank for human embryonic stem cells, the same concept would benefit mesenchymal cells, allowing for quick and accurate matches specific to the host. Due to the combined benefits of cord-derived cells, it would seem that extensive funding and research would yield many advances and discoveries in gene therapy as well as the field of regenerative medicine. It is also important to note that the nature in which these cells are derived pose no ethical quandaries, as these stem cells are derived from discarded placenta. Additionally, they are relatively easy to isolate and can be utilized in the lab at minimal cost.

2. SCAFFOLDING

While cells may proliferate and grow, they need to be guided and assembled in a proper dimensional order. This can only be achieved with the proper scaffold to direct it. Primarily, to meet the specific needs of the cell, the scaffold must be porous, adhesive, biodegradable, and include a mechanism for nutrient supply.

Porosity insures that nutrients can get to the cell and waste can be removed. Adhesion will allow for the cells to adhere to the scaffold so that it may grow in the direction desired. Additionally, in vivo, this prevents encapsulation of implanted tissue, thus allowing nutrients and wastes to be exchanged. Biodegradability will insure that the scaffold will disappear so that remnants do not become toxic and mechanical functions perform properly. Finally, the scaffold should be made of materials that function to supply nutrients to growing cell so that all cells that adhere are being fed.

It is crucial to note, however, that simply seeding the cells on a scaffold will not suffice. Cells operate and communicate with each other from early on, and even though cells may be of the same lineage, they have adapted different roles in any given system. For example, hepatocytes have different functions based on their location in relation to their oxygen gradient. Some tasked with being glycolytic hepatocytes can be transformed into gluconeogenic hepatocytes. Therefore, in a lab environment where oxygen perfusion is at a 100% homogeneously, it would result in cells not functioning to their specific characteristics (Lenas et al. 2008). A thorough understanding and study of cell mimetics is important in order for scaffolding to be effective.

BIOLOGICAL SCAFFOLDING

Through the process of decellularization, cells are stripped of the extra cellular matrix leaving behind just fibers and collagen. When applied to fully formed tissue or complete organs, the only left behind is a translucent, or ghost, extracellular matrix. This is ideal, as within the extracellular matrix all the intricate vascular and collecting networks are left in place. This allows for the reseeding of those areas to be more specific and accurate. Additionally, this

process serves to leave all mechanical functions working properly, and retains all the natural mechanics of the organ. In fact, in a study published by Nature Medicine, after eight days of reseeding a decellularized rat heart, it was able to conduct complete electrical myocardial impulses resulting in complete ventricular contraction as was demonstrated on an EKG (See Figure 1).

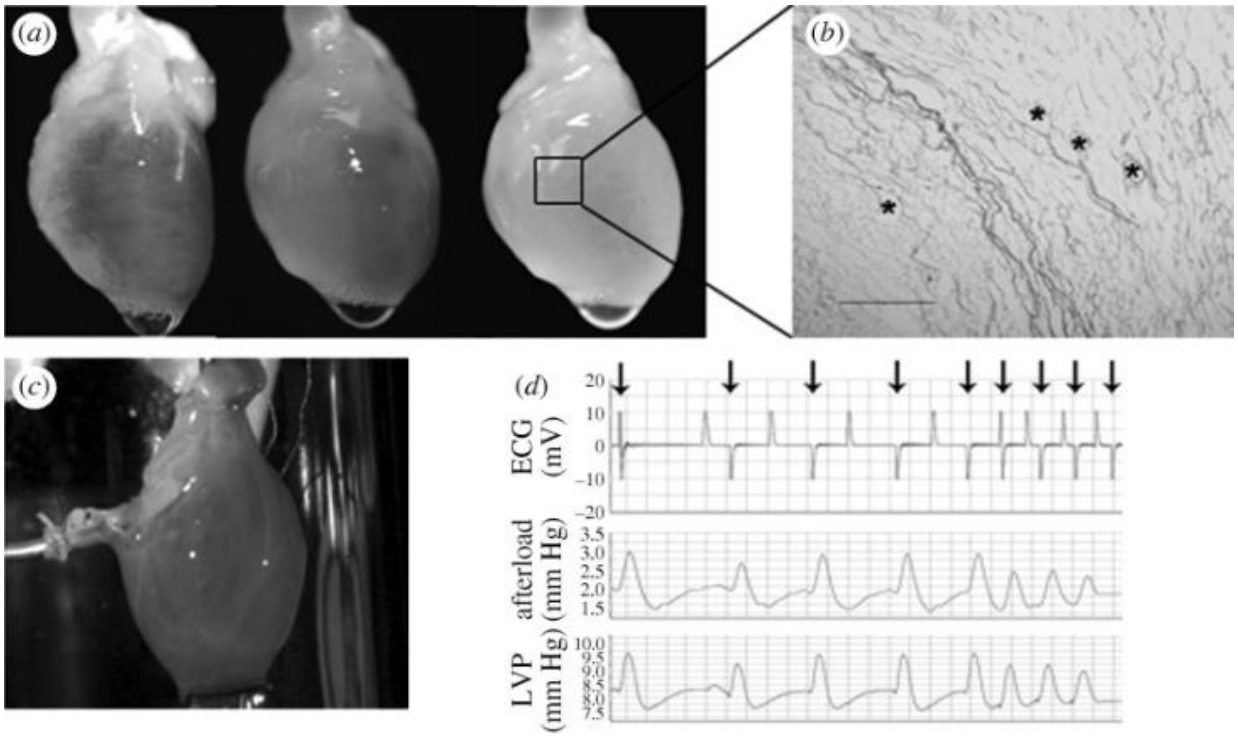

Figure 1: Photographs of cadaveric perfusion decellularization of whole rat hearts perfused with sodium dodecyl sulphate (SDS) over 12 h. (a) The heart becomes more translucent as cellular material is washed out from the right ventricle, then the atria and finally the left ventricle. (b) Haematoxylin–eosin staining of thin sections of SDS-treated heart showing no intact cells or nuclei. Maintenance of large vasculature conduits (asterisks). Scale bar, 200 µm. (c) Formation of a working perfused bioartificial heart-like construct by recellularization of decellularized cardiac extracellular matrix. Recellularized whole rat heart at day 4 of perfusion culture in a working heart bioreactor. (d) Representative functional assessment tracing of decellularized whole heart construct paced in a working heart bioreactor. Tracings of electrocardiogram (ECG), aortic pressure (afterload) and left ventricular pressure (LVP) of the paced construct on day 8 after recellularization, and on day 8 after stimulation with physiological (B50–100 mM) doses of phenylephrine. Source: Ott et al. 2008

The cells are stripped of the extracellular matrix through the utilization of detergents – most commonly sodium dodecyl sulphate. However, it has been discovered that sodium dodecyl sulphate may destroy up to 80 % of the collagen found in the matrix (Petersen et al. 2012). To counteract the chemical destruction of part of the extracellular matrix, TritonX-100/sodium-

deoxycholate was added. In a study done by Drexel University, researchers were able to combine sodium-deoxycholate with sodium dodecyl sulphate to achieve minimal to no extracellular matrix loss (Fitzpatrick et al. 2010). Additionally it was discovered through the use of electroporation, cells were able to be lysed without damage to the extracellular matrix. Electroporation is defined as a "non-linear biophysical process in which the application of pulsed electric fields lead to an increase in permeability of cells, presumably through the creation of nanoscale pores in the lipid bi-layer. At low pulsing energy, this permeability is reversible and cellular health and function is maintained. Once a critical electric field intensity threshold is surpassed (approximately 500 to 700 V/cm for ninety 50 μs pulses at 4 Hz in the brain and eight 100 μs pulses at 1 Hz in the liver, respectively),the cell membrane is unable to recover and cell death is induced in a precise and controllable manner with sub-millimeter resolution" (Sano et al. 2010). This process is referred to as non-thermal irreversible electroporation (N-TIRE). Clinical successes include the first woman to receive a tracheal transplantation seeded with her somatic mature adult progenitor stem cells in 2011.

However, the main drawback to biological scaffolding is the limited supply of donor organs to strip cells of. Even more importantly, specific dimensions needed for implantation is not possible with already formed biological scaffolding. In the case of a damaged right ear that needs to be matched with the remaining left one, exact dimensions and proportions are required. Clearly this would not be possible with scaffolding that is already shaped. It should be noted though that decellularization has had the most success with tissues that do not require specific dimensions, like a trachea.

SYNTHETIC SCAFFOLDING

Synthetic scaffolds can be comprised of either biological (natural) polymers such as chitosan, collagen, and polycaprolactone (PCL), or synthetic polymers such as poly(α-hydroxyacid) and polyglycolic acid (PGA). Most utilize some form of polylactic acid (PLA) and their copolymers (PLGA). There are a variety of techniques that have been utilized and reviewed for processing three-dimensional porous scaffolds. (Ikada 2006). Although it has been shown that synthetic polymers have advantages over natural polymers, such as straightforward control of bioabsorption rate and tunable mechanical properties, their surfaces are hydrophobic, thus preventing cell and tissue adhesion and requiring proper treatment to insure that these synthetic scaffolds absorb culture medium (Tamada et al. 1986). Additionally, the rate of degradation can be controlled and timed to coincide with cell to cell adherence when scaffolding support is no longer necessary (Jovanovic et al.). This process of hydrolytic degradation can be obtained via two methods. One is done by bulk degradation, and the second method used is through the process of surface erosion. "Bulk degradation can be observed if during random chain scission, an overall decrease of molar mass is exhibited. Surface erosion, is the process where hydrolysis removes only polymeric chains from the outer layer of the material thus leaving the bulk of the material untouched" (Yang et al. 2007). Surface erosion is favored for many applications of polymeric biomaterials due to the material properties remaining virtually intact, as degradation proceeds through removal of very thin layers of the material. This insures that the scaffold is no longer present to hinder any possible affects on mechanical function or toxic interference with cell communication. Most importantly, synthetic scaffolding offers the obvious advantage of precise and custom dimensional properties. This precision clearly is a need for partial tissue or organ repair that requires specific sections to be replicated. Additionally, exact proportions might be utilized for aesthetic reasons as well, such as seeding a scaffold with cartilaginous tissue in order to build one ear so that it complements the other ear. Furthermore, availability is a non

issue as supply is not dependant on any other factors other than procurement of the materials needed. The biggest obstacle that needs to be overcome is functionality. The scaffold designed must meet all the requirements outlined in order to allow the cells to proliferate and migrate in the proper direction. Of the many scaffolding that has been experimented with, only a select few have had much success in the clinical phase. With many materials to experiment with, the process is mostly done by trial and error with eventual successful results. It is with this fact that it seems the next big breakthrough is bound to happen relatively soon.

CONCLUSION

There is no doubt that the science of biomedical engineering still has much to accomplish in making the technology a widely accessible application in the clinical setting. Though cord, or placental, stem cells seem to offer the most promise for continued groundbreaking in the regenerative field because they lack many of the obstacles faced by embryonic and adult stem cells, currently, the use of mature adult progenitor cells is the only one approved by the FDA for cell therapy. A beneficial step forward for stem cells would be to create a human leukocyte antigen bank that has all cell type/markers available so that regenerative growth can be done quickly with no need for immuno-suppressive drugs due to human leukocyte antigen matching. Cells, when combined with synthetic polymer scaffolding, can proliferate and take up their three-dimensional shape freely. Indeed, the proper treatments of the scaffold must be maintained, and biodegradation via lysozymal activities and pathways must be closely monitored to insure proper profusion with minimal toxicity. With clinical trials currently underway, the near eventuality of full organ replication looks promising.

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CD4+CD25+ REGULATORY T CELLS AND THEIR ROLE IN SYSTEMIC LUPUS ERYTHEMATOSUS

Sara Shilcrat

INTRODUCTION

Systemic lupus erythematosus, or SLE, is an autoimmune disease that currently has no known cause or cure (Postal et al. 2012; Okamoto et al. 2011). According to the Lupus Foundation of America, 1.5 million Americans are thought to be suffering from SLE. It is found in females ages 15-44 but may also been seen in men, teens, and children (Lupus Foundation of America 2012). It is characterized by the loss of the immune system's ability to discern between "self" and foreign antigens. This leads to autoantibody production, abundant production of proinflammatory cytokines, stimulation of complement, and "immune complex depositions" (Okamoto et al. 2011). Multiple organs and tissues are affected due to the dysfunction of the immune system. Patients with SLE show an increased mortality rate compared to the population at large (Okamoto et al. 2011), although the five year survival rate is currently at 90% (Postal et al. 2012). The disease displays periods of advancement, remission achieved through drug therapy, and relapses. Genetic, hormonal, and environmental factors are thought to interact in the initial development of the disease, which can manifest itself in a variety of ways. Current treatment options include corticosteroids and immunosuppressants, which have resulted in some toxic effects in clinical use. To find a cure for SLE and improve patient prognoses, it is necessary to better understand the pathology behind the disease in order to formulate new drug therapies (Okamoto et al. 2011; Postal et al. 2012).

During the formation of T cells, autoreactive T cells, or T cells that target the body's own tissues as opposed to foreign antigens, are generally prevented from maturing and entering into circulating T cell defenses. However, at times, autoreactive T cells developing in the thymus may slip past the body's checks and enter into the circulation. Recent research has found that it is the job of regulatory T cells, or Tregs, to prevent these cells from attacking body tissues, a function known as peripheral tolerance (Okamoto et al. 2011; Venigalla et al. 2008). In effect, the Tregs are aptly called the "mediators of peripheral tolerance and potent suppressors of excessive immune responses" (Okamoto et al. 2011). More knowledge is needed to find out about these Tregs, how they function to prevent autoreactive attacks by responder T cells, and how they mediate cytokine production. A specific group of Tregs, namely the CD4+ CD25+ Tregs, has been avidly studied for their role in peripheral tolerance and autoimmune diseases like SLE. Understanding how these Tregs function, or sometimes fail to function properly, will allow for breakthroughs in SLE treatments and patient prognoses and is the impetus for this literature review (Okamoto et al. 2011).

The experiments discussed here include studies done on amounts and percentages of Tregs in SLE patients' peripheral blood compared to healthy controls (Baráth et al. 2007), how the number of Tregs affects the activity of the disease (Baráth et al. 2007), the lack of suppression by Tregs over responder T cells and responder T cells' cytokine production, including TNF-α and IFN-γ, in the blood and skin lesions of SLE patients (Carneiro et al. 2008; Valencia et al. 2007; Venigalla et al. 2008), and finally, the effects of rituximab treatment on the ratio of Th1/Th2 CD4+ T cells (Tamimoto et al. 2008). These experiments give a broad understanding of how the amounts of Treg cells differ between healthy controls and SLE patients and how the Tregs display altered suppressive functioning in SLE patients leading to an

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improved understanding of the course of the disease and possible routes for new treatment options.

DISCUSSION

Amounts and percentages of Tregs in SLE patients' peripheral blood

Multiple studies have been conducted to determine the amounts of Tregs cells in SLE patients with differing results (Crispin et al. 2003, Liu et al. 2003, Fathy et al. 2005, Alvarado-Sanchez et al. 2006, Miyara et al. 2005 cited in Venigalla et al. 2008). Baráth et al. did a study using flow cytometry that matched results from prior studies (Crispin et al. 2003, Liu et al. 2003 cited in Baráth et al. 2007) showing that the percentage of CD4+CD25+FoxP3+ Tregs was decreased in SLE patients compared to controls (in percentage per liter of peripheral blood; controls: 4.55%, n=32; SLE patients: 3.21%, n=44) as well as the numbers of Treg cells (controls: 0.038±0.017 G/L; SLE patients: 0.012±0.006 G/L).

Correlation between disease activity and amount of Tregs after plasmapheresis treatment

Five patients suffering from advanced forms of SLE that were not responding to conventional treatments were subjected to repetitive plasmapheresis treatments to determine the treatments' effects on counts of Treg cells (CD4+ CD25+ FoxP3+ T cells). An inverse correlation was seen between the SLEDAI, or disease activity, and the number of Treg cells, with the disease regressing and the number of Treg cells increasing ($r=-0.96$, $r^2=0.92$, $P=0.008$) (Baráth et al. 2007).

Treg functioning in the suppression of responder T cells and cytokine production

In one study, responder T cells (Tresp) of active[1] SLE patients were found resistant to the regulatory effects of CD4+ $CD25^{high}$ $CD127^{-/low}$ regulatory T cells (Tregs) that suppress their proliferation (Venigalla et al. 2008). The researchers built their study off of past research (Crispin et al. 2003; Liu et al. 2003; Fathy et al. 2005; Alvarado-Sanchez et al. 2006; Miyara et al. 2005) that differed as to whether SLE patients displayed differences in the percentage or overall amount of Treg cells. The researchers attributed the differences in the results to the lack of proper purification of the CD4+ $CD25^{high}$ Treg cells. SLE patients are known to have an abundance of an effector T cell called CD4+ CD25++ that is similar to the CD4+ $CD25^{high}$ Treg cells. These similar CD4+ CD25++ T cells cause contamination of counts that are meant to quantify CD4+ $CD25^{high}$ Treg cells. A culture of CD4+ $CD25^{high}$ Treg cells that shows proliferation is thought to be contaminated with the CD4+ CD25++ effector T cells since CD4+ $CD25^{high}$ Treg cells do not proliferate (Venigalla et al. 2008).

Procedure for procuring pure Treg samples

To alleviate the contamination problem, the researchers used a simultaneous staining procedure that targeted two T cell antigens: the CD25 and CD127 antigens. The researchers based their procedure on findings that indicated that the antigen CD127 allows for effective sorting between effector and regulatory T-cells; effector T cells express this antigen, or are known as CD127+, while regulatory T cells show little to no expression of this antigen, $CD127^{-/low}$ (Liu et al. 2006, Seddiki et al. 2006, Hartigan-O'Connor et al. 2007 cited in Venigalla et al. 2008).

The authors proved that past research that aggregated 'Treg cells' by selecting for CD4+ $CD25^{high}$ T cells via flow cytometry used contaminated cultures that contained more than just Treg cells. By testing the proliferation of cells characterized as CD4+ $CD25^{high}$ versus cells with CD4+ $CD25^{high}$ and the additional $CD127^{-/low}$ parameter, results showed significantly fewer of

[1] Active refers to the disease in a state of advancement while inactive refers to the disease being in a remission state (Valencia et al. 2007; Venigalla et al. 2008).

the latter type of cells. Solidifying the results further, the percentage drop in proliferation seen between the CD4+ $CD25^{high}$ 'Treg cells' and the highly purified CD4+ $CD25^{high}$ $CD127^{-/low}$ Tregs cells of SLE patients (P =0.0003) approached the percentage drop seen between these two types of cells in healthy controls (P=0.004) (Venigalla et al. 2008).

For further verification that the cells collected from the purification procedure were actual Tregs, the researchers compared the amounts of CD4+ $CD25^{high}$ $CD127^{-/low}$ Treg cells to counts done of verified regulatory T cells, or CD4+ $CD25^{high}$ FoxP3+ Treg cells. FoxP3, or forkhead box P3, is a transcription factor that is critical for normal Treg cell development (Fontenot et al. 2003 cited in Venigalla et al. 2008). The researchers could not use the FoxP3 transcription factor as a limiter for isolating true Treg cells with flow cytometry (instead of antigen CD127) due to the fact that FoxP3 is an intracellular marker. However, counts of Treg cells with FoxP3 (or FoxP3+) were done as a check to compare to amounts of $CD127^{-/low}$ Treg cells collected. If the values between the two types of cells showed a significant positive correlation, it could be concluded that the CD4+ $CD25^{high}$ $CD127^{-/low}$ T cells were the true Treg cells. When comparing the purification results of the CD4+ $CD25^{high}$ $CD127^{-/low}$ Treg cells to the CD4+ $CD25^{high}$ FoxP3+ Treg cells, a high positive correlation was seen between the total amount of each type of cell both for the SLE patients ($r^2 = 0.77$, P=0.0009, n = 13) and for the normal controls ($r^2 = 0.70$, P<0.0001, n = 17), proving the likelihood that the $CD127^{-/low}$ cells were indeed classic Treg cells (Venigalla et al. 2008).

Use of CD4+ CD25+ FoxP3+ Treg cells from control and SLE patients, both active and inactive cases, also highlighted that active SLE patients had an increase in the average percentage of CD4+ $CD25^{low}$ FoxP3+ T cells and CD4+ $CD25^{high}$ FoxP3+ T cells compared to healthy controls and patients with inactive SLE. Both types of cells, however, did not show significantly increased amounts when measured in comparison to a set volume of peripheral blood mononuclear cells (Venigalla et al. 2008).

Effects of Tregs on Tresp cells in SLE patients

After procuring pure samples of the Treg cells (CD4+ $CD25^{high}$ $CD127^{-/low}$), the researchers determined the effects of CD4+ $CD25^{high}$ $CD127^{-/low}$ Treg cells on T cell responders (Tresp). The researchers cultured measured amounts of the Treg cells with the Tresp cells. Different ratios of cells were created (Treg:Tresp), spanning from 1:1 to 0.03125:1 ratios. Results of the experiment from the 1:1 ratio of control Treg cells to control Tresp cells showed an 80±2% (n=9) inhibition of proliferation of the Tresp cells. With the decreasing amount of control Treg cells compared to the set value of control Tresp cells, the percentage of inhibition gradually decreased. On the other hand, the experimental group consisting of participants with active SLE showed decreased Treg-related suppressive functioning, with baseline inhibition of Tresp proliferation at a 1:1 ratio of only 53±6% (n=9). This statistically significant decrease in inhibition of proliferation ranged from the 1:1 ratio down until the 0.125:1 ratio (P=0.0006). Similar findings to a lesser extent were found when controls were compared to patients with inactive SLE (88±1% (n=8) versus 77±2% (n=8), ratios from 1:1 down until 0.25:1, P=0.0006) (Venigalla et al. 2008).

Responder T cells of SLE patients were not sensitive to the regulation of the Treg cells; two opposing hypotheses were conjectured to understand where the decreased sensitivity to the Treg cells stemmed from. The first hypothesis ascertained that the Treg cells in SLE patients were dysfunctional and lacked the ability to suppress the Tresp cells. Conversely, the second hypothesis implicated that the Tresp cells became resistant to the signals sent by the Treg cells and proliferated of their own accord. To test these two opposing theories, Tresp cells of active

SLE patients were grown with control Treg cells and vice versa. Tresp cells of active SLE patients cultured with control Tregs allowed for the following conclusions; if the suppression of the propagation of Tresp occurred by the control Treg cells at the elevated rate (around 80%), the problem could be isolated to the malfunctioning of the SLE patients' Treg cells. If, however, the suppression remained at the same level as that seen normally in active SLE patients, the SLE patients' Tresp cells were thought to have become immune to the mediation of SLE Treg cells. When testing the reverse (culturing Tresp cells of controls with Treg cells of active SLE patients), if the inhibition of the proliferative effects of the Tresp was very high as in the control patients, then the Tresp cells of the SLE patients' were thought to be resistant to SLE Treg cells. Just the opposite, if the reduction in Tresp proliferation was lower as in the active SLE patients, then the Tregs of the SLE patients were thought to have lost their mediating capacity. In the first case (control Treg cells + active SLE Tresp), the control Treg cells were unable to suppress the proliferation of the Tresp cells of the SLE patients, implicating a problem with the Tresp (74±5%, n=15 for control Treg cell/control Tresp cells vs. 48±6%, n=15 for control Treg cells/active SLE Tresp cells; P=0.001). The results were further confirmed to be related to the development of resistance by the active SLE patients' Tresp cells in the second experiment (active SLE Treg cells + control Tresp cells) where the active SLE Treg cells could suppress the control Tresp cells (48±6%, n=15 active SLE Treg cells/active SLE Tresp cells vs. 63±6%, n=15 active SLE Treg cells/control Tresp cells; P=0.03) (Venigalla et al. 2008).

From the testing, the Tresp cells were found to be resistant to the mediation of the Treg cells in active SLE patients. Other testing was conducted that further solidified the view that the Tresp were indeed not responsive to the Treg inhibition in active SLE patients. Statistically, the degree of inhibition of Tresp cell proliferation was inversely proportional to the level of activity seen in the disease, or more diseased states showed a decrease in the ability of the Treg cells to control the Tresp proliferation ($r^2 = 0.37$, $P<0.0001$, n=28). Finally, the researchers used ELISA during the assay on the control and active SLE T cells that showed the production of interferon-gamma (IFN-γ), a Th1 class of cytokines produced by Tresp cells and regulated by Treg cells, with the following results:

Treg cells of:	IFN-γ production by:	Able to suppress IFN-γ production?
Control	Control Tresp cell	Yes
Control	Active SLE Tresp cell	No (not as well suppressed)
Active SLE	Active SLE Tresp cell	No (unable to suppress)
Active SLE	Control Tresp cell	Yes

These results further prove the lack of Tresp cells of active SLE patients to respond to Treg cell mediation, whether it is the cell directly (i.e. via proliferation) or the products that the cell makes (i.e. IFN-γ production). An interesting supposition that the authors mention is the possibility that cytokines, like IFN-γ, may initiate the active SLE patients' Tresp cells to 'retaliate' and build resistance to the regulation of the Treg cells. To determine the voracity of this hypothesis, the role of the cytokines must be determined (Venigalla et al. 2008).

Contradictory research proving Tregs are malfunctioning. CD4+ $CD25^{high}$ Treg cells help in the maintenance of "immunologic homeostasis" and prevention of autoimmunity (Valencia et

al. 2007). Deficient Tregs, like those seen in SLE, attack target cells, increase cytokine production, increase cell-to-cell contact, and lead to apoptosis of target cells. In another study on Tregs (Valencia et al. 2007), the researchers hypothesized that the characteristic breakdown of "self" tolerance in SLE patients was due to altered Treg functioning. Findings from their research were in line with their hypothesis. Active SLE Tregs showed a decrease in FoxP3 mRNA and protein expression compared to controls and inactive SLE participants (controls: mean 85±5%, n=40; inactive SLE: mean 64.4±15%, n=8; active SLE: mean 45±10%, n=10 [P=0.003 comparison between controls and active SLE; P=0.2 comparison between inactive SLE and controls]). Decreases in this gene expression were not mentioned in Venigalla and colleagues' experiment (2008). Differing from results seen with Venigalla et al. (2008), Tregs were implicated as malfunctioning, doing a poor job of inhibiting the proliferation of CD4+ CD25- T responder (Tresp) cells and their cytokine secretions. The significant decrease that was evident in the suppressive functioning of the CD4+ $CD25^{high}$ Treg cells from the active SLE patients extended to both Tresp from controls and "self" (active SLE) Tresp cells. Further supporting the premise that the Tregs were defective, the problem could not be contributed to resistance conferred on the Tresp cells (like the findings of Vanigella et al. in 2008), as Tregs from the controls were able to suppress active SLE Tresp cell proliferation. With respect to inactive SLE patients, their Tregs were markedly different from those of the active SLE patients, displaying similar suppressive capabilities as control Tregs (Valencia et al. 2007).

Reversible loss of Treg suppressive functioning

The researchers also found that the loss of suppressive abilities of the active SLE Tregs could be reversed, not studied in the subsequent experiment done by Venigalla et al. in 2008. Incubating the Tregs in vitro with anti-CD3 led to activation of the Tregs. As a result, the Tregs exhibited an increase in FoxP3 mRNA and protein expression and restoration of their suppressive functioning on Tresp cell proliferation and IFN-γ secretion (Valencia et al. 2007).

Phenotypic changes in Tregs of SLE patients. Valencia et al. (2007) also studied the phenotype of the Treg cells harvested from controls and SLE patients. The Treg cells from active SLE patients differed phenotypically from Tregs collected from controls and inactive SLE patients. In particular, TNFRII, which has been linked to another autoimmune disease (Valencia et al. 2006 cited in Valencia et al. 2007), was increasingly expressed. TNFRII is part of a signaling pathway that downregulates CD4+ $CD25^{high}$ Tregs' suppressive function on CD4+ CD25- Tresp cells. Since active SLE Treg cells displayed an inability to regulate Tresp proliferation, the researchers tested whether TNF moderated the suppressive capacity active SLE Tregs. Results from the experiment displayed that adding anti-TNFRII antibodies or TNF stopped control and anti-CD3 activated active SLE Tregs from being able to inhibit CD4+ CD25- Tresp cells. The cells lost their regulatory capacity, indicating that expression of TNFRII affects the Tregs ability to contain Tresp multiplication (Valencia et al. 2007).

Correlations between SLE disease activity and Treg phenotype and suppressive capacity

Two notable inverse correlations were discovered in this study. The first correlation was found between SLE Treg FoxP3 expression and SLEDAI score, the systemic lupus erythematosus activity score[2]. Interpreting the correlations, a patient with a more active form of the disease had decreased FoxP3 expression and vice versa. A second inverse correlation was found between the percentage of suppression that the Tregs exhibited over the Tresp and the SLEDAI score. A patient with a more active form of the disease displayed Tregs with a lessened

[2] To be classified as an inactive SLE patient, the SLEDAI score needed to be less than 3, while an active SLE patient scored at or above 3 (Valencia et al. 2007).

capacity for suppressing the proliferation of their Tresp cells. Interestingly, no correlation existed between therapeutic drugs (glucocorticoids) and Treg functioning, indicating that malfunctioning of the Tregs was not related to the therapeutic treatment (Valencia et al. 2007).

Treg suppressive functioning and cytokine secretions in SLE patients' skin lesions. It is evident that proper control over Tresp and their cytokine secretions is lacking in SLE patients, whether it is due to the malfunctioning of the Tregs or the resistance of the Tresp to the Tregs' regulation. Cytokine secretions are increased, such as TNF-α and IFN-γ, due to the lack of regulation over the Tresp that produce these two cytokines.

Imminunologic disorders in several inflammatory diseases have been characterized according to the dominant cytokine pattern of the infiltrating CD4+ T cells. A Th1 pattern is characterized by predominance of interleukin-2 (IL-2) and interferon- γ (IFN- γ), whereas a Th2 pattern is characterized by predominance of IL-4, IL-5, IL-6, IL-10, IL-13 (Carneiro et al. 2011).

In a study by Carneiro et al. (2011), the researchers sampled epidermal keratinocytes of SLE patients extracted from areas of unaffected skin and skin lesions commonly found in SLE patients (both discoid and acute). Using PCR, the researchers quantified the amounts of 4 cytokines (IFN- γ, IL-2, IL-5, TNF-α) found in the skin cells. The results showed overexpression of at least one of the four cytokines in 47% of the 38 skin samples. Examining IFN- γ in particular (which is produced by the Tresp cells), 8 out of the 38 biopsies taken showed overexpression of this cytokine (7/8 of the biopsies were from acute/discoid lesions while the 8th came from an unaffected area of skin). TNF-α was overexpressed in only 3 samples taken from areas with skin lesions. From the results showing overexpression of IFN- γ specifically, the authors indicated that this suggests that Th1 class of CD4+ T cells, related to Treg cells and their functioning, may play some role in SLE's skin-related pathology (Carneiro et al. 2011).

Effects of rituximab on the ratio of Th1/Th2 CD4+ T cells

One drug on the market for SLE that was created to target B cells called rituximab has shown an effect on the ratio of Th1/Th2 CD4+ T cells (Postal et al. 2012). Rituximab has a tendency to tip the ratio in favor of Th1 CD4+ T cells over Th2 CD4+ T cells and may yield these effects by decreasing serum levels of TNF-α[3] (Tamimoto et al. 2008), mentioned previously with regard to the ability of this cytokine to inhibit Tregs' suppressive capacity over Tresp cells (Valencia et al. 2007). The study of rituximab, showed its safety, but more importantly, the drug generated remission in approximately 89% percent of the patients (n=9). Improvement in SLE patients leading to remission is, therefore, linked to changes in T cells and cytokine secretions (Tamimoto et al. 2008).

CONCLUSION

Treg cells are an important area of research in helping treat SLE patients. SLE patients suffer from having fewer of these important regulatory T cells, which help prevent autoimmunity. Other concerns include the changes in the phenotype of the remaining Treg cells and the production of TNF affecting the suppressive capacity of Tregs over Tresp (Valencia et al. 2007).

However, it is encouraging that plasmapheresis treatments in patients not responding to conventional SLE treatment showed increases in their Treg counts and decreases in their SLEDAIs, or disease activity (Baráth et al. 2007). Another promising finding is the similarity between inactive SLE patients and healthy controls with regard to appropriate Treg functioning

[3] The ratio for Th1/Th2 CD4+ T cells was calculated from flow cytometry of the CD4+ T cells. Th1 cells were determined as the percentage of IFN-γ+ cells while Th2 cells were determined as interleukin-4 positive, or IL-4+ cells (Tamimoto et al. 2008).

(Valencia et al. 2007). This indicates that patients in remission, which could be brought about by use of rituximab (Tamimoto et al. 2008), may generate restored Treg functioning. It is also interesting to hypothesize whether activation of Tregs, ex or in vivo, can be used clinically to restore proper Treg functioning in SLE patients like the in vitro findings seen in Valencia et al.'s experiment in 2007.

Review of this research is important for clarifying how Treg cells in particular relate to the current knowledge of SLE pathology and bring the researchers closer to finding which mechanism can be targeted as a cure for this disease. From the research presented, it is evident that impairment of proper Treg functioning, whether on the part of the Tregs or the Tresps, leads to the detrimental proliferation of Tresp cells and their respective cytokines that is characteristic of the autoimmune disease. It is necessary to conduct more research to determine whether Tregs or Tresps are malfunctioning, as the research differed on this point. Determination of the source of increased Tresp cells and cytokine production will allow for the creation of treatment options that target the deficient cells.

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DO PHOTOPERIODIC CHANGES IN MELATONIN SECRETION DETRIMENTALLY AFFECT THE FEMALE REPRODUCTIVE CYCLE?

Riki Szlafrok

ABSTRACT

Melatonin, better known as "the hormone of darkness," is secreted by the pineal gland during the night and helps us fall asleep. Because its internal regulation depends on light, melatonin is part of chronobiology, the study of biological mechanisms and their adaptations to lunar and solar related rhythms (Klein et al. 1991). Therefore, photoperiod changes greatly impact melatonin concentration, influencing changes in neuronal and hormonal mechanisms of the photo neuro-endocrine systems, namely reproduction. Research has shown that a disruption in the circadian rhythm of melatonin due to photoperiod changes detrimentally affects the rhythmic function of the female reproductive cycle. Research from journals, articles, and printed books has shown that both exogenous and endogenous features contribute to the reproductive cycle and that the internal mechanisms are entrained by environmental cues. Photoresponsiveness of the reproductive system is mediated by the internal biological clock, transcriptional factors, period genes, photic input, GnRH (gonadotropin-releasing hormone) neurons, and melatonin secretions. Specifically, melatonin secretions directly affect reproductive function either through stimulatory or inhibitory pathways. Seasonally breeding animals interpret photic information according to melatonin secretions. This clearly points to a relationship of melatonin with photic and reproductive qualities. Research is ongoing with various species of animals in hope of uncovering the mystery of the connection between light, melatonin, and reproduction, which may be helpful in understanding the effects of photoneuroendocrinology on the female human.

INTRODUCTION

In the industrial world of the 21st century, work at night seems to be unavoidable. Erhard Haus claims that 17% of the working world is involved in some kind of rotating shift work, permanent night shift, or trans-meridian travel (Haus and Smolensky 2006). Nurses work rotating shifts, wholesale food producers drive through the night, bakers bake in the wee hours of the morning, medical school students intern around the clock, security guards work night duty, and traveling businessmen fly many miles a month. What they all have in common is that they are on arrhythmic sleep schedules. The diabetic frequent flyer knows to keep insulin medication handy because glucose levels can fluctuate in response to confusing messages of night and day the body receives when traveling across several time zones, for traveling eastward shortens the day while traveling westward lengthens the day (Keystone and Kozarsky 2011). The body runs on an internal rhythmic schedule and may not adapt easily to extreme changes in the timing of surrounding environments. The World Health Organization has blamed shift work for the increasing occurrences of cardiac, gastrointestinal, and reproductive disorders and certain cancers (Longo et al. 2012). Melatonin is known to inhibit estrogen dependent disorders, including breast cancer. If sleep schedules are compromised, melatonin may not be able to guard the body from the invasion of cancer. Epidemiologic studies on women show a correlation between shift work employment and the incidence of breast and colon cancers. Melatonin disturbances have shown to affect proper reproductive function as well. Flight attendants and shift work employees, two groups of people who suffer from disrupted day/night schedules and

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increased variance in their melatonin production, have reported changes in their menstrual cycles. These changes may account for fertility issues and the inability to carry a pregnancy through nine months (Dawson et al. 2006). Therefore, women in the working world should be educated about the effects their schedules may have, if any, on their reproductive function if they intend to lead healthy reproductive lives.

This paper will focus on the effects of photoperiodic changes in melatonin concentration on female reproductive function. Although the estrous cycle of female rats is not the same as the menstrual cycle in the female human, experimental results and scientific reviews on rats are analyzed as a means of uncovering trends in animals that may be possible in humans as well.

MATERIALS AND METHODS

The information in this research paper was located using Touro College Library search engines, multiple databases, printed books, science journals, and articles. This paper offers an in-depth analysis of the information collected from these sources. Most of the information is based on experiments and research on the rodent reproductive cycles because not much effort has been made to test human subjects. It is not within the scope of this paper to come to a complete conclusion about the effects of light and the pineal hormone on the menstrual cycle, but this paper will attempt to unveil the mechanisms involved in these processes and offer possible reasons as to why certain effects may or may not be evitable. The role and function of melatonin will be discussed first, followed by a description of the menstrual cycle and the photoperiod. Then, the effects of the photoperiod and melatonin on reproduction will be examined. In addition, the role of the endogenous circadian system, or internal clock, and its impact on the female reproductive cycle will be discussed and compared to the exogenous factors influencing reproduction. Lastly, the far-reaching effects caused by an imbalance of melatonin and possible solutions to eliminate them will be discussed.

DISCUSSION

The Function of Melatonin and its Relation to the Female Reproductive Cycle at the Hypothalamic Level

The suprachiasmatic nucleus (SCN) of the hypothalamus receives photoperiodic information from the environment and uses it to controls biorhythmic and neuroendocrine functions. The suprachiasmatic nucleus is the body's master clock and is the prime controller of the body's circadian rhythm, the rhythm of various functions, processes, and mechanisms that occur in a 24-hour day. Through its retinohypothalamic connection, the suprachiasmatic nucleus interprets photic stimuli and sends this information to the brain and other organs in the form of chemical messages. The main target, another organ in the photo endocrine system, is the pineal gland, also called the diencephalic or deep photoreceptor (Klein et al. 1991). The pineal gland is the body's 'third eye' because it reacts, by secreting melatonin, based on what it sees from the outside environment (Cagnacci et al. 1995). In fact, pinealocytes and photoreceptors of the eye share common origin and components of signal transduction pathways (Lolley et al. 1992). Melatonin, produced exclusively at night, is secreted by the pineal gland after an adrenergic stimulus at the pinealocytes (Cagnacci et al. 1995). Research has shown that ablation of the suprachiasmatic nucleus destroys the circadian rhythm of pineal N-acetyltransferase activity, which can inhibit the proper functioning of the hormone melatonin (Klein et al. 1991). Many studies claim that melatonin inhibits the luteinizing hormone (LH).

Luteinizing hormone is secreted during the follicular phase of the menstrual cycle and is necessary for follicle development and subsequent ovulation. During the follicular phase of the eumenorrheic menstrual cycle, the hypothalamic releasing factor, gonadotropin-releasing hormone (GnRH), stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone from the anterior pituitary. Luteinizing hormone spikes immediately prior and during ovulation, creating a surge of luteinizing hormone mid-cycle, which is followed by the luteal phase and an increase in the ovarian steroid progesterone (Dawson et al. 2006). Consequently, if melatonin inhibits luteinizing hormone, it can inhibit ovulation which is necessary for proper reproductive function and fertilization. In addition, seasonal breeding, favoring long summer days, points to melatonin as a possible inhibitor of reproductive activity. An experiment conducted in Finland, by the Department of Obstetrics and Gynecology of the University of Oulu, sought to discover a connection between seasonal and menstrual secretions of melatonin and gonadotrophins in women. The study found that during a midcycle night, luteinizing hormone was 76% higher in the summer, a 22-hour day, than in the winter, a 5-hour day. Melatonin, on the other hand, was about 50% higher in the winter than in the summer. It is possible that melatonin inhibited luteinizing hormone and, therefore, allowed increased levels during the summer when the day is longer than night (Kivelä et al. 1988). However, these results do not address the fact that luteinizing hormone levels were high at night; if melatonin does inhibit luteinizing hormone, why was there an increase of luteinizing hormone at night, a period during which melatonin is high? It is true that, overall, the summer days have more hours of light and, therefore, luteinizing hormone peaks, but that does not explain why the luteinizing hormone peaked specifically at night when melatonin rises.

Melatonin and Sleep

Sleep disorders and their effects on menstrual disorders is another way to understand the possible effects of melatonin on reproduction. In researching the impact of sleep loss on health, Dr. Michael Thopy, sleep director of Montefiore Medical Center at Albert Einstein College of Medicine in New York, said," if you are supposed to sleep a third of the day, there has got to be a reason for it" (Neergaard 2012). In the results of an experiment done on 26 women by Kari Sveum of Northwestern University in Chicago, Illinois, twice as many subjects with delayed sleep syndrome reported menstrual irregularities than those without delayed sleep syndrome (American Academy of Sleep Medicine 2008). It is not clear from this experiment if sleep itself or a dysrhythmia of the light/ dark perception associated with sleep disorders is connected to menstrual irregularities. This paper will not consider the actual phenomena of sleep to be a key factor in influencing reproductive function; rather the body's interpretation of night and day by the photo endocrine system and subsequent melatonin secretions will be considered a key factor.

Melatonin and Specific Hormones

Melatonin directly impacts the female reproductive cycle by stimulating the release of gonadal hormones according to the information it receives from the suprachiasmatic nucleus. The suprachiasmatic nucleus itself also has a connection to the reproductive pathways via GnRH neurons of the hypothalamus. In contrast to the previous belief about the inhibitory effect of melatonin on luteinizing hormone in the menstrual cycle, an experiment done at the University of California showed an increase of luteinizing hormone following the administration of exogenous melatonin. During two

consecutive days of both the follicular and luteal phases of six normal cycling women, blood assays were checked for melatonin, luteinizing hormone, and other gonadal hormone concentrations. Exogenous melatonin or a placebo was given at 8:00 a.m. on the first days of each phase, and blood was drawn every 20 minutes from 9:00 a.m. to 5:00 p.m. on all four days in order to detect hormonal levels throughout each day. The results found that exogenously administered melatonin increased luteinizing hormone during the follicular, but not luteal, menstrual phase; the stimulatory effect of melatonin was selectively exerted in the follicular phase of the menstrual cycle. Melatonin levels were the same in both the follicular and luteal phases, but only affected luteinizing hormone in the follicular phase. The study acknowledges that this increase may not be felt when looking at the overall luteinizing hormone production during the entire menstrual phase, so it is premature to conclude that this melatonin truly has a stimulatory effect on reproduction (Cagnacci et al. 1995). In addition, the possibility of errors in the experimental procedure and analysis leave room for doubt as to whether melatonin can be categorized as a stimulant. One cannot base a theory on the cycles of only six women and simply categorize the follicular and luteal phases by the first two days of their cycles. In addition follicle-stimulating hormone, which is part of the positive feedback mechanism in increasing luteinizing hormone for mid cycle ovulation (Dawson et al. 2006) (Figure 1), was not affected at all by the exogenous melatonin. Thus, just stimulation to LH in the follicular phase may not be enough to really stimulate menstrual function. In researching the melatonin levels during the menstrual cycle of normal cycling women, Cagnacci reports a delayed melatonin phase in the luteal phase compared to the follicular phase (Dawson et al. 2006). Although melatonin levels were the same in this experiment, it is possible that because melatonin levels are delayed in the luteal phase, they do not affect LH as much as in the follicular phase. Thus, zooming in on LH in the follicular phase does not provide enough of a basis to prove that this melatonin is beneficial to the menstrual cycle as a whole. In addition, when drawing a conclusion about the body's secretion of melatonin and menstrual function, these results are irrelevant because the melatonin used in this experiment was not naturally secreted. Errors in the experiment as well as unnatural conditions make the results of this experiment questionable in reference to melatonin's stimulatory effects on the menstrual cycle.

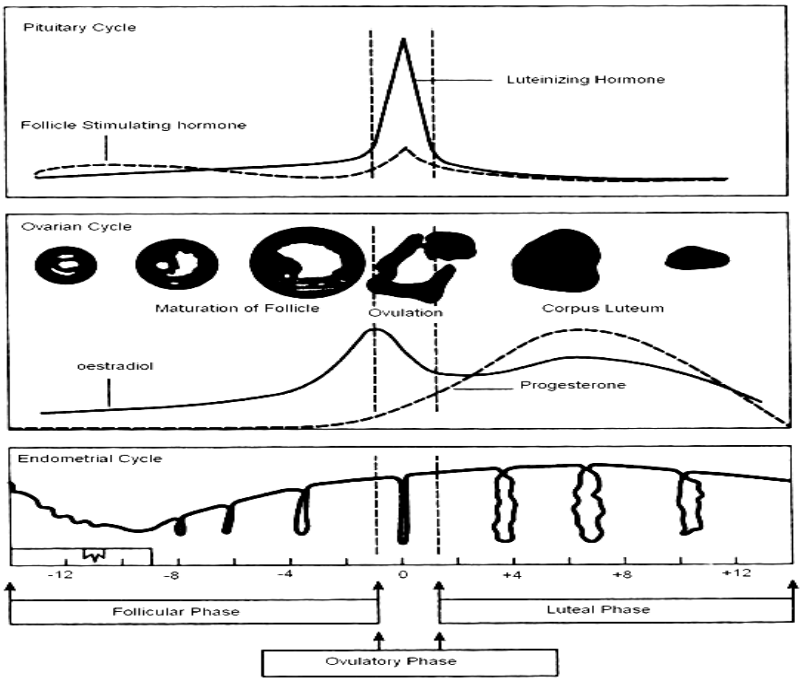

Figure 1: Stages of the menstrual cycle: hormones, in the ovaries, and in the uterus. Luteinizing hormone is needed for the LH surge at ovulation. Progesterone is necessary for proper growth of the uterus. Source: Dawson et al. 2006

High melatonin levels were detected in women with secondary amenorrhea, indicating that the pineal hormone may play a role in inhibiting menstruation. This condition is common in female athletes who strenuously train for sports on a low caloric diet, for exercise training that reduces body fat may lead to a reduced production of estrogen, thereby inhibiting proper function of the menstrual cycle (Dawson et al. 2006). Exercise induced amenorrhea is the body's way of telling itself to conserve energy where necessary and not put too much effort in functions that are not life threatening, like reproductive activity. The exact mechanisms and causes of secondary amenorrhea are not fully understood, but researchers see a relationship between melatonin and the amenorrheic women. Brezinski and Kadva report elevated melatonin levels in women with reduced gonadotropin levels "which may suggest a neuroendocrine pathology underlying this disorder" (Dawson et al. 2006). On the other hand, it is possible that melatonin itself does not reduce gonadotropin levels, but rather it increases in response to reduced gonadotropin levels. Another link between melatonin and the menstrual cycle is their shared ability to inhibit estrogen dependent disorders. Postmenopausal women are at a greater risk for developing breast cancer because of their lost menstrual cycle which is thought to prevent cancer by keeping estrogen levels in check. When circulating blood contains high levels of estrogen, breast cancer, an estrogen dependent disorder, can thrive.

Similarly, melatonin has been found to keep estrogen levels in check by reducing estrogen receptors, thereby decreasing the development of estrogen dependent disorders such as breast cancer and endometriosis. Melatonin's action on estrogen can be helpful, on one hand, in reducing the risk of estrogen dependent disorders but detrimental, on the other hand, if melatonin inhibits healthy levels of estrogen necessary for uterine tissue growth. The ovarian steroid estrogen, made from the precursor estradiol, is needed for preparing the uterus in case of fertilization. It is unclear how much melatonin is helpful and how much is harmful to estrogen in the human body. A study done at Al- Azhar University was designed to investigate the modulatory effect of melatonin on uterine estrogen and progesterone receptors and uterine contractility. Twelve non-pregnant Albino Wistar rats were classified into two groups, one of which was treated with melatonin daily for 15 days. The rats were then scarified to expose their uterine horns, treated with oxytocin, and carefully watched for contractility. In addition, a uterus section of four micrometers thick was mounted on a slide and stained to reveal estrogen and progesterone receptors. The results of melatonin treated rats showed a decreased effect of oxytocin on uterine contractility, decreased estrogen receptors, and increased progesterone receptors (Abu-Allah et al. 2003). These results, however, reflect the responses of the uteri of just six rats and, therefore, cannot be a strong enough basis to conclude the effects of melatonin on uterine receptors. Although it is still possible that melatonin may inhibit breast cancer development through its inhibiting the level of estrogen receptors, at the same time it may be decreasing estrogen receptors necessary for uterine lining development. This experiment does not mention if the decrease in estrogen was beneficial or harmful for healthy reproductive function.

Melatonin was helpful in increasing progesterone receptors, also necessary for uterine development. In this capacity, melatonin acts as a stimulant to reproductive activity. Melatonin's ability to elevate levels of progesterone is yet another connection between the hormone of darkness and menstrual function. Martensson suggests that melatonin is stimulatory to the menstrual cycle, because uterine activity is greater during the dark, the same time that melatonin levels rise (as quoted in Dawson et al. 2006). Progesterone,

meaning “good for gestation," is an ovarian steroid hormone which nourishes the uterine lining before fertilization and continues to nourish the developing embryo. In this sense, melatonin stimulates hormonal secretion at the level of the ovary. Dr. Len Lopez, a nutrition and fitness expert, explains that infertility and spontaneous abortion are common in women with low levels of progesterone because progesterone is needed to support menstrual function and pregnancy. He calls the adrenal gland a thief because it uses progesterone in order to produce the stress hormone cortisol, accounting for the stress-infertility connection (Lopez). However, although cortisol may inhibit progesterone levels, it has not been found to directly affect the menstrual cycle in any other way. A study done on patients diagnosed with fibromyalgia (FM), a rheumatoid like disease with various components on muscular, psychosomatic, and psychoneuroendocrine levels, investigated the effects of fibromyalgia on the H-P-G (Hypothalamic- Pituitary- Gonadal) and H-P-A (Hypothalamic- Pituitary- Adrenal) axes. The experiment consisted of 105 normal cycling women, of whom 63 were diagnosed with fibromyalgia, and 38 women who served as the control. After a night's fast, blood assays were collected and checked for FSH, LH, prolactin, cortisol, progesterone, and estradiol. Cortisol levels were significantly lower in patients compared to controls, while gonadotrophic levels were unaffected, providing no indication that the cortisol levels had an effect on progesterone levels. However, in addition to reduced levels of cortisol, there was an increase in the luteinizing hormone of patients with fibromyalgia also suffering from sleep disturbances. Once again, a relationship can be seen between sleep and melatonin disturbance affecting the synthesis of luteinizing hormone (Gur et al. 2004). It is clear from this experiment that cortisol levels do not affect gonadotrophic levels alone, but can possibly affect LH levels when melatonin levels are altered as well. Alternatively, it is just the melatonin levels that affected the luteinizing hormone and not the cortisol levels at all. Results are questionable because blood assays were taken only once. Because hormones may not suddenly appear in the blood from one minute to the next, extensive monitoring may be required in order to be used as experimental evidence.

PHOTOPERIODIC AND GENETIC CONTROL OF MELATONIN SECRETION AND REPRODUCTIVE FUNCTION

Photoperiodic Responsiveness and Reproduction

Extensive research on the secretion of melatonin and subsequent reproductive function has postulated a balanced relationship between exogenous and endogenous controls. On one hand, light, interpreted by the suprachiasmatic nucleus and hypothalamus, enhances gonadal growth, reproductive hormones, and successful breeding. This indicates that the night hormone melatonin may play a role in inhibiting reproductive function. However, many experiments connect reproductive function to endogenous controls, such as genetics and internal biological clocks, effectively ruling out light and photoperiods as being possible effectors of reproduction. Thomas Dickmeis of the Institute of Toxicology and Genetics in Germany links the two components and suggests that clock gene expression is controlled by light and the suprachiasmatic nucleus (Dickmeis 2009). Strong evidence in both directions demands the answer that both exogenous and endogenous factors control reproduction, and results differ depending on the species in question. For example, when exposed to certain sequences of light and dark, the gametes of some coelenterates mature and are released. This direct reaction to light is unique to this specific

species and not a reflection of the reaction of other animals to light exposure (Scharrer 2006).

In an article, David Kennaway describes the connection between the suprachiasmatic nucleus, melatonin, and reproduction. Studies have found that the suprachiasmatic nucleus vasopressin release stimulates gonadotrophin releasing hormone and, consequently, the surge of LH in the ovary (Kennaway 2005). Suprachiasmatic nucleus projections were found in GnRH immmunoreactive neurons and estrogen receptor cells, suggesting that the circadian system regulates the timing of some menstrual activity (De la Iglesia et al. 1995). Melatonin concentrations change according to SCN activity, and melatonin receptors in the SCN provide an additional link between the circadian timing system and seasonal changes in fertility. Kennaway adds that puberty was delayed in rodents experiencing long hours of darkness, indicating that melatonin probably effects the reproductive maturation of certain animals (Kennaway 2005). Research has shown the direct effects of light on gonadal growth, hormones, and puberty. Because melatonin is the marker for SCN function, and the SCN interprets the light information, responses to light are associated with circulating melatonin levels. The Institute of Zoology in London conducted an experiment on seasonally breeding Syrian male hamsters, involving photoperiodic changes. Both wild type and tau-mutant hamsters were maintained in stimulatory photoperiods for reproduction. The wild type hamsters were allowed 16 hours in the light and 8 hours in the dark (16L: 8D), the tau-mutants were allowed 12 hours in the light and 8 hours in the dark (12L: 8D) (the tau mutation shortens the rhythm by approximately 4 hours resulting in a circadian period of 20 hours instead of 24) (Stirland et al. 1996). The hamsters were then exposed to increasing hours of darkness for 84 cycles, resulting in testicular atrophy and complete regression at 11 hours dark for the wild type and 10 hours dark for the tau-mutant. This inhibition of gonadal growth in increased dark hours seems to show an inhibitory effect of the night hormone melatonin on reproduction (Stirland et al. 1996). Only because Syrian hamsters are long day breeders, prolonged exposure to darkness was detrimental to their reproductive system. These results, however, do not prove that darkness is an inhibitory factor of reproduction in different species of animals. Similar results were found during an experiment with male Djungarian hamsters and photoperiodic changes on gonadal growth. Newborn hamsters from a breeding colony in a long day setting were either kept in a long day (LD) setting or transferred out to a short-day (SD) environment after birth. In order to create four treatment groups that would reflect a variance in photoperiodic changes, at 30 days old, half of long day hamsters moved to the short day environment , and half of the short day hamsters moved to the long day environment (1. LD 2. SD 3. LD-SD 4. SD-LD). Blood samples were taken after 40 days, and at 40, 60, and 90 days of age, 4-6 hamsters in each group were sacrificed and had their testes weighed and coronal brain sections analyzed for GnRH cell bodies. Photoperiod greatly affected testis weights and follicle-stimulating hormone levels while it showed no relation to increased GnRH perikarya. The testis weights of rats transferred from short days to long days was the same as those reared in long days from the start (Figure 2). These results prove that long days are beneficial for the male Djungarian reproductive system. By 90 days, the testes of hamsters reared in long days or transferred to long days were the size of adult testes while SD/ LD-SD hamsters' testes fully regressed. Serum LH levels were unaffected by photoperiods, while FSH increased in LD groups, especially in the SD-LD group (Yellon

1994). Interestingly, the greatest increase in follicle stimulating hormone was found in the SD-LD group, signifying the effects of a variant environment, which ended in a stimulatory setting, as being the most helpful for reproductive function. How exactly light affected reproductive function is unclear because GnRH cell bodies did not increase in response to the light. It was thought that the GnRH immunoreactive cell bodies would increase in response to light, but they did not. Nevertheless, the authors suggest that photo treatment may control post-translation modification of GnRH molecules and effect reproduction (Yellon 1994). A study done on three strains of laboratory male rats, ACI, BUF, and PVG showed photo responsiveness which enhanced gonadal growth. Four groups of rats were raised. Two groups were subjected to the long day (LD) setting, which included 16 hours of exposure to light and 8 hours in darkness (16L: 8D), and the short day (SD) setting, which included 8 hours of exposure to light and 16 hours in darkness (8L: 16D). The other two groups were LD subjected rats with food restrictions (LD+ food restriction) and SD subjected rats with food restrictions (SD+ food restriction). Food restriction groups were fed 70% of the food given to the regular groups. The rats were sacrificed and weighed. In all three strains, short day settings resulted in a slower increase of body and reproductive organ mass. These two studies reflect the stimulatory effects of light on the reproductive function of certain species, unlike other rats that prefer short photoperiods for reproductive growth. The experiment done on the three rat strains, ACI, BUF, and PVG compared the effects of long day photoperiods on reproductive growth to the stimulatory short day photoperiods of Fischer and Brown Norway rats. The results in this experiment showed that long days were preferred in all three strains, indicative of a genetic component involved in these species that controls reproductive responsiveness to photic information (Francisco et al. 2004) (Figure 3). Although the results indicate long photoperiods as stimulatory for these three rat strains, no control of a short photoperiod was actually used in the experimental setup, which would have strengthened the results of this experiment. In all three experiments, light profoundly affected gonadal growth in males, without promise of similar effects on female reproductive organs. Still, the evidence provided in these experiments indicates a direct connection between the exogenous factors, the photic environment, and reproductive function.

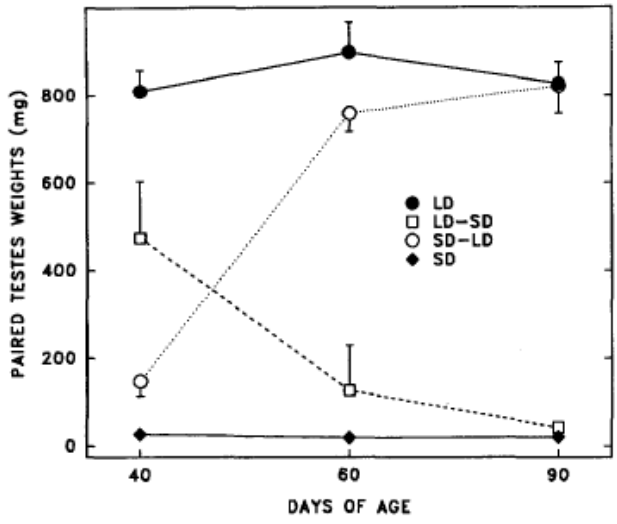

Figure 2: Long day conditions for both LD and SD-LD favored higher testicular weights than SD or LD-SD. Notice the immediate drop in weight of rats switched from LD-SD after only 10 days in their new conditions. Source: Yellon 1994

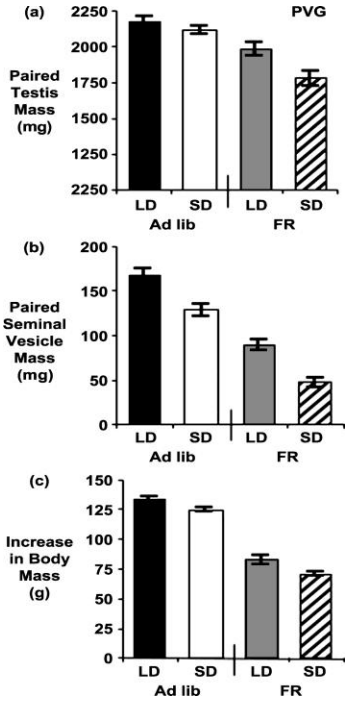

Figure 3: Weight of body and reproductive organs increased under conditions of long days, and normal food. Table for PVG strain; results of both ACI and BUG followed a similar pattern. Source: Francisco et al. 2004

Perspective on Endogenous Controls: Genetics, Peripheral Clocks, and Internal Timing System

How do the endogenous timing system and genetics enter into the scene of light and the SCN controlling melatonin and reproductive function? The answer is that the biological clock reacts according to photoperiodic change. In a sense, the SCN is entrained by the biological clock and genetic components of a specific organism. Animals were created with the ability to adapt to their environment for ultimate survival, and they time anticipated parturition in a season with optimal food availability to increase chances of survival. Therefore, long daylight hours may be beneficial for hamster reproduction but inhibitory to sheep reproduction (Foster and Kreitzman 2004).

Light itself is not necessarily the marker of growth and reproductive function; rather it tells the organism what time it is so that it can perform its various seasonal functions. An experiment was done on pinealectomized hamsters under conditions of constant dim light. Two groups received melatonin concentrations which corresponded to either the winter or summer months: more melatonin corresponded to the long winter night and less melatonin corresponded to the short summer night. Hamsters, long day breeders, treated with summer-like melatonin infusions were reproductively active while the winter group was not. A similar experiment done on sheep, which are short day breeders, showed a higher reproductive rate in the winter rather than the summer (Foster and Kreitzman 2004). These findings prove that reproductive response to external cues is at the genetic level and differs across various species. Notice how the hamster and sheep reacted in response to the melatonin concentration; these animals used the blood melatonin level as a marker of the season and became reproductively active depending on their endogenous timing system. This evidence strongly suggests that melatonin mediates the effect of photoperiod changes on reproductive function because the animals take cues from their melatonin levels. Melatonin levels change according to input from the environment, thus additionally proving how the suprachiasmatic nucleus controls reproductive function. Colin Pittendrigh

advocated for the consideration of the biological endogenous clock, its mechanism and entrainment by light as factors working together to control various functions. He remarked, "there are common mechanisms—built of different concrete parts—in circadian systems and photoperiodic effects everywhere" (as quoted in Foster and Kreitzman 2004).

A study conducted by researchers at the University of Illinois was designed to investigate the effects of SCN lesions on melatonin secretion and subsequent reproductive activity. The evidence of the study strongly suggests that the effects of photoperiod on reproductive function are controlled by melatonin secretions. Ovary intact ewes were subjected to unilateral or bilateral lesions of the suprachiasmatic nuclei, or sham lesions, placebo lesions which served as the control (sham lesion is when the identical brain surgery is performed, but does not target the brain tissue that the experimental group is testing). After recovery from the surgery, the ewes were exposed to alternating short day (9 hours of exposure to light and 15 hours in darkness) and long day (16 hours of exposure to light and 8 hours in darkness) photo regimens. Melatonin secretions in the unilateral and sham lesioned ewes remained normal, high at night and low during the day, while the bilateral lesioned ewes experienced disrupted and abnormal melatonin secretions. Almost all reproductive activity, with few exceptions, followed the pattern of melatonin—normal in unilateral and sham lesioned ewes and abnormal in bilateral lesioned ewes— indicating that melatonin is a direct effector of reproductive function (Scott et al. 1995). Like the previous experiment, this study strongly highlights the direct link between melatonin and reproduction, which are both controlled by photic messages. However, the experimental procedure in the study of the ewes is questionable, rendering the results questionable as well. An experiment must have only one variable in the experimental group which is not in the control group in order to accurately test the effect of the variable. In this experiment, the SCN lesion was the variable between the experimental and control group of sham lesions, but the alternating short and long days may have interfered with the experiment at hand. It is possible that the bilateral SCN lesions were responsible for the altered melatonin secretions, but because there was also `a constantly changing photoperiod, it is also possible that the disrupted light patterns caused altered melatonin levels and subsequent disruption in cyclicity. The authors mention that this experiment does not prove that the SCN generates reproductive function, because even ewes with abnormal cyclicity ovulated, but they do not address the fact that the SCN lesion may not have impacted melatonin at all. It is unclear if the SCN lesion was the sole cause of disrupted melatonin secretion because additional environmental factors added to the variability.

The rhythmicity of the SCN is traced back to transcriptional factors, *Clock* and *Bmall*, which code for period genes *per1*, *per2*, and *per3*, which are believed to play a role in fertility (Boden and Kennaway 2006). An experiment recorded in the Journal of Reproduction reports how cellular rhythmicity and reproductive function were affected in various mice knockouts. Mice with the *per1* and *per2* gene knockout maintained normal reaction to a light/dark cycle and did not show changes in reproductive activity, while the period of the *per3* knockout was shortened by .5 hours. When transferred to constant darkness, all rhythmicity was lost in the *per1* and *per2* knockout mice. Mice with the *Clock 19* knockout were also entrained to the normal light dark cycle and lost all rhythmicity when placed in total darkness. LH levels were normal in the *Clock* knockout mice, but prolonged and irregular estrous cycles did not allow for appropriate embryonic development and pup survival. The *Bmall* knockout mice had disrupted rhythmicity of the

SCN in a normal light/dark cycle, and they also showed reduced activity because of an inability to adapt to dark periods. Their reproductive functions were also harmed by delayed puberty, small ovaries and uteri, and the inability to establish viable pregnancies (Boden and Kennaway 2006). After analyzing the results stated in Boden's article, it can perhaps be concluded that the *Bmall* gene is a necessary endogenous feature that controls reproduction in response to environmental cues. All gene knockouts lost their rhythmicity in darkness, but only *Bmall* knockouts showed arrhythmic patterns even in a normal light/dark cycle. The *Bmall* knockout was the most affected by reproductive issues, while the other knockouts showed no or little effect on reproductive functions. It is possible that certain genes, more than others, are involved in regulating reproductive functions in response to environmental light. In regard to the effects of disrupted circadian rhythms in shift workers, an article in "Cancer Causes Control" attributes the desynchronization of the SCN to a delayed adaptation of certain genes to new environmental stimuli. The anterior portion of the suprachiasmatic nucleus adapts faster than the posterior portion, and it is possible that this delayed adaptation is responsible for circadian desynchronization at the genetic level (Haus and Smolensky 2006). These genetic factors contribute to an organism's responsiveness to light and reproductive activity.

An experiment done on female golden hamsters questions the genetic component of estrous cyclicity. Two groups of hamsters, one with normal hamsters and the other with tau mutant hamsters, which were kept under constant lighting conditions, exhibited identical estrous periods of 96 hours. The authors of this experiment suggest separate mechanisms controlling circadian periodicity and estrous periodicity because of the identical estrous period found in groups with differing circadian genes (Refinetti and Menaker 1992). From these results we conclude that light may play a major role in controlling reproduction, because two genetically different strains reacted identically in their reproductive cycle. Nevertheless, it can be suggested that genetics still does play a role in photo responsiveness but was not seen here because the mutated Casein kinase 1e (from the tau mutant) may not have an effect on the hamster's SCN interpretation and reproductive activity. The fact that estrous periods were prolonged in constant light reflects the hamster's identity as a long day breeder that is reproductively active during long daylight hours and does not discredit the endogenous timing system that controls reproductive function.

Research on the endogenous biological clock reveals mechanisms other than the suprachiasmatic nuclei, or specific genes that control various functions. A study done on glucocorticoid production and the circadian clock shows an adrenal circadian clock involved in adrenocorticotropic hormone release, besides for stimulation from the primary circadian oscillator (Dickmeis 2009). Control at the level of the adrenal gland proves how circadian rhythmicity exists in some peripheral organs and may function with or without input from the retinohypothalamic tract (Figure 4).

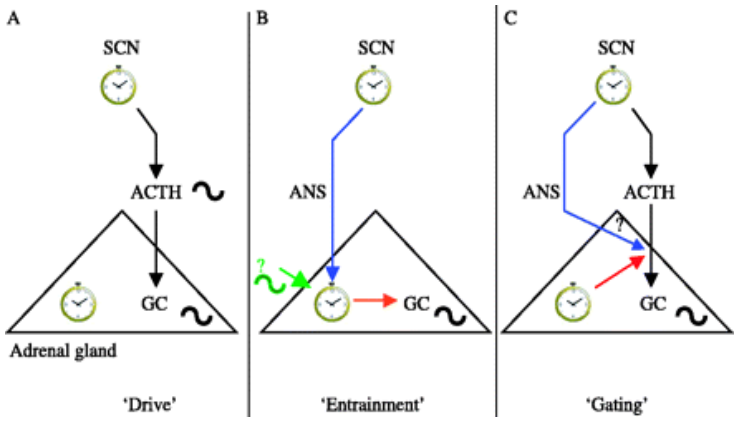

Figure 4: Glucocorticoid synthesis is stimulated by the 1. Master clock=SCN. 2. The adrenal gland's internal clock via input from the autonomic nervous system. 3. A combination of a & b. Source: Dickmeis 2009

Additionally, there may be other endogenous features controlling circadian rhythmicity even though sources of these mechanisms are unknown. In an article titled, "Stability, Precision, and near 24-hour period of the human circadian pacemaker," Czeisler reports that there is endogenous circadian rhythmicity even in the absence of periodic cues from the environment. An experiment was designed to lengthen the daylight hours of human subjects by delaying bedtime by four hours. After about a month on this schedule, the estimated intrinsic periods of melatonin, cortisol, and core body temperature were measured and proved to be similar to standard levels. The intrinsic circadian periods for all 24 subjects estimated between 24 and 24.35 hours, even though subjects experienced a 28-hour "day" (Czeisler et al. 1999).The results presented here reflect the workings of an internal biological clock that was not tricked by outside factors and continued functioning according to its internal circadian rhythm. Similarly, it is possible that reproductive function is also controlled by endogenous rhythmicity like the melatonin levels in this experiment, but this specific experiment did not test gonadal hormones. The authors also mention a study done on submariners who experienced 18 hours of artificial light while undersea on naval duty for six weeks; melatonin levels were only about .01 hours longer than the subjects in the present study and, once again, provide a basis to assume that an internal rhythm regulates hormonal function aside from the SCN.

Lee and McClintock from the Committee on Biopsychology at the University of Chicago, present a study on the seasonal variation in fecundity in female rats under constant lighting conditions. In the absence of environmental cues, which were always thought to be a trigger for reproductive function, these rats displayed increased reproductive activity in long summer days. The results of this experiment performed in two laboratories once again point to an endogenous feature controlling the rhythmicity of life functions aside from the level of the photoreceptor. Those conducting the experiment were under the impression that the female rats would respond like male rats had in a previous experiment in which rats exhibited seasonal variation in physiological measures of pineal and gonadal activity in a controlled lab. Five cohorts of reproductively mature Sprague-Dawley female rats of different ages chosen from a colony of 200 entered the experiment at different points of the year. Stimulatory light conditions of 14 hours exposure to light and 10 hours in darkness were constant throughout the two years of the experiment, as well as temperature, barometric pressure, and circulating fresh air (modified by a heating/cooling unit in the lab). Fecundity was measured by short, regular, and variant cycles which increase the chances of fertilization. Even though the rats had no indication of the time of year, fecundity peaked in the summer months with the appearance of shorter and more regular estrous cycles (Figure 5) and higher rates of mating. The second laboratory housed 3000 Wistar rats under 12 hours exposure to light and 12 hours in darkness for 19 months. Again, reproductive success and fecundity peaked between May and August, proving that these laboratory rats display the same seasonal fecundity as wild rats, probably because of an endogenous mechanism controlling reproductive function. It is well established that these rats prefer long days for reproductive success, but the fact that fecundity peaked during the summer months (even though stimulatory lighting conditions were available all year round) proves that an endogenous feature controls the reproductive function in these female rats. However, like the authors suggested, it is possible that changes in humidity levels throughout the experiment were indicative of the time of year and may have been the trigger of increased fecundity. In addition, male reproductive activity and its response to

constant environmental factors must be taken into consideration when investigating the fecundity and mating success of the female rats (Lee and McClintock 1986).

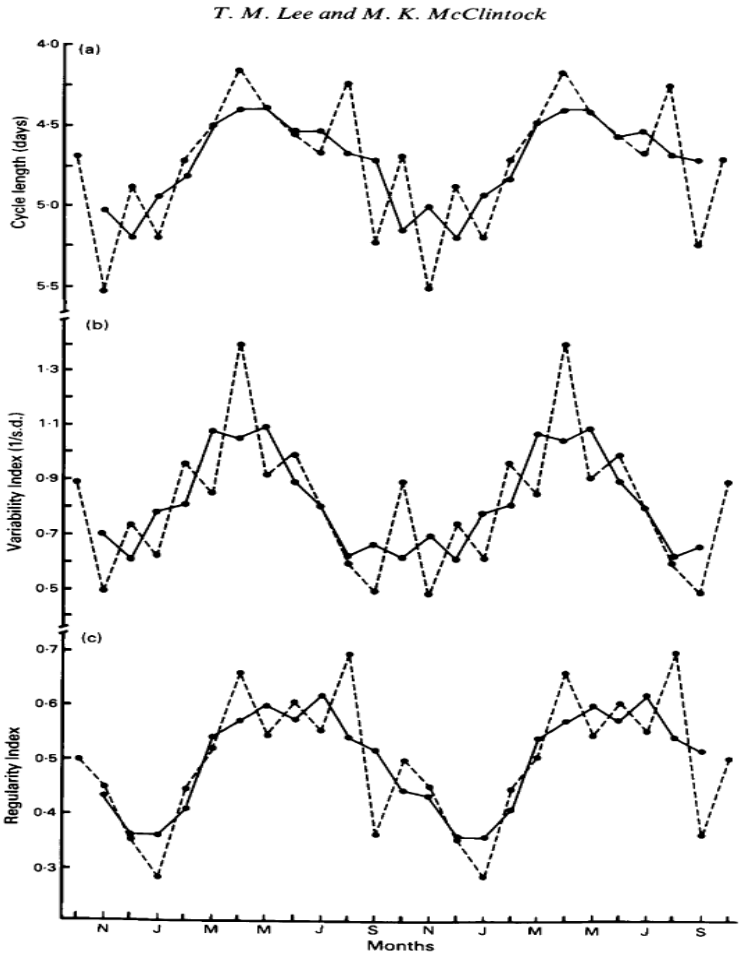

Figure 5: Fecundity peaked between May and August in both years of the experiment. In lab 1, Fecundity was measured according to regularity, variability and length of estrous cycle. Bold lines are the average, and dotted lines are the surrounding range. Source: Lee and McClintock 1986

FURTHER RESEARCH:

Both endogenous and exogenous components are inherent in the photo responsiveness of melatonin and subsequent reproductive function. Disrupted light/dark cycles, melatonin secretions, and genetic factors can contribute to reproductive malfunction and disordered cyclicity. Besides for light telling the body what time it is and keeping rhythmic functions running in a healthy manner, sunlight can also help fertility because of the vitamin D it gives off to the environment. An experiment done on female Holtzman rats compared the reproductive capacity and fetal development of healthy and vitamin D deficient rats. Although the vitamin D deficient rats were capable of reproduction, overall fertility was reduced by 75% and litter sizes by 30% (Halloran and Deluca 1980). We can hypothesize that sunlight, as the main source of natural vitamin D, may be helpful for reproductive function, although no direct experiment relates actual sunlight to fertility. Workers who use the daytime for sleeping to compensate for a night lost sleep may be harming their chances of sunlight exposure which directly and indirectly helps fertility. There have been very few experiments done on humans testing the effects of the light/dark cycle on melatonin secretion and reproductive function, because it is difficult to keep

human subjects under constant light or dark conditions for long periods of time. Therefore, scientists use epidemiological studies to investigate the effects of disrupted light exposure on melatonin and gonadal activity (Kennaway 2005), although results of these studies are not as direct as human experimental results would be.

CONCLUSION

Photoperiodic changes in melatonin concentrations affect female reproductive function because of photic and genetic factors. Limited research has been done on human subjects to test the effects of constant photoperiodic changes on the female menstrual cycle, but evidence from experiments on animals may hint to trends that can be evident in the female human as well. However, as mentioned before, each species runs on its unique biological clock that interprets environmental information based on endogenous features, so experimentation on animals does not really provide conclusive evidence on human subjects. Nevertheless, the mechanisms involved in melatonin secretion and reproductive function can be similar in animals and humans and can be used as a springboard for further research on the photoresponsiveness of human reproduction. Researchers suggest that workers minimize their exposure to constantly changing schedules in order to avoid possible disorders that may arise from the desynchronization of circadian function.

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NORMAL PRESSURE HYDROCEPHALUS: HOW CAN IT BE TOLD APART FROM NEURODEGENERATIVE DISEASES OF THE ELDERLY?

Raphael C. Zohn

ABSTRACT

Normal pressure hydrocephalus (NPH) affects more of the older population than people recognize. The underestimation of this neurological condition is due in most part to the overlap of its symptoms to other forms of dementia as well as many other geriatric conditions. The objective of this paper was to research and contrast various methods of differentiation in the diagnosis of normal pressure hydrocephalus as well as find pretreatment indicators of successful surgery. Methods included reviewing of articles and studies done to evaluate which symptoms are most commonly presented in normal pressure hydrocephalus and their subtle differences from the symptoms of other neurodegenerative diseases. There are also comparisons of different theories as to the prevalence of normal pressure hydrocephalus and which, if any, symptoms are indicative of a correct diagnosis. Conclusions were as follows: there are guidelines, although controversial, that can be followed in trying to distinguish normal pressure hydrocephalus; there are some symptoms that are better prognosticators of successful surgery than others, and while surgery is often followed by the subsequent relapse of symptoms, this is possibly due to the comorbidity of other disorders with normal pressure hydrocephalus. Surgery should therefore be approached cautiously while weighing the risks versus the benefits. Normal pressure hydrocephalus seems to be fairly prevalent and when appropriate, some older people might be able to reverse their symptoms.

INTRODUCTION

If an elderly man were to walk into his doctor's office complaining of walking difficulties, urinary incontinence, as well as having trouble with memory and hard thinking, the doctor might initially presume the man has Alzheimer's disease. Upon further review, he would be greatly mistaken. Extensive testing can lead to a drastic change in the diagnosis. Intermittently elevated levels of cerebrospinal fluid (CSF) pressure in the lateral ventricles of the brain along with ventriculomegaly (enlargement of the ventricles without a noticeable amount of cortical atrophy) would tell the doctor that the man is suffering from hydrocephalus. Unfortunately, this example is very common. Despite the fact that a patient might be presenting the abovementioned classic clinical triad of symptoms for hydrocephalus, errors all too likely. Normal pressure hydrocephalus (NPH) is a subcategory of communicating hydrocephalus, one of the four main categories of the disease. NPH has primary (idiopathic) and secondary (known) causes. Secondary causes include complications following head trauma, subarachnoid hemorrhage, meningitis, brain tumor, or previous neurosurgical procedures. While people of all ages are affected by secondary NPH (sNPH), idiopathic NPH (iNPH) distinguishes itself as a disease of the elderly. About 50% of NPH cases are idiopathic and half come from a known event. When most think of hydrocephalus, the swelled head of a child comes to mind. Few realize how widespread iNPH is and that many older patients suffer from it. Many doctors don't even notice it and diagnose it as something else. However, if caught early on, iNPH can be treated with a much greater chance of success. The problem arises when it comes to pinpointing all the characteristics of iNPH that can aid in its diagnosis. Its symptoms are not specific to the diagnosis and occur commonly in many other neurodegenerative conditions, such as Parkinson's and Alzheimer's disease (Klassen and Ahlskog 2011). Furthermore, it is often comorbid with other neurological disorders that are actually present in geriatric patients. This doubt makes it

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difficult to elect for surgery and is the primary reason why 80% of cases of hydrocephalus go unrecognized and untreated (Kiefer and Unterberg 2012). Studies done on its prevalence, along with case studies and accounts show that it is far more common than would appear at first glance due mainly to mistakes and the fact there is little awareness of its commonality. The very fact that iNPH is believed to be uncommon, leads to false diagnoses, which in turn causes skewed statistics. While this disease is perceived to be rare, attempts to identify all patients with iNPH overlook a large number of cases (Conn 2011). This paper will explore just how prevalent it is, as well as discuss literature on ways it can be better recognized and diagnosed. Studies indicate that certain symptoms are more indicative of iNPH than others. There are many characteristics of NPH that are specific to it, and can aid in its diagnosis. How can we differentiate a potentially reversible disorder from the more common forms of dementia?

BACKGROUND OF NPH

HISTORY

The first mention of hydrocephalus dates back all the way to 2500 BC where references can be found in ancient Egyptian medical literature. It was not until 1000 AD, however, that an operative procedure was performed. An Arab surgeon at that point in time clearly describes the removal of spare cerebrospinal fluid from the skulls of hydrocephalic children. He writes that "the volume of the skull then increases daily, so that the bones of the skull fail to close" (Aschoff et al. 1999). A real treatment for the condition did not enter the scene until the 20th century when shunts and other neurosurgical procedures became popular. The first description of NPH came in 1965 when a small group of patients presented with various neurological symptoms, ventricular enlargement, and what looked like normal cerebrospinal fluid pressure during lumbar puncture. After shunting, their symptoms improved (Vacca 2007). The shunt treatment does not fully heal the patient though, and, to this day, there is no complete cure.

ANATOMICAL DEFINITION

Hydrocephalus can be broadly defined as an abnormal expansion of the lateral ventricles in the brain caused by an accumulation of cerebrospinal fluid. Cerebrospinal fluid cushions the brain and spinal cord in the subarachnoid space between the arachnoid and pia maters that cover them. Cerebrospinal fluid is produced by specialized capillaries known as the choroid plexus. Cerebrospinal fluid flows from the two lateral ventricles through their respective foramina of Monro into the third ventricle. From there it passes through the aqueduct of Sylvius into the fourth ventricle, which is located in the posterior fossa, and then the central canal of the spinal cord. It enters the space in between the meninges through small openings in the ventricular system and covers the brain and spine, acting as a cushion and protecting the brain from shock. From the subarachnoid space, cerebrospinal fluid is absorbed by clusters of arachnoid villi, sometimes called arachnoid granulations, close to the top of the brain, and eventually drains into the venous system from the superior sagittal sinus (Tortora and Derrickson 2006). The cerebrospinal fluid in a normally functioning person flows around the superior sagittal sinus and gets reabsorbed by the arachnoid villi due to pressure gradient differential. When cerebrospinal fluid is being blocked at any point during this flow cycle, it causes the ventricles in the brain to get stretched and become enlarged, affecting portions of subcortical brain tissue as well as white matter. There is approximately a pint of cerebrospinal fluid produced in the brain each day and the turnover rate of the cerebrospinal fluid is more than three times a day. However, because the production of the fluid is independent of the absorption, a lack of absorption results in an accumulation of cerebrospinal fluid in the ventricles (See Figure 1).

ETIOLOGY

There are several different types of hydrocephalus. Some are born with it, while others develop it much later in life. It can be genetic or acquired through physical or mental trauma. It can make one's head swell up like a balloon or shrink the brain in some cases. There are different causes for hydrocephalus and thus several different categories. Reasons could be either disrupted flow of the fluid, a problem with reabsorbing the fluid, or too much production. The most common is flow obstruction. There are four main categories of hydrocephalus: congenital, acquired, non-communicating, and communicating. NPH is a form of communicating hydrocephalus meaning there is no obstruction in the actual pathway and is therefore extremely difficult to detect. The etiology of NPH is unclear. While some cases can be attributed to known neurological injuries like subarachnoid hemorrhage and meningitis, most are idiopathic. Its symptoms most likely are due to ventricular dilation. The leading theory behind this dilation is the lack of reabsorption of cerebrospinal fluid into the venous system. Others suggest that the cerebral vasculature may have a role in the pathogenesis of NPH (Siedlecki 2008). Although the intracranial pressure gradient increases between the fluid in the subarachnoid space and the ventricles, it remains normal. It is this increasing pressure that leads to cerebral ischemia and a stretching of the periventricular white matter. Scarring and fibrosis on the arachnoid granulations can disrupt the absorption of cerebrospinal fluid.

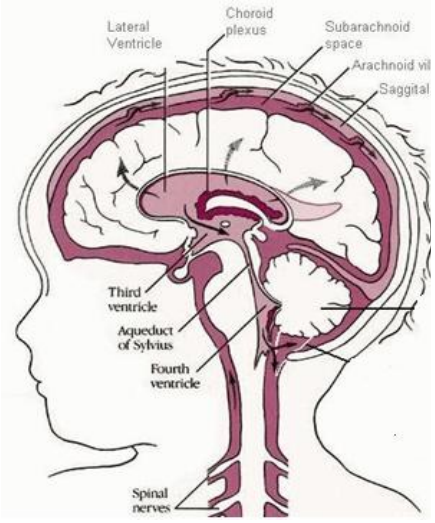

Figure 1: Anatomy of the ventricle system. Source: National Fibromyalgia Research Association

DIAGNOSIS

NPH is characterized by the chronic elevation of intracranial pressure due to increased cerebrospinal fluid becoming stable and therefore not exhibiting some of the usual hydrocephalic symptoms, such as headaches, vomiting, nausea, or altered consciousness. The big three symptoms, referred to as the Hakim triad after the doctor who first described them, include gait impairment, cognitive dysfunction, and urinary incontinence. NPH can be considered when the triad of symptoms is present. All three symptoms, however, are not required to suspect NPH. In the past, NPH would be diagnosed only if presenting the complete triad. Now, however, it can be diagnosed and treated in the presence of only two cardinal symptoms or even just one. This change in attitude resulted from the recognition that the longer NPH remains untreated, the worse the prognosis gets, with the complete triad always signifying an advanced stage of the disease (Kiefer and Unterberg 2012). Typically, gait impairment occurs first, with cognitive impairment and urinary incontinence occurring later. The difficulty with gait is usually described as a shuffling or magnetic walk and an exaggerated wide stance. It is also usually first to improve after shunt surgery. If left untreated, gait may deteriorate until the patient can no longer walk and is limited to a wheel chair. Dementia, when present, is subcortical. Cortical dementia, such as Alzheimer's has characteristics such as severe memory loss, an inability to recall words and understand common language. In contrast, subcortical dementia presents as inattention, latency in recall, and lack of spontaneity (slowness in response and reaction). These are known as abulic traits (Byrd 2006). The urinary disorder associated with NPH starts with urinary frequency or

urgency and then develops into complete loss of bladder control in the later stages of the disease. Incontinence often will not appear in early stages, and may go unreported as patients will attribute it to other prostatic issues and normal aging. It is also possible that what is chalked up to incontinence is merely an inability to reach the bathroom because of the slowed gait. These three clinical manifestations are likely caused by pressure on the structures adjacent to the ventricles. For example, pressure on the frontal lobes and their interconnections, including structures such as the limbic system, may cause symptoms of dementia. Pressure on the cortical center for bladder control may be the reason for incontinence, and pressure on the corticospinal tract, whose fibers lateral to the ventricles supply motor function to the legs, may cause the gait disturbance. The fibers of the corticospinal tract passing closest to the lateral ventricles in the corona radiate may be the reason why gait is first to improve.

The earlier on NPH is diagnosed, the more reversible it seems to be. Physical examination, assessment of cortical and subcortical function, and careful analysis of gait, along with a complete record of previous health, are the most important tools used in diagnosing NPH. Diagnoses aren't simple because these symptoms overlap with other disorders. Today, radiographic imaging studies are used as the main determining factor. In patients with clinical features of NPH, CT scans or MRIs should be used to measure ventricular size, rule out ventricular obstruction, and look for other possible causes like tumors, infections, and structural abnormalities. Diagnostic guidelines for monitoring symptoms have been published to pick out probable NPH patients from the possible (Siedlecki 2008). Screening of prospective surgery candidates is done to decide if shunt surgery would successfully reverse their symptoms. There have been conflicting reports as to the effectiveness of shunting procedures. However, cerebrospinal fluid monitoring and a trial of controlled, continuous cerebrospinal fluid drainage of 150-200mL per day has proven to make diagnoses more accurate (McGirt et al. 2005). Invasive diagnosis includes a spinal tap test: lumbar puncture with the removal of 30 to 70 mL of cerebrospinal fluid. This can be repeated for two or three days in a row. Modern forms of treatment lead to improvement in 70-90% of patients according to some studies (Kiefer and Unterberg 2012).

TREATMENT

The foremost method of treatment is the surgical installment of a ventriculoperitoneal shunt, a tube that connects the ventricles of the brain to an alternate drainage site, usually the abdominal cavity. A small hole is made in the skull and a catheter is placed in one of the lateral ventricles. It is then tunneled subcutaneously behind the ear and down to the abdominal cavity. The shunt contains a cap and valve ensuring there is no back-flow. In a way, some non-communicating hydrocephalic patients have an advantage because the only issue is the blockage of the subarachnoid space. In such cases, the shunt need only bypass that blockade and can empty into the subarachnoid space. Patients that have shunts implanted need life-long monitoring to ensure that it is doing its job and there is no recurring hydrocephalus. Shunt surgery is a very invasive procedure and is not without risk. Included in these risks are hemorrhage and stroke. There are some drugs that help delay the need for surgery: acetazolamide, furocemide, and digoxin, to name a few. Symptoms tend to improve upon removing the excess fluid at least for the short-term. It has been found to be the most effective available treatment for NPH. It is believed that shunting improves symptoms by removing the pressure from the parenchymal absorption pathway at the areas of the ventricles where cerebrospinal fluid is produced, reducing interstitial swelling and pressure, which then reduces ischemia.

iNPH

Hydrocephalus is special in that it is one of the few reversible causes of dementia. iNPH mainly affects people over 60 years of age. The big problem with iNPH is that it goes unnoticed and thereby untreated in many cases. Folks who think there is no cure for their dementia would be shocked to find out that it may actually be quite reversible. Diagnosis is made very difficult by the fact that there is overlap with many other diseases of the elderly and the surgery is quite invasive and can create complications. Often, after surgical implantation of a shunt, the symptoms will return, leading to questions whether NPH was the problem to begin with. It further complicates things when NPH is actually comorbid with some of the diseases that it is confused with, such as Alzheimer's and Parkinson's. It is estimated that more than 750,000 Americans have NPH, but less than 20% receive an appropriate diagnosis and treatment. This is largely due to its misdiagnosis in the first place and it is believed that an astounding 5 to10% of people living with dementia, in fact have NPH (Siedlecki 2008).

PREVALENCE

The true prevalence of NPH is debatable. The available epidemiological data are inconsistent partly because there is no one set of diagnostic criteria. Moreover, 75% of those with NPH also have vascular dementia or Alzheimer's disease. A study done recently in Germany found one person in 80 to be demented. About 250,000 people receive a new diagnosis of dementia each year and NPH is thought to account for about 6% of all cases of dementia. A study of demented patients in nursing homes found that 9% to 14% of them had the symptoms typical of NPH. The incidence of NPH is approximated at 0.2 to 5.5 new cases per 100,000 people per year. Its prevalence is said to be 0.003% in patients under 65, and 0.2% to 2.9% for 65 and up. The prevalence of NPH rises significantly with age (Kiefer and Unterberg 2012). In one community-based study, data suggest that real clinical and imaging features that strongly suggest NPH are relatively uncommon and that most patients originally suspected of having NPH do not respond to a cerebrospinal fluid removal trial. The authors come to the conclusion that despite "relatively high prevalence figures (0.4%–14%)…studies that simply tabulate gait disturbance, dementia, incontinence, and ventriculomegaly without broader reference to the overall clinical context probably overestimate NPH prevalence" (Klassen and Ahlskog 2011). This is interesting because they seem to partially dismiss the triad of symptoms and neuroimaging showing enlarged ventricles as being indicative of NPH. Most other studies find that differential diagnosis based the clinical triad of symptoms and reading of imaging tests is more subtle. It is possible that their sample sizes are too small to be able to draw these conclusions from them.

DIFFERENTIAL DIAGNOSIS

The diagnosis of NPH and how its symptoms manifest themselves is controversial to say the least. Many other diseases have similar clinical characteristics to NPH, either by themselves or together with others. The most closely related are Alzheimer's disease, Parkinson's disease, diffuse Lewy body disease, vascular dementia, frontotemporal dementia, chronic alcoholism, carcinomatous meningitis, subdural hematoma, lumbar canal stenosis, and endocrine disorders such as hypothyroidism and Addison's disease. It is possible that many diseases can be alternate causes of symptoms of NPH as well as coexist with it. For example, spinal stenosis, a gait-associated condition, a cognition-associated condition such as subdural hematoma, and urinary diseases such as benign prostatic hypertrophy together at the same time in a patient and will appear to be NPH. The following is a contrast between the symptoms of NPH and other neurological disorders in numerous steps to be followed during diagnosis:

IMAGING AND CEREBROSPINAL FLUID STUDIES

Features on CT scans that suggest NPH are the presence of bigger temporal horns, overly enlarged ventricles (ventricles tend to enlarge with age anyway), and brain tissue shrinkage. Ventricles enlarge with age, but if the ratio of the maximum width of the anterior ventricular horns to the maximum width of the calvarium is greater than 0 .3 (called the Evans ratio), the ventricles are considered abnormally enlarged (Factora and Luciano 2008). NPH can be characterized by findings on MRIs such as transependymal resorption, T2-weighted increased intensities in brain parenchyma next to the ventricles, and preservation of hippocampal tissue. Typical findings of NPH also include a coronal section at the level of the posterior commissure revealing a narrowed subarachnoid level surrounding the brain, called a tight convexity, and narrow medial cisterns. In addition to the lateral ventricles, the third ventricle is usually enlarged as well, while the fourth may or may not be enlarged. In addition, a higher level of cerebrospinal fluid flow through the aqueduct of Sylvius and a forceful cerebrospinal fluid flow through the foramina in the cerebrum. Spinal tap using a radionuclide isotope introduced into the subarachnoid space through a lumbar puncture has been one of the most used tests for diagnosing NPH. The test detects increased resistance to cerebrospinal fluid absorption via the arachnoid granulations, as seen in NPH. The standard spinal tap test collects between 20 and 50 mL of cerebrospinal fluid, measures the opening pressure, and conducts cerebrospinal fluid analysis. Normal cerebrospinal fluid protein and glucose levels, white blood cell count, and an opening pressure in the range of 60 to 240 mm H2O, is highly suggestive of NPH. In all, there are six cerebrospinal fluid-related tests that can be used to figure out if NPH is present and also help determine if the patient is likely to respond to shunting. These include the standard spinal tap, large-volume spinal tap, temporary external lumbar drainage, extended intracranial monitoring, cerebrospinal fluid outflow assessment, and measurement of aqueductal cerebrospinal fluid flow (Osei-Boamah 2011). A particularly effective prognosticator of surgery outcome is the continuous spinal drainage of 150 to 200 mL of cerebrospinal fluid per day for 2 to 7 days. This test is considered to be positive if the number of steps taken in a 10-meter gait test, and the time needed to walk 10 meters, are reduced by 20% or more and/or there is an improvement of at least 10% in psychometric tests.

GAIT DYSFUNCTION

Diseases that share similar gait symptoms with NPH include: Parkinson-plus syndromes, Parkinson's disease, vascular Parkinsonism, visual impairment (cataracts, glaucoma, and macular degeneration), lumbar canal stenosis, large joint osteoarthritis, and peripheral neuropathy deconditioning (Factora and Luciano 2008). How can NPH's specific gait defects be told apart from the walking difficulties older people with these illnesses encounter? NPH patients initially complain of dizziness, trouble walking on stairs, and difficulty getting up from or sitting down on a chair. As the disease progresses, the patient's gait deteriorates significantly, becoming slow, broad-based, short-stepped, and glue-footed. Freezing during walking and difficulty with turning or starting to walk also suggest disease progression. Often, as a result of these gait abnormalities, frequent falls will call for the need of a cane or walker. In the later stages of NPH, motor deficit is usually worse because of the cognitive deficits. Sometimes, it is so severe that the patient can't walk at all. Of major importance for the differential diagnosis, are the following: Externally rotated posture of the feet, particular difficulty turning on the body's long axis, and absence of apraxia (Kiefer and Unterberg 2012). This is confusing because in the past, some actually referred to the symptom as gait apraxia because it looks like the person has forgotten how to walk and has trouble with basic components of walking (DiCecco 2009; Osei-Boamah 2011;

Siedlecki 2008). Apraxia is a disorder of motor planning and should not be confused with ataxia, a lack of coordination of movement, or abulia, the lack of desire to carry out an action. One doctor, however, writes in his findings that the short stride, slow gait, and difficulty with turning present challenging similarities between the gait of NPH and that of Parkinson's disease and cerebellar ataxia. Ways to help identify NPH include the absence of extrapyramidal signs such as cogwheel rigidity and no response to levodopa. In addition, NPH patients do not exhibit a resting tremor. Unlike patients with Parkinson's disease, NPH patients do not respond to visual and acoustic cues. Cerebellar ataxia is different in that it has other features such as dysarthria, gaze-evoked nystagmus, and appendicular dysmetria. NPH has none of those symptoms (Osei-Boamah 2011). NPH patients also have poor posture with a forward rounding of the shoulders and arms that hang loosely at their sides. They appear to always be watching their feet as they attempt to move. This limpness of the extremities is a telling sign of NPH, whereas Parkinson's is usually coupled with rigidity and stiffness of the limbs and trunk (Siedlecki 2008). In addition, the gait with Parkinson's disease tends to be more narrow-based, and the balance problems and disequilibrium are not as apparent (Ropper and Brown 2005). Many believe that a detailed gait history is extremely important for an accurate diagnosis. Specifically, the onset of the gait problems should be examined. Was the onset a progressive decline or more sudden? Typically, gait impairment in NPH is more gradual. Did the patient exhibit symptoms of tremor or bradykinesia (a general slowness of movement) suggesting Parkinson's? If the patient has back or cervical neck pain, it suggests the presence of lumbar canal stenosis or cervical myelopathy respectively (Factora and Luciano 2008). Parkinson-plus syndromes have features such as gaze palsy or autonomic dysfunction in addition to the shuffling gait that reduce the likeliness of diagnosis of NPH. In the elderly it is more likely to have multiple etiologies for these gait symptoms. For example, patients who have symptoms of NPH also can have lumbar canal stenosis. Therefore, one should always consider which of the disorders is more likely to be the cause for the impeded gait. In this case, it should also be considered that lumbar canal stenosis can interfere with a trial of cerebrospinal fluid removal used in predicting shunt outcome in NPH and limit potential for improvement after shunt placement (Factora and Luciano 2006).

COGNITIVE IMPAIRMENT

Disorders with similar cognitive manifestations as NPH include: Alzheimer's disease, diffuse Lewy body disease, vascular dementia frontal lobe dementias, depression, and prion disease. Cognitive impairment associated with NPH is often mild, and might initially be attributed to normal aging. The problems are mainly subcortical in nature. Short-term memory loss, short attention and concentration spans, apathy, and slow processing are some results of subcortical dementia. In other words, difficulty forming words, being unable to carry out simple tasks sequentially, or difficulty interpreting sensory stimuli are all cortical features and do not appear in NPH dementia. Any of these symptoms would distinguish NPH from cortical dementias, such as Alzheimer's disease (Byrd 2006). If other signs of cortical dementia, such as aphasia, agnosia, or apraxia are present, Alzheimer's disease should be considered. Dementia is also the dominant clinical feature of Alzheimer's disease. Alzheimer's and NPH can occur together, however, and the incidence of Alzheimer's disease in patients with suspected NPH is between 33% and 50% according to one study's estimate (Savolainen et al. 1999). Vascular dementia (stroke, multi-infarct dementia, vertebrobasilar insufficiency) and dementia with Lewy bodies are other causes of dementia to consider. Vascular dementia usually presents as a stepwise decline over an extended period, with greater loss of higher-order cognitive functions such as visuospatial perception and executive function. Lewy body dementia is especially hard to

tell from NPH, as it has both gait and cognitive deficits. However, its hallmarks are hallucinations and cognitive fluctuations which are rare in NPH patients (Johnson and Graham 2010).When the comorbidities are associated with subcortical deficits like in vascular dementia, depression, and frontotemporal dementia, it is much more difficult to separate those characteristics that imply NPH. Table 1 shows the differences between cognitive deficits one comes across in NPH and the symptoms Alzheimer's disease and vascular dementia classically present (Factora and Luciano 2006). The timing of the cognitive impairment's development compared to that of gait is useful diagnosing NPH and determining if shunting will be successful. Normally dementia does not precede gait impairment in NPH, and when it does it signifies a weaker response to surgery. As previously discussed in regards to Alzheimer's disease, in cases where dementia is the predominant feature of all symptoms, consider evaluation for another neurodegenerative disease (Siedlecki 2008). History of cognition in a patient plays a role as well. A history of slowly progressing fading of memory and other areas of cognition bad enough to affect daily activities supports a diagnosis of Alzheimer's disease. These symptoms may also appear years after the onset of Parkinson's. A known history of strokes and a stepwise decline with each might mean vascular dementia. Mental status testing is used to confirm cognitive impairment is present and see how severe it is. The Mini-Cog and the Short Portable Mental Status Questionnaire are used to screen for the presence of cognitive impairment. The Folstein MiniMental State Examination can also be used to quantify how severe the impairment is. A clock-drawing test is sometimes used to assess visuospatial skills and executive function.

Table 1: Comparison of dementia characteristics.

	Alzheimer's disease	**Vascular dementia**	**Normal pressure hydrocephalus**
Memory impairment	X	X	Impaired retrieval
Executive dysfunction	X	X	X
Impaired visuospatial process	X	X[a]	
Impaired language	X	X[a]	Bradyphrenia
Impaired complex motor skills	X	X[a]	
Psychomotor slowing			X
Impaired attentiveness			X

a- Can occur based on location of infarction (tissue death)

These are not problems present in NPH patients according to most. Use of a screening test such as the Geriatric Depression Scale can weed out patients with depression that can affect cognitive function. Neuropsychological tests can be very useful in distinguishing between the

cortical and subcortical dementias (Factora and Luciano 2008; Siedlecki 2008). Early cerebrospinal fluid shunting can improve the cognitive deficits in as many as 80% of patients with NPH, but improvement is far less likely if the patient also has vascular dementia or Alzheimer's disease (Kiefer and Unterberg 2012).

URINARY INCONTINENCE

In the elderly population, incontinence is very prevalent. Causes include benign prostatic hypertrophy resulting in bladder outlet obstruction, retention coming from neurogenic bladder, either from long-standing diabetes or related to Parkinson's, pelvic relaxation that helps lead to stress incontinence, and cystitis. Identifying the specific type of incontinence (urge, stress, overflow, or functional) can help find the true cause. The use of cystoscopy and urodynamic testing can be used to eliminate other diagnoses (Factora and Luciano 2006). Incontinence in NPH comes from detrusor hyperactivity which results from absence of central inhibitory control but there is no evidence of defective bladder sphincter control. It starts as increased urinary frequency. It later develops into urge incontinence and then permanent urinary incontinence. Fecal incontinence is rare in NPH and points to another type of disorder. Urinary symptoms usually come later than the other two symptoms and are lacking entirely in some patients. In patients with NPH, the slow gait can compound the problem, making it difficult to reach the bathroom in time and can make it difficult in determining the real cause (Byrd 2006). Diuretics can cause urinary frequency, alpha-agonists obstruction, and some medications with anticholinergic side effects may produce urinary retention. Evaluation of urinary incontinence can target certain symptoms. A urinalysis can easily be done to exclude the presence of a urinary tract infection. Getting a postvoid residual can identify those with urinary retention who are experiencing stress symptoms or any sensation of incomplete void. Urodynamic testing to evaluate urge incontinence has not been helpful in distinguishing NPH from other causes of detrusor instability. Cerebrospinal fluid shunting can improve bladder dysfunction in as many as 80% of NPH patients if surgery is done early on, but if performed in an advanced stage less than 50% to 60% respond positively (Kiefer and Unterberg 2012).

PROGNOSIS

The prognosis for NPH depends on a variety of factors, including age of onset and timing of surgery. Overall, most reports say people with suspected NPH have a 50% chance of benefiting (for the long term) from the installation of a shunt (Longe 2006). That figure includes those with known causes. iNPH in particular, as previously discussed, has several indicators of good shunting outcome that include early diagnosis, gait as the predominant symptom, and a positive response to large-volume cerebrospinal fluid draining. The presence of B waves during the majority of continuous intracranial monitoring, cerebrospinal fluid outflow resistance of more than 18 mm Hg/mL/min., and increased aqueductal cerebrospinal fluid flow are also indicative of potential success with shunting (Osei-Boamah 2011). A study by Malm et al. (1995) found that the number of patients with iNPH who improved from surgical implantation of the ventriculoperitoneal shunt went from 64% at 3 months to 26% at 3 years. This showed that a shunt may be effective only temporarily, lasting from 1 to 3 years. However, shunting can still make an enormous difference in quality of life for many of these patients. In the short term, the success rate is usually reported between 50% and 90% with all patients suffering a decrease over the following years. The high variability in reports is likely due to differences in selection, method of shunting, care in following up, and how success is defined. Much failure over time is caused by the development or progression of other neurologic or systemic disease (Factora and Luciano 2008). Some reports of symptom improvement after ventriculoperitoneal shunt surgery

are as low as 30% in patients with iNPH and 50% to 70% for patients with sNPH (Johnson and Graham 2010). Studies have shown that up to 93% of patients have major improvements in gait shortly after surgical treatment of NPH, as opposed to only 50% improving in cognitive symptoms (Yusim et al. 2008). Indeed, many seem to think the gait symptom is the primary indicator of NPH as well as ventriculomegaly (See Figure 2). In one study, among 41 patients with suspected NPH, all had gait dysfunction, 30 had cognitive impairment, and 14 had urinary incontinence. Twenty out of forty one presented with two of the symptoms, while twelve had all three parts of the triad. Nineteen were found to have at least moderate postural instability (defined by a positive pull test). All 40 patients with available results had documented ventriculomegaly. Only 13 of these underwent shunt placement, as it was offered only to those with gait improvement after a trial of cerebrospinal fluid removal, and only 4 of these had all elements of the classic NPH triad (Klassen and Ahlskog 2011). However, despite these positive symptoms, more than half did not respond well to the shunt in the long run. Of note is the fact that no patient with postural instability showed definite improvement after shunting, and 4 out of 5 (not including one who died during surgery) were later given an additional diagnosis. It may be that this symptom is a trademark of underlying neurodegenerative disease. Shunting may not be the best course of action for these patients (Klassen and Ahlskog 2011). Many feel that the best predictor of ventriculoperitoneal shunt outcome is external lumbar drainage; it is said to be about 85% accurate in identifying patients that would benefit from surgery, and is becoming more and more popular with neurosurgeons (Byrd 2006). Any surgery in the elderly population is going to have risks. Studies have risks as high as 30% to 40% in shunting, and severe morbidity or death at 6% to 8% (Factora and Luciano 2006).

CONCLUSIONS

NPH is a lesser-known medical condition for which a relatively small amount of research is conducted to improve treatment for. It was once thought that its prevalence hovered around 0.5% (Brean and Eide 2008). Recent studies show that it is closer to being between 1 and 2%. The reason for the discrepancy seems to be an oversimplification of the terminology of the symptoms (Conn 2011), and there do seem to be more subtle tells when it comes to diagnosing NPH. Identifying symptoms in the triad is simple, but determining which patients truly have NPH and will benefit from shunting is not straightforward. Shunting outcomes are not quite related to which patients had NPH to begin with due to comorbidity with other diseases. Although patients need not have all three symptoms for NPH to be considered, the gait deficit seems the most crucial for diagnosis. The mere presence of enlarged ventricles does not seem to be a valid indicator as they tend to enlarge with age. There are multiple conflicting theories and therefore many disagreements as to the true symptoms of NPH. Thus, the differential diagnosis of NPH can be quite difficult. The criteria for a diagnosis of NPH seem clear to everyone only with the following findings that make the diagnosis of NPH less likely: intracranial pressure above 25 cm H2O (this rules out iNPH by definition), age under 40, cortical deficits, progressive dementia without gait disturbance, and lack of progression of symptoms. The progression of symptoms is a controversial point, as

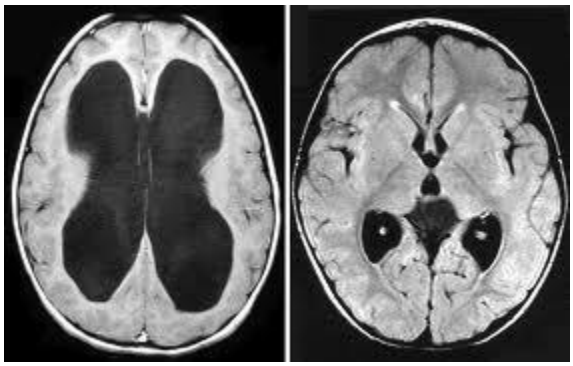

Figure 2: CT scan of ventriculomegaly vs. normal in adult

authors differ on what is considered a normal time of progression. A patient is considered a potential ventriculoperitoneal shunt candidate if he or she has symptoms consistent with NPH (not explained by other diagnoses) and has enlarged ventricles on imaging. The doctor should try to rule out as many other possible disorders as possible, and consider surgery by carefully weighing the risks and potential benefits as well as factoring in the chance for success.

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ARTIFICIAL DEVICES AS A VIABLE ALTERNATIVE TO THE CONVENTIONAL HEART TRANSPLANT

Hadassa Radzik

ABSTRACT

The human heart is one of the most vital organs in the body. It distributes blood throughout the body, providing the body with oxygen and nutrition, and contributes to metabolism. When the heart fails, blood flow is impaired, thereby limiting the exchange of oxygen within the cardiopulmonary system as well as diminishing oxygenation and nutrition to the other major organs and periphery. The only current proven treatment for advanced heart failure is cardiac transplant. Given the heart's importance and the scarcity of donated organs, modern medicine has experimented with the creation of an artificial heart. Because the heart is primarily a pump controlled via electrical impulses, it lends itself to artificial replication, and advancements in modern engineering and medicine have turned this theory into reality. Currently, there are mechanical devices which can act as a bridge to transplant and, in many cases, improve the quality of life of their recipients. Due to a scarcity of available donor hearts as well as the high cost and complications associated with conventional heart transplants, it is imperative to do an analysis as to whether left ventricle assist devices or the total artificial heart are viable alternatives to conventional cardiac transplants. If the artificial devices can be as productive as a transplanted heart without any overt risk, they greatly expand and improve the prognosis of patients in end stage of heart failure. This paper will weigh the benefits of artificial devices as viable alternatives for the conventional heart transplant within the different aspects of treatment for end stage heart failure.

INTRODUCTION

The body requires that all its organs and supporting systems work symbiotically to achieve its optimum level of health and wellness. Failure of any organ can have an effect on the entire system. When an organ such as the heart fails, all other bodily functions suffer. Given the role of the heart in oxygenation and nutrition, it is quite obvious why heart failure leads to decreased quality of life and eventual death. It is, thus, imperative that every effort be made to take care of this very necessary organ and to try to preserve its form and function for as long as possible.

Heart disease is rapidly becoming one of the top killers of American men and women. Poor dietary habits and lack of exercise tend to lead to high cholesterol and buildup of plaque in the arteries, paving the way toward heart disease. Statistics show that every 30 seconds, someone dies of heart failure (Debakey 2000). Such staggering numbers beg a question. Why can these patients not be saved? The answer is that those who are dying have generally entered advanced stage IV heart failure. Once this stage has been reached, there is little a doctor can do aside for transplanting a new living heart. It is for this reason that research into the creation of a feasible, functional artificial heart is so crucial.

Conventional heart transplantation is still considered the gold standard in treatment for end stage heart failure due to its associated successes and the overall improvement in the quality of life experienced by the recipients (Jovic 2011). Studies show that the survival rate for transplant recipients is 86% in the first year and 64% after 5 years (Jarvik 2011). Therefore, before analyzing the effectiveness of artificial devices as a replacement for conventional heart transplant, a complete understanding of the conventional transplantation procedure as well as its advantages and disadvantages must be gained.

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CONVENTIONAL HEART TRANSPLANTS

The conventional heart transplant involves surgically removing the damaged or diseased heart and replacing it with a healthy donor heart. Although simple in theory, this procedure is difficult to execute due to the scarcity of donor hearts as well as the high cost associated with it. In order for a heart to qualify for donation, it must meet extremely stringent requirements. The potential donor must be one who has been declared brain dead but still remains on life support and satisfies established criteria in regard to age, medical condition, cause of death, and psychosocial history (Massad 2004). Additionally, the donor heart must match the blood and tissue types of the recipient almost perfectly in order to prevent rejection. Furthermore, once the organ is removed from the donor, it is packed in ice in order to preserve it, leaving the surgeon with a maximum of four to six hours to implant it into the recipient. Thus, it is crucial that the recipient be ready for surgery at a moment's notice. The convergence of all these prerequisite conditions is quite rare to the extent that in 2008 there were 3,500 heart transplants performed worldwide, while 800,000 patients were in stage IV heart failure waiting for donors (Korfer 2007).

The procedure of replacing a failing heart with a donor heart is complicated and invasive. Time, technique, and skill are of the essence. Once the donor heart is identified and allocated to a recipient based on certain criteria, the timetable from removal to implantation is kept to a minimum. At the time of procurement, the healthy donor heart is arrested by injecting into it two liters of potassium and covered with ice before removal. Once it is excised from the mediastinum, the heart is inspected for any septal defects or abnormalities. After determining that no defects are present, the heart is then placed in a sterile bag with slush and saline and transported to the transplant center. Upon the heart's arrival at the transplant center, the recipient is placed on pulmonary bypass, and a cardioectomy is performed using the Lower and Shumway technique to remove the native heart and attach the new heart to the native left and right atrial cuffs (Massad 2004).

Even if all the details of the transplant surgery are executed successfully, the transplant of a living heart remains fraught with many dangers and difficulties. The threat of contracting graft versus host disease is constant. Therefore, the patient is placed on immunosuppressive drug therapy to suppress the production and activity of T-cells which would attack the foreign organ (Jovic 2011). Consequently, routine endomyocardial biopsies must be conducted in order to ensure that the medication is effective and rule out acute cellular rejection. While immunosuppressive medication diminishes the threat of graft resistance, long-term use of immunosuppressive drugs is associated with renal failure as well as thromboembolic disease (Atasever et al. 2006). Additional complications experienced in postoperative patients include cardiac failure, pulmonary and systemic hypertension, and opportunistic infections such as pneumonia (Jovic 2011). In a study of 34 post-transplant patients, conducted by the Ege School of Medicine, researchers found that nearly one third of heart transplant patients die of pulmonary complications. In this particular study, two patients died of cardiac failure in the early postoperative period, and another 10 patients developed severe pulmonary complications, most commonly in the form of pneumonia (Atasever et al. 2006).

ETHICAL ISSUES DUE TO SCARCITY OF DONOR HEARTS

Due to the stringency of the criteria that a donor heart must meet in order to be suitable for transplant, donor hearts remain a rare commodity. Due to issues of supply and demand, ethical questions arise as to whether age, physical illness, mental capacity, or value to society should be considered in evaluating a potential transplant recipient (Jarvik 2011). If any of these

factors are taken into account, there is an inherent selection bias that is present. Furthermore, it leads into other ethical quagmires, such as determining whose life is more valuable. Therefore, there are strict guidelines set by the National Health and Medical Research Council regarding the criteria and procedures that must be adhered to in this grey area. The Australian National Health and Medical Research Council put forth a set of guidelines by stating that "there should be no discrimination between potential recipients on the basis of… race, nationality, religious belief, gender, marital status, sexual orientation, social status, disability or age, except where conditions associated with the persons age directly determine the likelihood of a poorer outcome" (Macdonald 2008). Yet, many believe that these factors should be taken into consideration when screening potential recipients. They are, therefore, fighting to further narrow the spectrum of eligible recipients.

Although conventional heart transplants are the preferred cure for advanced heart failure, there are many disadvantages associated with it, mainly high cost, scarcity of donor organ, and postoperative complications. Therefore, research into an alternative artificial device is crucial.

ARTIFICIAL HEART IMPLANTATION

Mechanical circulatory devices have the potential to eliminate the problems that plague conventional heart transplants and are changing the face of heart failure therapy (Frazier et al. 2009). Because they can be mass-produced in a factory, the scarcity of donor hearts becomes a non-issue, and their cost is lower. To date, approximately 15,000 artificial devices have been implanted worldwide, and the numbers are projected to increase (Copeland 2011).

DEVELOPMENT OF ARTIFICIAL DEVICES

As early as 1960, researchers embarked on the road to find a permanent solution to the heart problem. They simultaneously pursued a technique for heart transplantation as well as a proposal for an implantable mechanical circulatory device that would replace the failing organ. Research into artificial devices focused on two types of pulsatile devices, one to replace and the other to aid the failing heart. One device was the total artificial heart (TAH), which would completely replace the failing heart, and the second device researched was the ventricular assist device (VAD), which would assist the failing heart in blood circulation. Both devices would be pulsatile and attached to an internal and external battery source to propel the motor. Additionally, they would have sensors to monitor and adjust the pressure at which the device pumps blood in order to allow for a natural beat (Morlacchi and Nelson 2011) (See Figure 1).

Figure 1: Cardiowest TAH.
Source: Platis and Larson 2009

PULSATILE TOTAL ARTIFICIAL HEART

The total artificial heart was designed to replace and mimic the natural heart, with one component pumping deoxygenated blood from the heart to the lungs via the pulmonary artery and a second component pumping the oxygenated blood from the lungs to the heart via the pulmonary vein. The mechanical valves provide unidirectional flow and work complementary to the diaphragm during the cardiac cycle to eject the blood of the right and left ventricles. An attached external drive console provides controllers that give the device the ability to adjust the heart rate, systolic pressure, and vacuum for the ventricle. Devices like these, such as the Cardiowest temporary Total Artificial Heart, could produce a cardiac blood output of more than nine liters per minute and allow normal metabolic functions to continue. However, the earliest versions of these devices did not function efficiently, causing many complications, such as clot formation, bleeding, hypertension, and death due to failure of mechanical parts (Platis and Larson 2009). Two major technical problems associated with these devices were the lack of appropriate materials that would not be rejected by the body and the large size of the device, making it poorly suited for long-term use. The device, with two components surgically implanted into the chest and another two into the stomach, was extremely large and heavy, requiring a lot of space to be implanted into the body (Morlacchi and Nelson 2011). Additionally, other components, such as the large external power console located exterior to the body, confined patients to the hospital and inhibited improvement in their quality of life. Therefore, this model was phased out of use.

LEFT VENTRICULAR ASSIST DEVICES

Due to the failures associated with the total artificial heart, researchers turned their focus to the smaller ventricular assist device in the 1980s. The device is most commonly referred to as left ventricular assist device (LVAD) because the majority of the pumping work of the heart is done by the left ventricle. The objective of this device was to act as an alternative to organ transplant and aid the heart in its pumping of the blood and regulation of the heartbeat. This was accomplished through implantation of the device near or within the heart, with a direct connection to the heart, causing a complete bypass of blood flow to the left ventricle. This technique allowed the blood to flow through the pump, eliminating the symptoms caused by ventricular failure, thereby providing complete support of the circulation of blood within the patient's body (Morlacchi and Nelson 2011).

Because the left ventricular assist device is less technically demanding, researches and developers have been able to achieve quick advancement in a relatively short amount of time. There have been major improvements in its size, durability, simplicity, and ease of implantation within the last 20 years. A very significant improvement in the device has been the implementation of self-contained mechanisms that have eliminated the need for an external driver (Morshuis et al. 2010). The small size of the device allows for the pumps as well as the additional components to be implanted into the body. Additionally, due to its small size, the implantation procedure does not require a complete opening of the chest, reducing the amount of recovery time following surgery (Morlacchi and Nelson 2011). Moreover, the introduction of batteries as a rechargeable external power source allowed patients to have a small external battery and controller attached to them, thereby promoting mobility and allowing patients to return home, improving their quality of life.

The design of the left ventricular assist device has evolved from the first-generation pulsatile volume displacement pump to the more commonly used second-generation continuous flow pump. The pulsatile volume displacement pump was designed to simulate the natural

heartbeat during the circulation of blood from systemic circulation to pulmonary circulation and vise versa. It mimicked the natural pulsing action of the heart with its own pulsatile action, sucking the blood from the left ventricle into the pump and then forcing it out into the aorta. It consisted of inflow and outflow valves as well as a diaphragm to exert pressure on the blood, causing a natural pulse. The typical stroke volume associated with these pumps was 50-70ml, with the afterload of the device depending on the amount of blood that filled the device during the preload period (Copeland 2011).

The more recent continuous flow device (see Figure 2) uses either an axial flow pump or a centrifugal pump to provide pulseless circulation of blood throughout the body. Both pumps use rapidly spinning rotors to enable continuous blood flow. The axial pump design includes a magnetically suspended motor which rotates the axial pump without any physical contact. In this design, the rotor is the sole moving part, and the additional components of the motor, the two stators, are "passively stable" and are "controlled according to the rotors position so as to levitate the rotor" (Okada et al. 2003). These motors can spin up to 8000-10000 rpm, thereby facilitating the blood in reaching all the necessary destinations in systemic circulation (Copeland 2011).

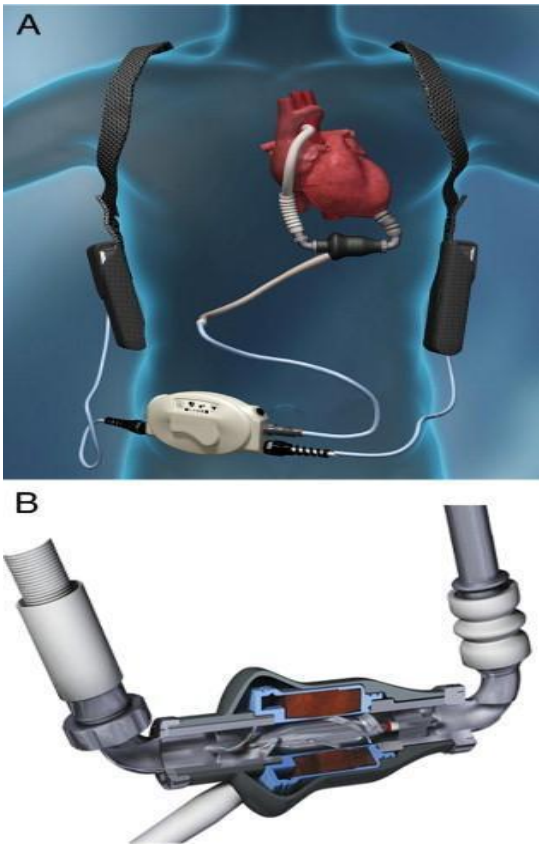

Figure 2: Components of the Continuous-Flow LVAD. Source: Pagani et al. 2009
(A) The inflow cannula is inserted into the apex of the left ventricle, and the outflow cannula is anastomosed to the ascending aorta. Blood exits through the left ventricular apex and into the left ventricular assist device (LVAD), which pumps throughout cardiac diastole and systole into the ascending aorta.
(B) The LVAD pump is placed within the abdominal wall or peritoneal cavity. A percutaneous lead carries the electrical cable to an electronic controller and battery packs, which are worn on a belt and shoulder holster, respectively.

The centrifugal pump design is very similar to the axial pump with exception to its size. It is somewhat larger than its axial pump counterpart, but is more energy efficient. It has a circular shape and uses a rotating impeller, which can have numerous blades, to create flow. The average speed of the motor is 2000 rpm, generating an output of eight liters per minute, and the hydraulic flow is from the left side while the inflow is from the right. A negative side effect associated with both of these models is the formation of clots around the bearings of the motor that are lubricated by the blood (Anderson et al. 2000).

Researchers used the success of the continuous flow left ventricular assist device as a blueprint for a model of a new artificial heart that would completely replace the native heart with the implantation of two continuous flow devices working in tandem. This device is an improvement of the original total artificial heart in size, reliability, function, and energy efficiency. The continuous flow total artificial heart (CFTAH) consists of two rotary pumps which control the systemic and pulmonary circulation. Instead of mimicking the natural heartbeat by pumping the blood, they allow for the continuous flow of blood through the body using the mechanisms discussed above. Since little is known about the physiological effects of pulseless circulation of blood, it is currently undergoing phase II animal trials (Frazier et al. 2009).

DISADVANTAGES AND ADVERSE SIDE EFFECTS

The disadvantages and complications associated with the artificial models are numerous and include the biomaterial used to make the device, mechanical failures, and blood clotting. The procurement of a suitable biomaterial with which to make the artificial device has proven difficult, because antibodies in the blood tend to react with almost all manmade substances, causing infection. Therefore, developers are currently coating the internal and external surfaces of the device with polyurethane, which has demonstrated compatibility, to reduce the potential for infection. Additionally, researchers at the Milwaukee Heart Institute are attempting a method of lining the device with the patient's own endothelial cells. This is done by removing cells from the inner lining of the stomach through a procedure similar to liposuction, culturing them in a lab, and subsequently growing them on the device (Cecchin and Pfeiffer 1993).

Researchers are addressing the side effect of thrombi formation around the movable parts of the motors resulting in thromboembolism (stroke). The clotting is a result of the minimal clearance space between the device and the myocardial tissue, which interferes with the blood flow patterns. In order to prevent this, there is a need to improve the model of the motor by providing additional clearance space or with a mechanism to prevent clotting (Anderson et al 2000). Currently, patients with implanted left ventricular assist devices are prescribed long-term antithrombotic therapy in order to prevent the formation of clots (Pagani et al 2009).

Additional risk associated with implantation of mechanical devices is death due to failure of mechanical parts. Although the device is small in size, there are many mechanisms involved in the delivery of energy to the motor which powers the pump. If any of the components fail, there is a narrow window of time during which the patient can survive without replacing the component. Therefore, patients are given additional battery packs to prevent death due to failure of an external component. Nevertheless, if there is a glitch in any of the internal components or in case of a power failure, there is little that can be done for the patient at that point. Therefore, these mechanisms need continuous improvement in their reliability and durability to prevent such failures from occurring.

EXPERIMENTATION AND STUDIES

There are various models for artificial devices to replace or aid a failing heart. Therefore, experimental testing is needed to determine which designs are effective and what improvements must be made to ensure minimal adverse side effects. Once the development phase of the device is complete, there are various stages in the rigorous experimental process. Experimentation begins with phase I testing (i.e. in vitro), during which the device is placed in a simulated human body environment with a mock circulatory system in place (Morlacchi and Nelson 2011). The device is then tested in regard to implantation techniques, size, mechanical operation of parts, motor efficiency, battery life, durability, reliability, and adaptation to various stimuli. According

to the successes or failures of the various aspects of the device, certain modifications are made in the model.

Once phase I is complete, barring any complications, the researchers move forward to phase II testing, in which they implant the device in an animal with a failing heart and monitor the various improvements as well as complications that arise in the operative and postoperative stages. If the device passes animal trial, it proceeds to clinical trials and is usually implanted in patients who are not eligible for cardiac transplant. The clinical trials are primarily for testing of surgical implantation technique, effectiveness and durability of the devices, as well as any complications that may arise due to the device.

Jack G. Copeland and colleagues conducted a clinical trial to provide comprehensive analysis as to the efficacy of the CardioWest temporary total artificial heart, a pulsatile total artificial heart. The patients in the protocol group were in acute heart failure and at high risk of imminent death. These patients did not meet criteria for left ventricular assist devices for different reasons, and their last resort was to receive the total artificial heart while waiting for a heart transplant. In this particular study, 79% of the protocol group survived to receive a heart transplant versus 49% of the control group. Furthermore, immediately after implantation, the protocol group's mean arterial pressure rose and their mean venous pressure dropped significantly, showing the effectiveness of the device. Although the results of the analysis look promising, there were many ensuing adverse effects associated with the implantation of the total artificial heart, including loss of blood, device malfunction, the large size of the device (limiting the population size which can receive the implant only to those with enough space in their mediastinum), and death (Copeland et al. 2004). These results indicate that the total artificial heart is not the ideal artificial device to be used as a replacement for a failing heart.

Clinical trials for the HeartMate continuous flow left ventricular assist device (see Figure 3) began in 2003. The trials consisted of 18 months of follow up data on 281 patients who, in urgent need of heart transplant, underwent the implantation of the HeartMate left ventricular assist devices. The trial results showed that patients exhibited greater survival rates, less frequent adverse events, and improved reliability with continuous flow left ventricular assist devices as compared to pulsatile flow devices. At 18 months, of the 281 patients in the trial, 157 had meanwhile received heart transplants, 58 were living mobile lives with left ventricular assist devices in their bodies, seven had their left ventricular assist devices removed due to recovery of their hearts, 56 had died, and three had been removed from the trial after exchanging their devices for another type of device. The results demonstrated that heart function had significantly improved after six months of LVAD support as compared to the pre-LVAD baseline. Although the results seem promising, there were many side effects associated with this device, including sepsis, stroke, multi-organ failure, bleeding, and device malfunction. Therefore, additional improvement is called for in order for this model to be considered for the vast majority of end stage heart failure patients (Pagani et al. 2009).

Figure3: HeartMate II Left Ventricular Assist System. Source: FDA 2009

Phase II trials conducted by the Texas Heart Institute tested the physiological effects of the continuous flow total artificial heart implanted in a calf. The findings were significant. On postoperative day one, the calf was standing for several hours at a time, and by day three the calf was eating a full diet and had been weaned off the ventilator. Throughout the study, the calf gained six pounds and was able to recognize its keepers and sleep at regular intervals. The pumps operated without mechanical problem over the course of the study. The most promising success the calf had was running on the treadmill for 40 minutes with no sign of exhaustion. The left and right pumps increased in response to increased oxygen need without any external adjustments, and the calf's breathing remained normal. The calf demonstrated overall improved quality of life and overall improvement of metabolic functions. While this study had promising results, the calf was euthanized on day 48 due to a glitch in the left axial pump. Although the results of the study were promising to the extent of the device's success, the fact remains that the calf only survived 48 days, demonstrating that the device is a long way from being considered a viable replacement for the heart (Frazier et al. 2009).

CONCLUSION

Based on numerous studies that have been conducted, it is evident that the development of an artificial device that will completely replace the failing heart is still in its early stages. The various problems associated with the device prevent it from being considered a viable long-term solution for replacement of a failing heart. Although there is no current long-term solution for the problems associated with conventional heart transplants, the future for patients in end stage heart failure looks bright. Over the next few decades, researchers hope to perfect the model of the total artificial heart to the extent that it will be a long-term solution for those in need of a new heart. Additionally, successes associated with left ventricular assist device implantation as a temporary bridge, aiding the failing heart until a real heart is available for transplant, are allowing for more devices to receive FDA approval, subsequently leading to an increased number of implantations. Therefore, these devices should be considered while weighing the options for a patient diagnosed with advanced stage heart failure.

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CURRENT THEORIES ON THE HUMAN SEX RATIO

Yisroel Cofsky

ABSTRACT

The human sex ratio is skewed toward males, and this is the subject of much research. A review of some of the theories currently available was conducted, searching and analyzing the current scientific literature. Suggestions to explain the ratio include unequal proportions of X and Y sperm, the effect of maternal diet, maternal testosterone levels, and the natural fluctuations in the consistency of the cervical mucus. Although the difference in X/Y sperm proportions does not adequately explain all the data, the other theories do seem to explain the greater percentage of male births. However, there is still not enough information available to verify which theory is the most plausible.

INTRODUCTION

When asked what percentage of births is male and what percentage is female, most people will answer that it is 50-50%. However, the human sex ratio, or ratio of males to females, is not even; rather more males are born than females. The actual ratio of males to females is approximately 1.05:1 (Mathews and Hamilton 2005). This is against convention, and possible causes have been suggested. Understanding the causes may be of practical use to couples seeking to select the gender of their children. There is great interest among many couples to have a child of a specific gender, often for family balancing among couples who already have a few children of one gender (Belkin 1999). Understanding the natural causes for selection of gender would be of great benefit to such couples. If a cause for the higher ratio of males can be determined, it can be used to select for males, or avoided to select for females. However, the current theories do not offer a practical and guaranteed method for selecting gender.

DOCUMENTATION OF RATIO

The sex ratio has been well documented, initially using census data (Table 1). In the United States, the ratio for male births from 1943 to 2002 is reported as 105 (convention in reporting ratio gives the figure for males in respect to 100 females). There are variations based on race, ethnicity, and maternal age (Tables 2 and 3). Whites seem to have a male birth ratio around 105, and among African Americans, the ratio is around 103 (Mathews and Hamilton 2005). Among Americans of Asian descent, the ratio is around 107. Variation in the ratio is also seen in different countries. In fact, in some countries, a ratio as high as 107 has been observed, while other countries have reported lower ratios (Grech et al. 2002). It is important to note that in some countries, such as China and India, the sex ratio is extremely high (113 and 109, respectively). However, these high numbers are mainly a result of artificial factors, not natural ones. These countries have a culture that favors males, resulting in sex-selective abortion and infanticide. This paper will focus on possible causes for the natural sex ratio observed in countries that do not have a culture of sex-selection practices.

Year	2002	2001	2000	1999	1998	1997	1996	1995	1994
Males	2,057,979	2,057,922	2,076,969	2,026,854	2,016,205	1,985,596	1,990,480	1,996,355	2,022,589
Females	1,963,747	1,968,011	1,981,845	1,932,563	1,925,348	1,895,298	1,901,014	1,903,234	1,930,178
Ratio	104.8	104.6	104.8	104.9	104.7	104.8	104.7	104.9	104.8

Table 1: Number of male and female births and sex ratio at birth, in the United States, 1994-2002. Source: Mathews and Hamilton 2005

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White	Black	Japanese	Hawaiian	Chinese	Filipino	Mexican	Non-Hispanic white	Non-Hispanic black
105	103.2	108.9	107.5	107	107	103.8	105.4	103.2

Table 2: Sex ratio at birth by race and origin of mother, in the United States, 2002. Source: Mathews and Hamilton 2005

under 15	15-19	20-24	25-29	30-34	35-39	40-44	Over 45
105.1	105.2	104.7	105	104.9	104.2	103.7	109.7

Table 3: Sex ratio at birth by age of mother, in the United States, 2002. Source: Mathews and Hamilton 2005

SECONDARY AND PRIMARY RATIOS

The observed ratio, known as the secondary sex ratio, reflects the ratio of males to females at birth. Initially, it was suggested that the primary sex ratio, or ratio at conception, is equal, and only the secondary ratio is skewed due to a higher rate of miscarriages among female fetuses. The female was believed to be weaker, resulting in a higher ratio of live male births. However, when studying fetuses from miscarriages, researchers found the opposite to be true. They found that a greater percentage of males than females were spontaneously aborted (Klug et al. 2009). These findings suggested that the primary ratio was even higher than the observed secondary ratio. The data suggested that the primary ratio at conception was at least 120, and some researchers even suggest 160. These numbers demonstrate a great selectivity for males at conception, although the subsequent reduction in the ratio from 120 at conception to 105 at birth reflects a weakness among male embryos. This weakness may be partially explained as an immune reaction against the male fetus. Research on pregnant mothers diagnosed with toxemia, a condition among pregnant women that causes severe hypertension and risk of stroke, shows a greater proportion of toxemia among women carrying male fetuses (Toivanen and Hirvonen 1970). This suggests that toxemia, suspected to be an autoimmune reaction, is more sensitive to triggers in the male embryo than in the female embryo, resulting in more male embryos being spontaneously aborted than female embryos. This may be a possible explanation for the sharp decrease from the primary sex ratio to the secondary ratio.

However, the initial ratio, with greater rates of conception of males, is somewhat of a biological mystery. In humans, like all mammals, sex is determined by the sex chromosomes, the X and Y chromosomes. Females carry an XX chromosome, and males carry an XY chromosome. Since all females have XX, the haploid ovum will only contain an X chromosome, and the sex of an embryo will be decided by the sex chromosome contained in the sperm that fertilizes the ovum. If the sperm cell contains a Y chromosome, the resulting embryo will be male (XY), and if it contains an X chromosome, the embryo will be female (XX). Spermatogenesis, the formation and maturation of sperm, is a result of meiosis. Through meiosis,

one germ cell divides into four haploid gametes. This should result in an equal number of X-bearing and Y-bearing sperm, as the germ cell containing one X and one Y chromosome should be divided equally. If X and Y sperm rates are equal, no noticeable difference in the sex ratio should be observed, as the odds of fertilization by an X-bearing sperm should be the same as that of a Y-bearing sperm.

UNEQUAL SPERM RATIOS

There are those who argue that sperm are not equally divided into X and Y sperm. This would have to be a result of some factor in spermatogenesis resulting in higher rates of maturation for Y-bearing spermatozoa than X-bearing spermatozoa. Selective elimination of X-bearing sperm would result in an increase in the ratio of Y sperm, explaining the higher ratio of male births. However, there does not seem to be any clear mechanism explaining the selective elimination or failure of X-sperm maturation.

Some studies suggest that environmental factors may influence the ratio of X and Y bearing sperm, selectively eliminating X-bearing sperm. One study has linked the exposure of POPs (persistent organohalogen pollutants) to an increase in the ratio of Y-sperm to X-sperm in males. POPs include pollutants such as DDT (dichlorodiphenyltrichloroethane) and PCBs (polychlorinated biphenyls). Studies by Tiido et al. (2006) seem to show a positive association between the ratio of Y-bearing sperm and serum concentration of PCB-153. Swedish and Greenlandic men had high levels of PCB, and their sperm had a higher proportion (51.2%) of Y-sperm than expected. On the other hand, Polish men, who did not have elevated serum levels of PCB-153, had almost equal sperm distribution (50.3% Y and 49.7% X). These numbers suggest that environmental factors may play a role in the maturation of X and Y sperm, and may, in fact, be responsible for the observed sex ratio.

However, this theory does not adequately explain the sex ratio. If uneven X and Y sperm distribution is behind the greater proportion of live male births, then countries with low POP exposure (and, thus, even sperm distribution) would be expected to correlate with an even ratio of live births. This is clearly not the case. For example, in Poland, where males have been found to produce sperm with an even distribution of X and Y sperm, the sex ratio is still skewed towards males, with a reported ratio of 106 (Grech et al. 2002). Furthermore, equal proportions of X and Y sperm were found in males who had fathered three or more children of the same gender (Irving et al. 1999). The study, trying to explain the apparent altered sex ratio observed in these families, fails to find a correlation between X/Y sperm proportion and gender of the offspring. Men bearing only sons were found to have an almost equal amount of Y-sperm (49.7%) as X-sperm, and the same results were found among those who fathered only females. These findings, as well as the lack of correlation between observed sperm distribution and the actual sex ratio in counties like Poland, suggest that the factors causing the sex ratio are of maternal origin, not paternal origin. Maternal influences would likely play a role in increasing the odds of fertilization by Y-sperm, resulting in a higher ratio of males.

NUTRITION AS A POSSIBLE CAUSE

Some studies link the skewed ratio to maternal nutrition. Noorlander et al. (2010), studying Danish women, compared blood sodium (Na^+) and calcium (Ca^{2+}) levels with the ratio of males and females at birth. Noorlander et al. hypothesized that higher blood mineral levels result in a higher ratio of females, and that the observed sex ratio, with higher male ratios, is a result of low Na^+ and Ca^{2+} levels. To test the hypothesis, women who were planning on becoming pregnant were placed on a high calcium diet, and their serum Na^+ and Ca^{2+} levels were monitored for at least nine weeks prior to planned conception. The researchers used a

prediction rule to satisfy their requirements: women with Na^+ values of at least 140 mmol/L and an increase in Ca^{2+} levels by at least 0.1 mmol/L were predicted to conceive a female. The results showed that 73% of those who had satisfied the prediction rule had females, much higher than the expected 50%, suggesting a positive association between mineral levels and female conception. This would suggest that the overall sex ratio, with higher proportion of males, is an effect of low mineral intake. The role of Ca^{2+} in fertilization has been studied and found to play a crucial role in both capacitation of sperm and activation of metabolism in a fertilized ovum. However, it is not clear if it would affect X and Y sperm differently, and no mechanism for the link between Ca^{2+} levels and sex selection has been proposed.

MATERNAL DOMINANCE HYPOTHESIS

The maternal dominance hypothesis is the result of studies suggesting that women with more dominant personalities are more likely to conceive males. According to Valerie J. Grant (1996), a dominant personality is defined as authoritative or influential and is measured by personality tests. Although a theory based on difficult to measure personality traits may be considered suspect, this claim is based on a biological theory. It is postulated that the dominant personality is a result of higher testosterone levels, and this has an influence on the gender that is conceived. Grant suggests this to be responsible for the skewed sex ratio, as the maternal influence results in more male conceptions.

The elevated testosterone level that is the subject of this theory is not serum level; rather it is the level in the follicular fluid. Follicular fluid is secreted by granulosa cells in the secondary follicle surrounding a developing oocyte, and it contains steroids—both estradiol and testosterone—in concentrations that may be 40,000 times that of serum concentrations. The theory proposes that these higher testosterone levels influence the selection of sperm, causing greater rates of capacitance among Y-bearing sperm. However, studies on the effects of follicular fluid on sperm counts show that it does not result in a higher proportion of Y-sperm than in samples incubated in standard growth media (Bahmanpour et al. 2009). It is, therefore, difficult to claim an effect on the sperm itself by follicular testosterone. It is possible, however, that the effect is not on the incoming sperm; rather the effect is on the receptiveness of ova to X or Y sperm. It is thought that during the development of the zona pellucida, the glycoprotein layer that surrounds the ovum, its molecular composition may be somewhat influenced by the high testosterone levels. As a result of these subtle changes, the zona pellucida would be more receptive to a Y-bearing sperm, resulting in a higher ratio of male conceptions (Grant and Chamley 2010). It is important to note, though, that a difference in receptiveness to X or Y sperm would involve a biochemical difference between the two. To date, the only difference that has been found is morphological, not chemical, and it is not known if such a difference is sufficient to play a role in zona pellucida selectivity.

TIMING OF OVULATION

Another theory that suggests maternal cause of the skewed sex ratio is the effect of the timing of ovulation on the gender that is conceived. An often-cited study by Susan Harlap (1979) shows that the timing of coitus in respect to ovulation seems to influence the sex ratio. Other studies agree, their data showing a U-shaped curve, with coitus closer to ovulation reflecting an equalization of the sex ratio, and coitus farther away (both before and after ovulation) skewing the ratio (Gray et al. 1998). Coitus more than two days before or after ovulation resulted in males in more than 60% of conceptions, and inseminations closer to ovulation had a more even ratio of males to females, with some studies showing only 49.3% male for coitus within one day of ovulation. The overall result would be a higher ratio of male conceptions, as the even ratio at

time of ovulation is skewed towards males when the total number of conceptions, including those farther away from ovulation, is included.

It is tempting to explain this with the adage that Y sperm swim faster than X sperm, a result of the smaller mass of the Y-sperm. If ovulation would precede coitus, then the ovum is already in the oviduct, and the faster swimming Y sperm would be expected to fertilize the egg, resulting in a greater ratio of males. This is clearly not satisfactory, as the sex ratio of conceptions arising from coitus within one day of ovulation should display the same effect, as the faster swimming Y-sperm should have greater fertilization rates. Furthermore, coitus performed before ovulation should not result in a greater proportion of males, and this is not the case. In addition, it is now believed that faster swimming sperm do not have greater odds at fertilizing the ovum. The current hypothesis is that sperm have a window of competence for a brief time, and the timing and length of this capacitance depends on many factors, including location in the reproductive tract. In addition, sperm do not have the ability to undergo the acrosome reaction necessary for fertilization until they have spent some time in the environment provided by the female reproductive tract (Gilbert 2006). If the sperm that first arrive at the ampulla of the oviduct are not in the narrow time frame of capacitance, they will not be able to fertilize the ovum.

EFFECT OF CERVICAL MUCUS

A more plausible explanation for the correlation between timing of ovulation and the gender of the conceived embryo is the effect of the cervical mucus on sperm penetration. The cervical mucus, produced by secretory cells in the cervix, consists of glycoproteins and lipids, mixed with water. It acts as a plug that physically impedes the entry of foreign material (including sperm) into the cervix. The consistency and pH of the cervical mucus change during the ovulatory cycle, and these change appear to be affected by hormones, with estrogen and progesterone have opposing effects on the mucus. Prior to ovulation, the cervical mucus is viscous, and sperm penetration is poor. Close to ovulation, the mucus changes. The mucus becomes more alkaline (pH 8.5) and less viscous, resulting in the formation of channels through the mucus. This appears to be a result of rising estrogen levels. At ovulation, the mucus is very porous and watery, and sperm penetration is high. After ovulation, in response to the secretion of progesterone, the viscosity of the mucus increases, and the channels begin to close up (Linford 1974).

Y-bearing sperm have different morphology than X-bearing sperm. As the Y chromosome contains less genetic material, the Y-sperm contains less chromatin than the X chromosome (about 3% by mass), resulting in less dense sperm. This affects the shape of the sperm head of the Y-sperm compared with that of the X-sperm. The difference in shape has been observed to be a more streamlined Y-sperm, with a smaller width-to-length ratio than the X-sperm (McCoshen et al. 1994).

It has been postulated that the streamlined Y-sperm, with narrower heads, would pass through the channels in cervical mucus more easily than X-sperm. This difference in penetration would be more pronounced farther away from ovulation as the viscosity of the mucus begins to decrease. At that point, there would be channels in the mucus wide enough to allow passage of Y-sperm but not X-sperm, and Y-sperm penetration would be expected to occur at a greater rate than X-sperm penetration. More Y-sperm would enter the oviduct, making fertilization of a male more probable, effectively skewing the ratio in favor of males. However, this difference in morphology between X and Y sperm would play less of a role closer to ovulation, as the channels would be open wide enough to allow penetration of X and Y sperm at equal rates. At

that point, both X and Y-sperm would be present in the oviduct in equal numbers, and fertilization of a male would have the same probability as fertilization of a female. Consequently, an equal ratio of male to female conceptions would be expected for conceptions resulting from coitus on the day of ovulation (Martin 1997). (It is worth noting that when coitus is the same day as ovulation, conception rates for a male is slightly lower than the expected 50%. The observed 49.3% ratio might be explained by the greater preponderance of male fetuses to spontaneously abort, raising the possibility that when odds of fertilization are equal for both X and Y sperm, less male pregnancies will be detected due to an almost immediate termination of a small percentage of male pregnancies.)

This has been supported by studies on X and Y sperm penetration through cervical mucus samples. In one such study, samples of cervical mucus were obtained from women at different points in the ovulatory cycle. The samples were mixed with semen samples, allowing the sperm to swim up through the mucus. The samples were then studied, and a difference was noted in the different samples. In the samples that were taken earlier in the ovulatory cycle, the observed proportion of Y-sperm at the far end of the mucus samples was higher (70%) then the proportion of X-sperm. In mucus samples taken at ovulation, the ratio of X and Y sperm was about equal (Rohde et al. 1973). This is because mucus plays a role in selectively rejecting X-sperm, but only in mucus that is not at the stage of ovulation. This suggests that changes in viscosity and consistency of the cervical mucus play a role in the skewing of the sex ratio.

Further evidence for this theory is seen in the change of the sex ratio in conceptions following administration of clomiphene citrate. The observed sex ratio after clomiphene citrate administration is much lower than normal, with a larger number of females being born (Silverman et al. 2002). Clomiphene is used to treat female infertility through induction of ovulation. It is a selective estrogen receptor modulator which blocks estrogen receptors in the hypothalamus, effectively turning off the estrogen negative feedback loop. This results in rising estrogen levels, which will then cause the LH (luteinizing hormone) surge that triggers ovulation. The ovulation being induced has been ruled out as a possible cause of the lowered sex ratio, for the same effect on the ratio is not observed when ovulation is induced via other agents. The clomiphene citrate itself is, therefore, the most probable cause for the change in ratio. This phenomenon can best be explained with the cervical mucus theory. As estrogen affects the cervical mucus consistency, clomiphene citrate can be expected to cause the mucus to become less viscous, similar to mucus at the time of ovulation. This would have the effect of allowing the larger X-bearing sperm equal penetration of the cervical mucus, resulting in lowering of the ratio.

CONCLUSION

The skewed sex ratio, with a greater proportion of both male conceptions and male births, is the subject of various theories. The theory of uneven distribution of X and Y sperm fails to offer a satisfactory explanation for the observed ratio of live births. The theories of maternal nutrition and follicular testosterone levels fail to offer a mechanism of action. The explanation offered by the cervical mucus theory does seem plausible and also explains the effect that timing of coitus relative to ovulation has on the sex ratio.

The sex ratio is well documented, and data show differences among race, ethnicities, and even maternal age. Perhaps this data may be used as further evidence to any of these theories. If, for example, a difference in the cervical mucus consistency is found among different age groups, this difference can then be correlated with the observed sex ratio for each group and can be used to prove the link between cervical mucus and the sex ratio. Studying serum calcium levels

among different ethnic groups and comparing them to the sex ratios of those groups would provide evidence, either for or against, the maternal nutrition theory. Similar research can be done on follicular testosterone levels among different groups to strengthen or weaken the maternal dominance hypothesis. However, no such studies appear to be available at this time.

Influencing the gender of a child to be conceived has long been a subject of interest and the subject of many scientific and non-scientific theories. For parents looking to conceive a child of a specific gender, the current research does not offer much of a practical solution. Diet may be changed to raise mineral levels, and timing of ovulation may be taken into account. However, these methods, even if responsible for the sex ratio, will only skew the odds of conceiving a specific gender by minute amounts. Although a difference in ratio will be seen when documenting hundreds of thousands of births, the slight difference in ratios will not make a significant difference for any individual couple. For couples having a medical need to conceive a child of a specific gender (as in those who are carriers of an X-linked gene), technologies such as pre-implantation diagnosis in conjunction with in-vitro fertilization are available to guarantee the gender of the child. These technologies are usually not offered to those without a medical need, due to ethical concerns, so couples looking to balance their families will often have to rely on these less reliable methods (Grady 2007).

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TARGETED REINNERVATION

Yonatan Levi Moshayev

INTRODUCTION

Imagine living a life where even the simplest of tasks such as eating a grape or holding an egg required intense concentration and months of training. Until recently, this was the harsh reality for people with upper limb prostheses. Currently, the most common upper limb prosthetic technology being used is body powered. These devices capture remaining shoulder movements with a harness and transfer this movement through a cable to operate the hand, wrist, or elbow. With this control method, only one joint can be operated at a time. When the amputee has positioned one component, he or she can activate a switch that locks that component in place, and then he or she can operate the next component (Longe 2006; Miguelez et al. 2009; Edeer and Martin 2011). Until recently, this was the only technology available for someone using an upper limb prosthesis.

Over the past few years, myoelectric prostheses have been developed. These prostheses use electromyogram (EMG) signals (the electrical signals generated during muscle contraction) from remaining upper limb muscles to control motorized arm joints. Myoelectric signals are derived from the contraction of voluntary control muscles in the residual limb and are recorded by surface electrodes implanted in a prosthetic socket. Unfortunately, myoelectric prostheses are also restricted to one function at a time because of a lack of independent control signals (Longe 2006; Miguelez et al. 2009; Edeer and Martin 2011).

Current control strategies use the electromyogram signals from one or two agonist-antagonist remaining muscles to sequentially operate each function in the prosthesis. For example, an individual with transhumeral amputation uses the biceps and triceps muscles to control the elbow, wrist, and hand. For an individual with shoulder disarticulation the pectoralis major can be used to close the hand and supraspinatus to open it. The individual would then use a chin switch to toggle through the controls so that these same two muscles operate the wrist rotator and elbow. This type of operation is not always intuitive, because the residual muscles control physiologically unrelated movements (Longe 2006; Miguelez et al. 2009; Edeer and Martin 2011).

It is estimated that by the year 2020, the number of people in need of a prosthetic limb will reach 2.4 million (Nielsen 2002). Evident that prosthetic technology needed improvement to serve a growing demand and multitudes of frustrated patients, Todd A. Kuiken (2006) developed a better upper limb prosthetic alternative using a procedure called Targeted Reinnervation. The purpose of this paper is to assess the extent in which targeted reinnervations improve prosthetic functions, facilitate its use, and restore meaningful sensory feedback in patients.

With Targeted Muscle Reinnervation (TMR), it is possible to denervate regions of muscle that are not bio-mechanically functional in or near an amputated limb and transfer residual arm peripheral nerve endings to these muscles. Over time, the transferred nerves reinnervate these muscles. The surface electromyogram signals from the newly reinnervated muscles can be used as additional control signals for an externally powered prosthesis (Kuiken 2006; Hijjawi et al. 2006).

The amazing thing about using targeted muscle reinnervation to control prosthetic limbs is that it is intuitive. The patient thinks about closing his or her hand and it closes. This is achieved because the electromyogram signals from the reinnervated muscles are used to control

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functions in the artificial arm that the motor nerves naturally controlled before amputation. The motor nerve that usually sends the signal to close the hand contracts the reinnervated muscle. This produces a surface electromyogram signal that is processed and translated by the prosthesis and causes the prosthetic hand to close (Kuiken 2006; Hijjawi et al. 2006).

Even more impressive is that a similar procedure can be done for Targeted Sensory Reinnervation (TSR). With targeted sensory reinnervation, a section of skin near or overlying the targeted muscle reinnervation site is denervated and the remaining afferent sensory nerve fibers from the residual arm are transferred to it. Over time, the transferred nerves reinnervate this skin and can potentially provide meaningful sensations for amputee patients (Kuiken 2006; Kuiken et al. 2007a; Marasco et al. 2009).

In theory, targeted sensory reinnervation can offer patients a sense of texture, temperature, and pressure of the objects they touch with their prosthesis (Kuiken et al. 2007a; Marasco et al. 2009). If a patient touched a hot cup of tea, sensors in the prosthetic hand could process information about the texture, temperature, and pressure of the cup and use that information to apply proportional stimuli to the reinnervated skin. In theory, the patient should feel as if he/she were actually touching the cup with a normal hand.

METHODS

The data used for this research paper was compiled from, case studies, review articles, and proof of concept studies found in medical and scientific journals using the Touro College database. Several medical books and online sources were also used for data.

FIRST TARGETED REINNERVATION PATIENT (M1)

The first patient to undergo targeted reinnervation was a 54-year-old male working as a high-power lineman, who experienced severe electrical burns in May 2002 that resulted in bilateral shoulder disarticulation (Kuiken 2006). He was initially fitted with an externally powered prosthesis on the left side, that was controlled using touch pads in his shoulder socket and a chin switch, and a body-powered prosthesis on the right. The patient became relatively proficient operating his new prostheses after receiving extensive operational training (Kuiken 2006; Hijjawi et al. 2006).

The left limb was chosen for the targeted reinnervation procedure because that was the side of his externally powered prosthesis. Together, the patient and his medical team decided not to alter the operation of his right body-powered prosthesis because it worked very well for him (Kuiken 2006; Hijjawi et al. 2006).

The goal of surgery was to create four new physiologically appropriate myoelectric control inputs using the four major arm nerves from the brachial plexus. The left pectoralis major and minor muscles were denervated, and the proximal ends of the native nerves were ligated and sutured up under the clavicle to prevent them from reinnervating the pectoral muscles. The musculocutaneous, median, and radial nerves were transferred on to the clavicular head, the upper sternal head, and the lower sternal head. The pectoralis minor was moved out from under the pectoralis major and over to the lateral thoracic wall to serve as a fourth donor muscle segment for the ulnar nerve. In addition, this prevented the pectoralis minor electromyogram from interfering with the myoelectric signals from the other nerve transfers. All four of the residual plexus nerves were sewn onto the distal ends of the original pectoral muscle nerve fascicles and onto the muscle itself (Figure 1) (Kuiken 2006; Hijjawi et al. 2006).

Most of the pectoralis muscle's subcutaneous fat was surgically removed to optimize surface myoelectric recordings. Once the fat was removed, the recording electrodes were as close as possible to the muscle regions with the strongest surface electromyogram signals and the least cross-talk from adjacent muscles (Kuiken 2006; Hijjawi et al. 2006).

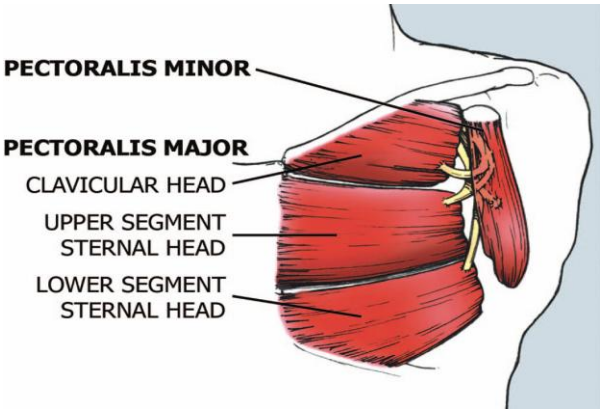


Figure 1: Diagram of first TR patient's surgical procedure. Source: Hijjawi et al. 2006

RESULTS

After five months of recovery, the patient was able to activate four different areas of his pectoralis major muscle when he tried moving his phantom hand or arm. Two distinct electromyogram signals could be identified in the mid-pectoral region where the median nerve was relocated. When the patient thought about closing his hand, a strong electromyogram signal could be identified on the lateral pectoral region. Surprisingly, when the patient tried to open his hand, an independent signal could be detected more medially. When the patient tried to flex his amputated elbow, it caused a strong contraction of the muscle just below the clavicle (Kuiken 2006; Hijjawi et al. 2006). This was consistent with musculocutaneous nerve reinnervation because the musculocutaneous naturally controls elbow flexion (Tortora and Grabowski 2003). Extension of his hand and elbow caused a substantial contraction of the lower pectoralis muscle, consistent with radial nerve reinnervation. Surprisingly, however, the transfer of the efferent ulnar nerve to the pectoralis minor was unsuccessful (Kuiken 2006; Hijjawi et al. 2006).

In addition to the motor reinnervation of the muscle, targeted sensory reinnervation occurred in the skin of the chest wall primarily over the musculocutaneous, median, and ulnar nerve transfers. When the patient's chest was touched in different places, he felt as though he was being touched on different points of his hand and arm. The patient acquired the sensation of touch, sharp/dull sensation, graded pressure sensation, and thermal sensation, all previously thought lost to him (Kuiken 2006; Kuiken et al. 2007a; Marasco et al. 2009).

He felt these sensations over an area 15 cm across × 17 cm high. The area was mapped using a cotton-tipped probe that indented the skin with 300 grams applied force (gAF) (Figure 2). He usually felt pressure in large areas of his hand when touched at a single point. These evoked sensations were localized to the palmar and dorsal aspects of his hand and forearm.

As seen in Figure 2, pressing the skin over the lateral ulnar nerve reinnervation site elicited sensations on the forearm, palm, digit four, and digit five. Pressing the skin over the superior median nerve transfer site stimulated sensations on the palm and first three digits. Similarly, pressing the skin over the inferior musculocutaneous and radial transfer sites generated sensations localized to the back of the hand and forearm. Such sensations corresponded well with the natural skin sensations provided by nerves in a normal hand (Figure 3).

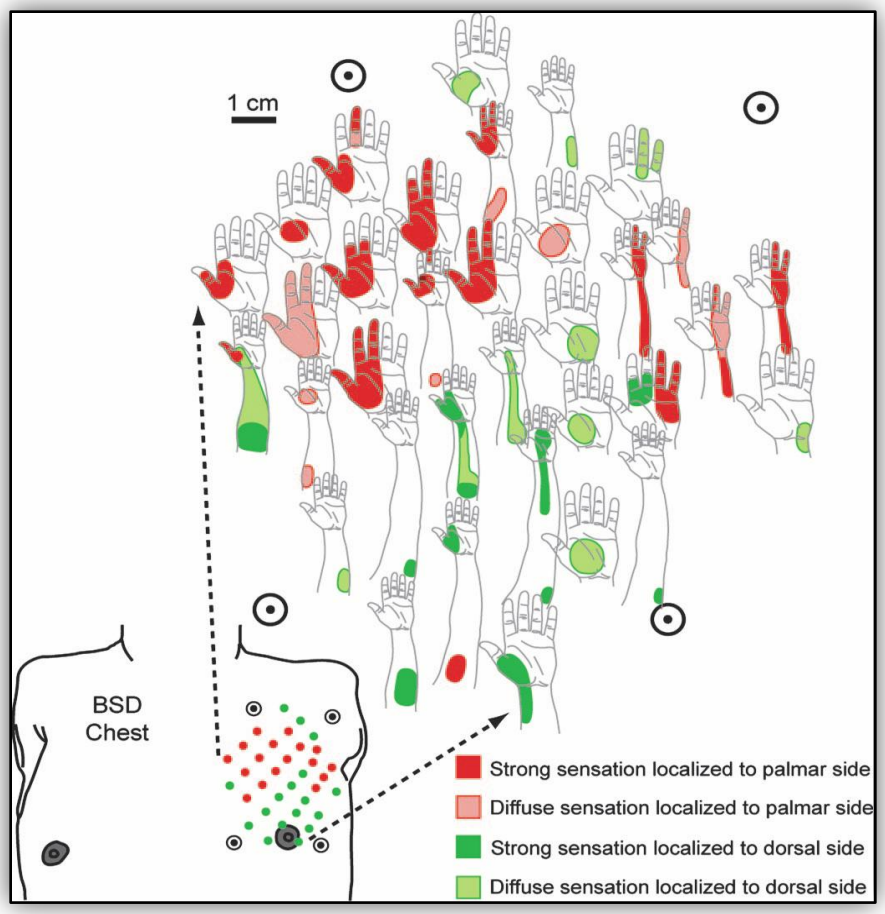


Figure 2: The reinnervated chest skin of patient M1 (BSD) showing sensations referred to the missing limb elicited by indentation of the skin by a cotton-tipped probe (300 gAF). Referred sensations localized to either the palm side (red) or the backside (green) of the missing limb. Circled points at the corners serve as registers to orient the diagram. Source: Kuiken et al. 2007a

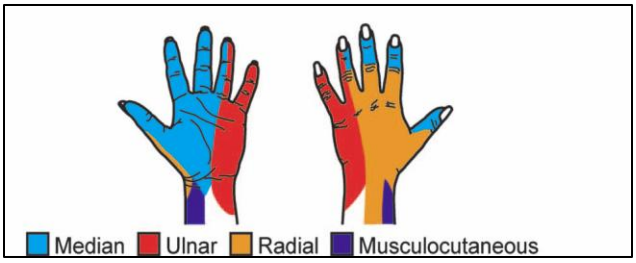


Figure 3: Diagrams of skin sensation provided by each nerve in the normal hand. Source: Kuiken et al. 2007a

The patient was fitted with an experimental 3 Degrees of Freedom (DOF) myoelectric prosthesis that consisted of a Griefer terminal device, a powered wrist rotator, a Boston digital arm, and a LTI-Collier Shoulder joint. An electronic lock also was added to the left shoulder joint, operated with a single touch pad in the apex of the left socket. The choice was made to use the three strongest electromyogram signals to control the experimental prosthesis (Kuiken 2006; Hijjawi et al. 2006).

The lateral hand close and wrist flexors portion of the median nerve reinnervation site was used to control hand closing, while the medial portion was used to control the patient's hand opening. The patient could then use a touch pad in the shoulder socket to switch from hook/hand function to wrist function and use the same electromyogram signals to rotate his wrist. The musculocutaneous reinnervation site was used for elbow flexion. The amount and speed that the prosthesis moved was dependent on the strength of the electromyogram signal controlled by the extent of muscle contraction. This type of control was preferred because it allowed the patient to

operate his elbow and terminal device intuitively and simultaneously (Kuiken 2006; Hijjawi et al. 2006).

TESTING

Two tests were performed comparing the function of the patient's experimental myoelectric prosthesis with his old touch pad prosthesis. The first test, the box and blocks test, is a standardized, validated assessment in which the test subject moves 1-inch-square blocks between two boxes separated by a short wall. The subject has to move as many blocks as he can from one box to the other in a one-minute period (Mathiowetz et al. 1985). The test was slightly altered, allowing the patient two minutes of moving the blocks.

After using his original touch-pad prosthesis for 20 months, the patient was able to move 5.7 boxes, averaged over three trials. After seven months of recovery and just two months of training, the patient was able to move 14 boxes using his new myoelectric prosthesis. That's a 246% increase in speed with his experimental prosthesis (Kuiken 2006; Hijjawi et al. 2006).

A second test, called the clothespin test was developed that required use of the elbow, terminal device, and wrist rotator unit. The patient has to take clothespins off a horizontal bar, rotate the pins, and place them on a higher vertical bar. The goal is to see how long it takes the patient to move three clothespins. The patient's speed improved 26% on this test (Kuiken 2006).

It is also important to note, that the patient reported that he strongly preferred his new myoelectric prosthesis and was able to do things that he could not do with his old prosthesis (Table 1).

Things patient can do *better* with myoelectric prosthesis	*New things* patient can do with myoelectric prosthesis
Take out garbage	Feed himself
Carry groceries	Shave
Pick up yard	Put on socks
Vacuum	Weed in garden
Dust mop	Water the yard
Pick up toys	Open small jar
Put on hat	Use pair of handicap scissors
Put on glasses	Throw a ball
Wash driveway	

Table 1: Patient M1's self-report of improved function with nerve-muscle graft controlled prosthesis. Source: Kuiken 2006

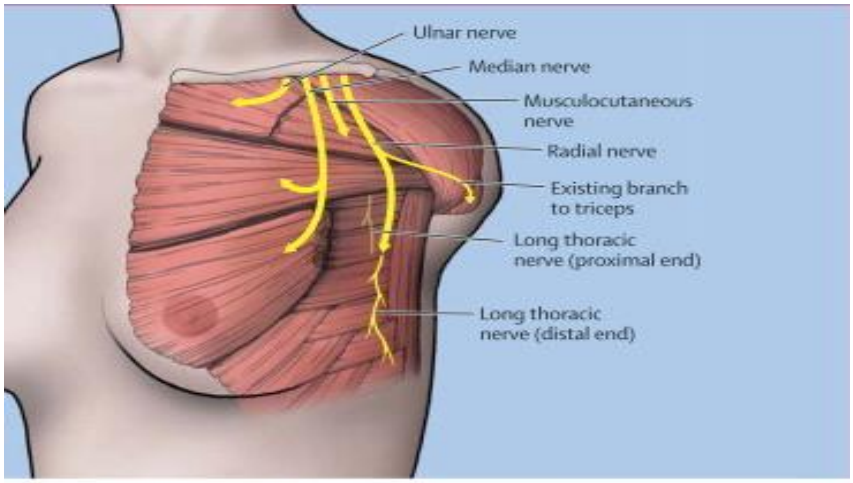
The one issue the patient did have, was that the prosthesis made him too hot and caused him to sweat. When the prosthesis got wet, it did not function as well and had to be taken off to dry (Kuiken 2006).

FIRST FEMALE TARGETED REINNERVATION PATIENT (F1)

The first female patient to undergo targeted reinnervation was a 23-year-old woman with a very short transhumeral amputation at the left humeral neck. In her case, the ulnar nerve had been transferred to the medial region of the upper pectoralis muscle, the musculocutaneous nerve was transferred to the lateral region of the upper pectoralis muscle, the median nerve was transferred to the middle and lower pectoralis muscles, and the distal radial nerve was transferred

to the serratus anterior muscle (Figure 4a). In addition, the supraclavicular cutaneous and intercostobrachial cutaneous nerves were cut and the distal ends were anastomosed to the ulnar and median nerves (Figure 4b).

A



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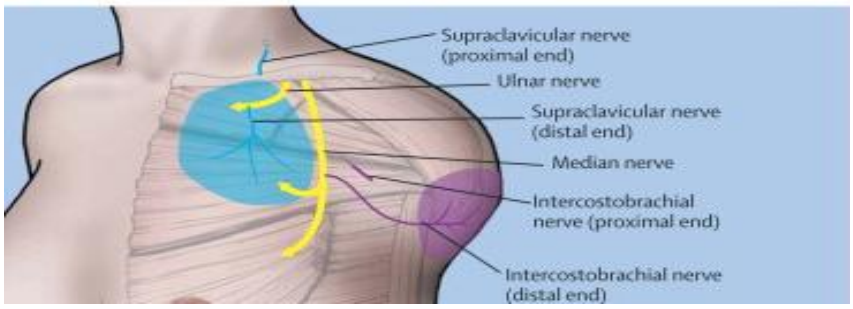

Figure 4: Diagram of first female patient's targeted reinnervation surgery (A) Targeted muscle reinnervation. The musculocutaneous, ulnar, and median nerves were transferred to separate segments of the pectoralis major muscle. The long thoracic nerve innervating the inferior three slips of serratus anterior was divided and the distal segment was coapted to the radial nerve. (B) Targeted sensory reinnervation. The supraclavicular cutaneous nerve was cut and the distal segment was coapted to the side of the ulnar nerve. The intercostobrachial cutaneous nerve was cut and the distal end was coapted to the side of the median nerve. Source: Kuiken et al. 2007b

A 4-cm diameter of subcutaneous fat was thinned over the clavicular head of the pectoralis muscle to enhance the surface electromyogram signal while not disfiguring the patient's breast. Similarly, a 4-cm circle of fat over the serratus muscle was removed through a separate incision (Kuiken et al. 2007b).

RESULTS

The targeted muscle reinnervation was successful, so a new experimental prosthesis was made comprising of a motorized elbow with a computerized arm controller, a motorized hand, and a motorized wrist rotator. Two pressure-sensitive pads were also mounted in the patient's socket, which were used to control her motorized wrist, allowing proportional, independent, simultaneous control of all three joints (Table 2).

	Control source	Nerve	Muscle
Elbow flexion	Electromyogram	Musculocutaneous	Lateral clavicular pectoralis major
Elbow extension	Electromyogram	Radial-triceps branch	Remnant triceps
Hand close	Electromyogram	Median	Sternal pectoralis major
Hand open	Electromyogram	Distal radial	Inferior serratus anterior
Wrist pronate	Anterior shoulder pressure button		
Wrist supinate	Posterior shoulder pressure button		

Table 2: Control pattern of targeted motor reinnervation prosthesis in this patient. Source: Kuiken et al. 2007b

Targeted sensory reinnervation was also successful. However, instead of feeling regular sensation the patient felt tingling in her arm in response to being touched on her target chest skin. With increased pressure, the patient felt an increased intensity of the tingling sensation. Her referred-touch sensation thresholds ranged from 0.4 to 300 gAF. In addition to graded pressure, the patient was also able to sense hot/cold sensations, distinguish between sharp and dull sensation, and was able to perceive vibration in her reinnervated skin (Figure 5). The patient was also able to perceive distinct sensation of each finger at different areas of the nerve transfer sites (Figure 6).

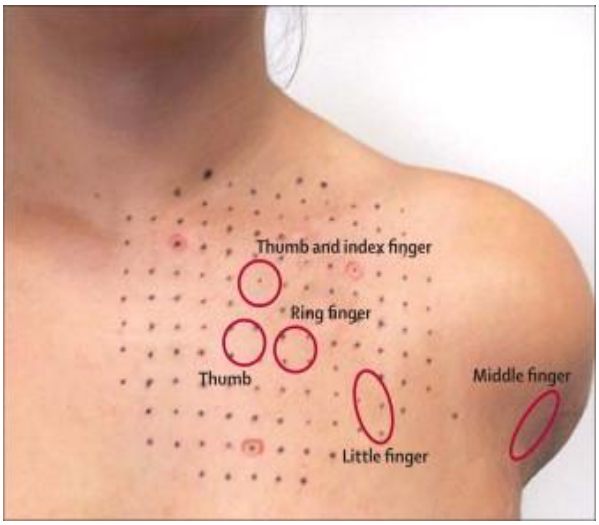

Figure. 5: The reinnervated chest skin of patient F1 (STH) showing sensations referred to the missing limb elicited by indentation of the skin by a cotton tipped probe (300 gAF). Red, referred sensation points localized to the palm side of the hand. Blue, points where a general diffuse feeling of pressure was felt within the hand. Circled points orient the diagram. P, proprioceptive sensation of fourth finger joint position. S, sensation of skin stretch. Double headed arrows, direction of stretch. Arrowheads, edge sensation. Source: Kuiken et al. 2007a

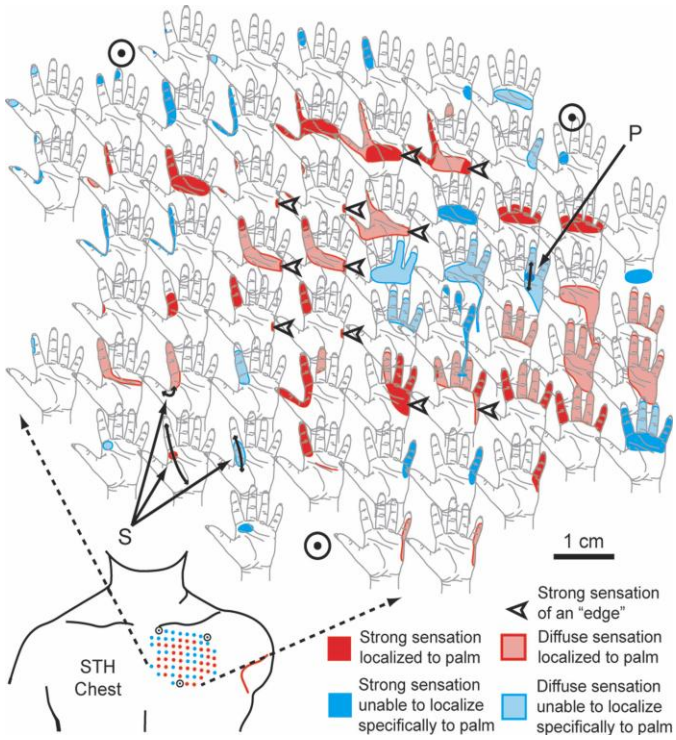

Figure 6: Map of areas that the patient F1 perceived as distinctly different fingers in response to touch. Source: Kuiken et al. 2007b

TESTING

Functional testing was done with her conventional myoelectric prosthesis, which she had been using for five months and then again with her new experimental prosthesis that she trained with for only seven weeks. A slightly modified box and blocks test was done, allowing the patient two minutes, instead of one minute, to move the blocks. The patient was allowed to practice for several minutes until she felt comfortable with the task. She then did the task three times with short breaks in between. With her old prosthesis, she moved an average of 4.0 blocks (SD-1.0). With her new prosthesis, she moved 15.6 (SD-1.5). That is almost a 400% increase in speed with her new prosthesis (Kuiken et al. 2007b).

The Assessment of Motor and Process Skills (AMPS) test was also done. The AMPS is an internationally recognized, occupational therapy–specific assessment of the quality of a client's activities of daily living performance (Merritt 2011) the test required the patient to do two tasks. For the old prosthesis, she had to prepare a peanut butter and jelly sandwich from beginning (gathering items) to end (returning items to appropriate storage) and iron a shirt from beginning to end. For the experimental prosthesis, she had to prepare a grilled cheese sandwich, and prepare and serve a tossed salad with four ingredients. For the old prosthesis, she got a single score of 0.30 for motor skill and 0.90 for process skills. For the new prosthesis, she got a score of 1.98 in both motor and process skill (Kuiken et al. 2007b).

ADVANCES IN PROSTHESIS DESIGN

In addition to being the first male targeted reinnervation patient, M1 was also chosen to try out advanced experimental prostheses. He was fitted with a 6 degree of freedom (DOF) prosthesis capable of shoulder flexion, humeral rotation, elbow flexion, wrist rotation, wrist flexion, and hand control (Table 3).

Location of input	Control input type	Controlled function
Median nerve transfer to middle pectoralis*	EMG	Hand open
Median nerve transfer to middle pectoralis*	EMG	Hand close
Musculocutaneous nerve transfer to superior pectoralis*	EMG	Elbow flexion
Radial nerve transfer to inferior pectoralis*	EMG	Elbow extension
Middle Deltoid	EMG	Internal humeral rotation
Latissimus Dorsi	EMG	External humeral rotation
Shoulder contact with anterior location of socket	Force sensing resistor	Wrist flexion/extension
Shoulder contact with posertior location of socket	Force sensing resistor	Wrist pronation/supination
Shoulder contact with superior location of socket	Digital rocker switch	Shoulder flexion/extension
EMG = electromyographic *Targeted reinnervation site		

Table 3: Location of targeted reinnervation sites and additional electromyographic and analog inputs used for control of the 6-DOF prosthesis. Source: Miller et al. 2008

The patient was able to independently operate all six of the prosthetic arm functions with good control. He was able to simultaneously operate 2 degrees of freedom of several different joint combinations with relative ease, and was also able to operate up to 4 degrees of freedom simultaneously, but with poor control. Overall, the patient's workspace was significantly increased and some of the timed tasks performed were faster with his new 6-DOF system (Miller et al. 2008).

Patient M1 was also fitted for a prosthetic arm with 7 degrees of freedom in January 2007. In addition Patients M1, F1, and one other patient where all fitted for a 10 degrees of freedom prosthesis in May, June, and July 2007 (Kuiken et al. 2009).

DISCUSSION

The cases presented show the successful use of targeted reinnervation in both men and women with different levels of upper limb amputation. The targeted sensory reinnervation procedure may possibly be utilized in lower limb amputations to provide patients with sensory perception of the floor they are walking on. Targeted muscle reinnervation can also provide the information necessary to control a powered joint in lower limb prosthesis.

However, despite such promising results, there is still much room for improvement and research. Further study is clearly needed to understand how long amputated nerves are viable.

Finding ways to provide more control signals is the key to operating more degrees of freedom. Using implantable myoelectric sensors (IMES) in place of surface electrodes can possibly enhance the amount and quality of control data collected from electromyograms (Merrill et al. 2010). By implanting the electrodes, crosstalk would be significantly reduced and the issue of sweat with patient M1 would be resolved. The size, weight, and battery life of advanced prostheses need to be improved in order to provide a more comfortable and practical experience for patients. Finally, a prosthesis that actually provides sensory feedback still needs to be developed.

The future for amputee patients seems more promising than ever. Who knows where this kind of technology might take us? Perhaps using targeted reinnervation, we can make prosthetic limbs so authentic, powerful, and efficient that even healthy people, with real limbs, will choose to wear them.

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BENEFITS VERSUS COSTS OF STATIN DRUGS

Sara Shilcrat

ABSTRACT

Statins have been prescribed to the masses as primary and secondary prevention for coronary disease caused by hypercholesterolemia after their initial discovery in the late 1980s. Their actions in reducing low-density lipoproteins and increasing high-density lipoproteins are well documented; however, many negative effects have been reported related to muscle pathology and kidney function. The goal of this study is to investigate whether the benefits of this class of drugs outweigh the costs. Intense review of the literature was conducted using scholarly articles with original research findings that were located via electronic databases such as Medline, Science Direct, Proquest Medical Library, and Google Scholar. Research findings on the benefits of statins extended beyond their lipid-related effects and included benefits to the immune system and inflammatory response, sepsis prevention, and improved endothelial cell functions, among others. Negative side effects of statins are many, including damage related to skeletal muscle tissue, such as rhabdomyolysis, myofiber necrosis, myotoxicity, myopathy, myalgia, reduced muscle resting chloride membrane potential (gCl), vacuolization of the T-tubule system, sarcolemma detachment, and targeting of the muscle's mitochondria. Differences between type I oxidative myofibers and type IIB glycolytic myofibers are discussed as well as the lipophilic and hydrophilic tendencies of the statins in relation to the damage inflicted on skeletal muscle tissue. In some rare cases of statin administration, motor neurons displayed Amyotrophic Lateral Sclerosis (ALS)-like symptoms that progressed up until muscle denervation. Additional negative side effects were seen to the circulatory and excretory systems, including altered chemical composition of both the blood plasma and urine, and rare renal failure due to rhabdomyolysis. The inquiry as to whether statins affect cardiac muscle as they do skeletal muscle is also addressed with the minimal findings that seemed to indicate that cardiac muscle is not targeted by statins.

After taking into account the benefits versus the costs of statins, in addition to the lack of a better drug on the market for combating coronary disease, it was suggested that statin administration should continue due to its proven cholesterol-related effects. However, statin users should be limited to patients with coronary disease triggered by high cholesterol. Patients with proven treatment options, such as patients with cancer or autoimmune diseases, were cautioned not to take statins for the possible benefits of unproven pleiotropic effects due to the likelihood of damage to skeletal muscle and kidney functioning. Monthly blood work and urinalysis were also suggested for patients on statins, and patients should be advised to speak to their physicians if they feel muscle pain or encountered changes in the ease of manipulating their muscles, as these are possible signs of muscle and nerve problems.

INTRODUCTION

In 1987, lovastatin, commonly known as Mevacor, Altocor, or Altoprev, was released into the public market. This new drug, isolated from the fermentation of the fungus *Aspergillus terreus*, was the beginning of a new class of drugs, marketed by the name of 'statins,' that focused on lowering cholesterol levels (Statin 2012; Torbert 2003). At the time, knowledge of the connection between cholesterol and the formation of plaques, or atheromas, in blood vessels was just beginning to develop. Although today it is common knowledge that cholesterol escaping from ruptured atherosclerotic plaques is pinpointed as the culprit of many heart attacks, this was

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yet unknown. Statins evolved from the skepticism that surrounded the lipid hypothesis, a controversial idea which associated coronary heart disease with increased levels of low-density lipoprotein (LDL) cholesterol and decreased levels of high-density lipoprotein (HDL) cholesterol (Statin 2012). Statins, or HMG-CoA reductase inhibitors, effectively stop the metabolic pathway ending in the synthesis of cholesterol. This class of drugs inhibits the functioning of an enzyme known as HMG-CoA reductase, and as a result, HMG-CoA, or 3-hydroxy-3-methylglutaryl-Coenzyme A, is not converted into mevalonic acid (Figures 1 and 2). Since this is the first step, also known as the rate-limiting step, in the pathway that leads to cholesterol, blocking this transformation will stop the entire cholesterol synthesis pathway (Figure 3). With the formation of cholesterol at a halt, fewer plaques will form, and subsequently rupture, decreasing the risk of heart attacks and other adverse effects of cardiovascular disease. However, in addition to blocking the formation of cholesterol which improves the cardiovascular disease prognoses, using statins also prevents the formation of other cholesterol derivatives such as isoprenoids and sterols including testosterone, estradiol, and cortisol, among others which may result in additional repercussions.

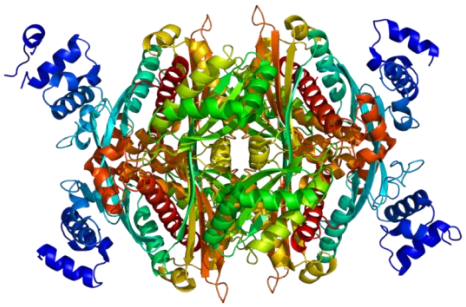


Figure 1: HMG-CoA Reductase Ribbon Model. Source: HMG-CoA reductase 2012

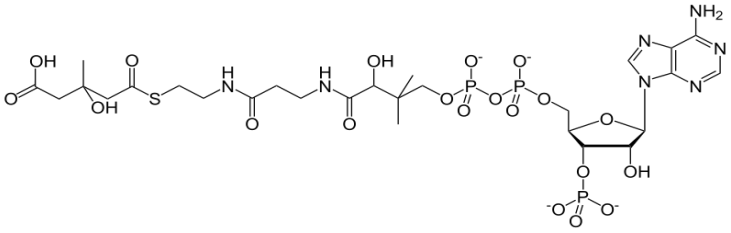


Figure 2: 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) Structure. Source: HMG-CoA 2012

Today, statins are commonly prescribed as primary and secondary prevention for cardiovascular diseases associated with elevated cholesterol levels, or hypercholesterolemia (Merx and Weber 2006; Statin 2012). Since the discovery of lovastatin, other statins have successively entered the market including simvastatin (1988, as Zocor and Lipex), pravastatin (1991, as Prevachol, Selektine, and Lipostat), fluvastatin (1994, as Lescol and Lescol XL), atorvastatin (1997, as Lipitor and Torvast), cerivastatin (1998, as Lipobay and Baycol), and rosuvastatin (2003, as Crestor) (Torbert 2003) (Figure 4).

Other prescription drugs on the market, such as Vytorin (simvastatin and ezetimibe), Advicor (lovastatin and niacin extended release), Caduet (atorvastatin and amlodipine besylate), and Simcor (simvastatin and niacin extended release) combine one of the statins with another drug for multiple therapeutic effects (Statin 2012). Also, statins may be prescribed with concurrent use of fibrates, immunosuppressants, corticosteroids, antifungals, blood thinners like warfarin, and other prescription drugs (Mohaupt et al. 2009; Nicholls et al. 2011).

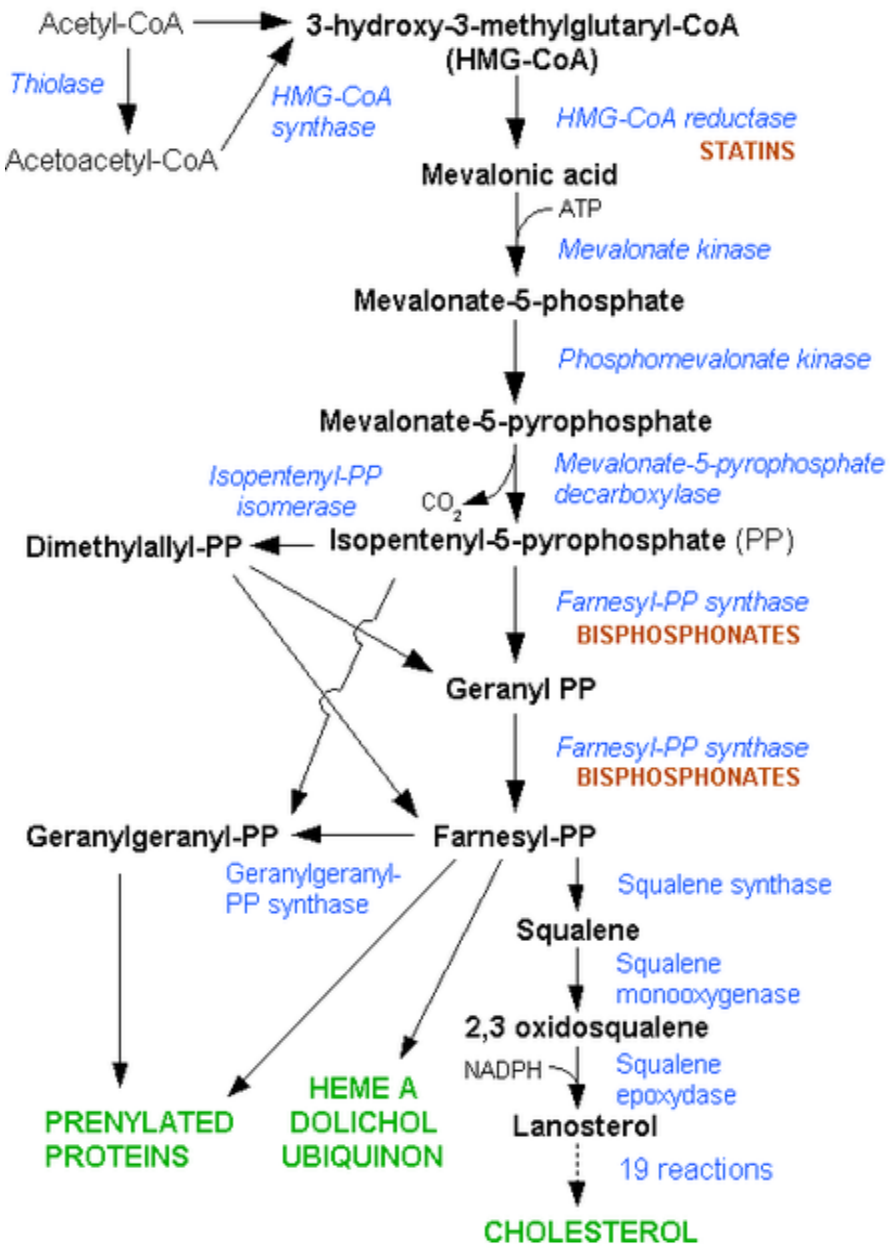


Figure 3: Cholesterol Synthesis Pathway. Source: Statin 2012

Each of the statins differs in its ability to reduce LDL cholesterol. Ranging from most effective to least effective, the statins can be arranged in the following order: cerivastatin > rosuvastatin > atorvastatin > simvastatin > lovastatin > pravastatin > fluvastatin (Statin 2012). Recommended dosages of the statin drugs differ based on their potency, with less potent statins prescribed at higher dosages and vice versa. Statins can also be classified on a continuum of being more hydrophilic or more lipophilic, affecting their LDL cholesterol reducing power. Differences in a particular statin's hydrophilicity are thought to cause different physiological effects on the body, especially when discussing the effects of statins on skeletal muscle (Pierno et al. 2006; Sidaway et al. 2009).

Examining the chemical structure of statins shows general similarities that exist in this class of drugs (Figure 4). Some key differences do exist, however, between statins that are derived from fermented natural substances (mevastatin, lovastatin, pravastatin, and simvastatin[1]) and laboratory-created synthetic statins (atorvastatin, cerivastatin, fluvastatin, pitavastatin, and rosuvastatin). Generally, the statins consist of two or more ring structures; in synthetic statins, one of the rings is a nitrogen-containing heterocyclic ring not found in naturally-derived statins. In addition, lacking in the fermentation derivatives, a fluorinated benzene ring is found in the synthetic statins as well as a seven-carbon fatty acid chain branching off a ring structure terminating in a carboxylic acid. The fatty acid chain is also characterized as a diol, with two hydroxyl groups coming off the chain at positions C3 and C5 counting from C1 of the -COOH group.. In the naturally occurring statins two fused cyclohexene rings share two carbon atoms and have double bonds located a single carbon away from one another. These statins lack the fatty acid chain and instead, a cyclic ester with a hydroxyl group attached 2 carbon atoms away is present. Additionally, they contain a second ester attached further away on the molecule that is followed by a *sec*-butyl group. Finally, the synthetic statins may contain one or more isopropyl groups branching off of the heterocyclic N-containing rings (Table 1).

The variations in statins' structures have generated a common assortment of side effects, but the individual structure of the statin has been shown to yield differences in the severity of the side effects when they are experienced. In 2001, cerivastatin was removed from the market due to the severe side effects, specifically rhabdomyolysis, or the breakdown of muscle tissue, which led to numerous deaths (Obayashi et al. 2011; Sidaway et al. 2009; Statin 2012). A few more common, less severe side effects seen in skeletal muscle after using statins include myotoxicity, myopathy, any abnormal condition or disease of muscle tissue, myalgia, or muscle pain, and limb weakness. Other adverse side effects seen with statins include increased creatinine kinase (CK) activity, increased ryanodine receptor 3 (RYR3) mRNA expression (Mohaupt et al. 2009), sarcolemma detachment (Mohaupt et al. 2009), vacuolization of muscle fibers (Mohaupt et al. 2009; Obayashi et al. 2011), increased myoglobinemia and myoglobinuria (Pierno et al. 2006) , reduced sarcolemma resting chloride membrane potential (gCl) (Pierno et al. 2006; Pierno et al. 2009), muscle fiber necrosis, neuromuscular damage with ALS-like (amyotrophic lateral sclerosis) symptoms (Edwards et al. 2007), and kidney damage (Campese and Park 2007).

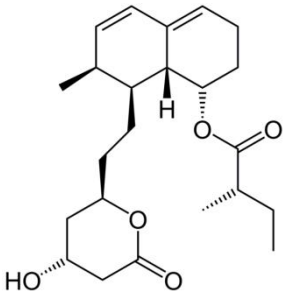
Despite these adverse effects, statins have been proven to be very effective at reducing LDL cholesterol and boosting HDL cholesterol. Other beneficial actions of statins include pleiotropic effects unrelated to lipid mobilization such as sepsis prevention (Hackam et al. 2006 cited in Merx and Weber 2006), increased KLF2 expression (T cells) (Bu et al. 2010), improved endothelium function including increased nitric oxide (NO) production (Liao and Laufs 2005; Merx and Weber 2006), and other possible applications for treating autoimmune and inflammatory disorders (Bu et al. 2010; Weitz-Schmidt 2003).

The sale of Lipitor™ (atorvastatin) swept the market, netting Pfizer an unprecedented yield in the pharmaceutical industry of more than $12 billion dollars (Statin 2012). Yet whether the benefits of statins are really worth the adverse side effects experienced is still under debate. Doctors seem bent on continuing to prescribe the statin drugs and tend to taper the dosage as needed to mitigate the side effects. But when do the ill effects of statins go so far that it is no longer possible to justify their use? To what degree must the body's chemistry be altered in order to stop using statins?

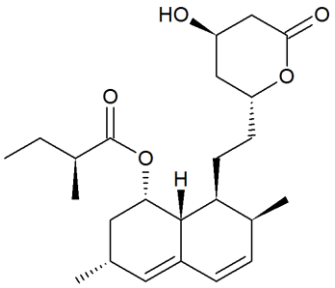
[1]Although simvastatin is actually synthetically made from a substance produced by fermenting *Aspergillus terreus*, it closely resembles naturally-derived statins in its structure.

Naturally-Derived Statins

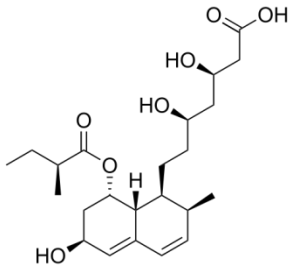
Mevastatin



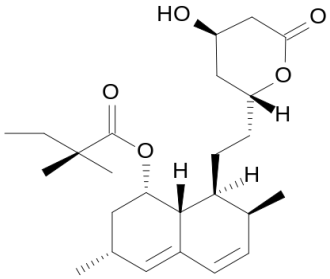
Lovastatin



Pravastatin

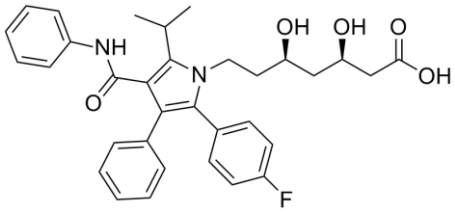


Simvastatin[1]

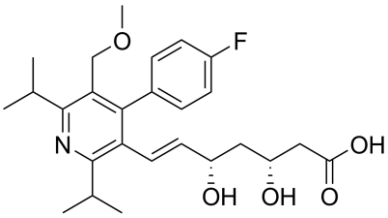


Synthetic Statins

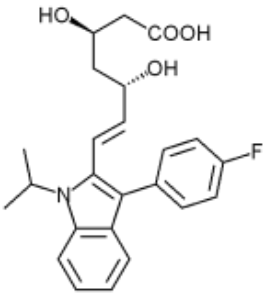
Atorvastatin



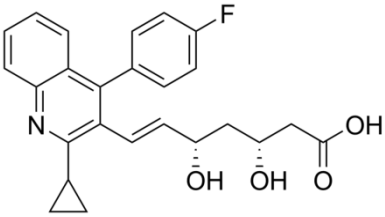
Cerivastatin



Fluvastatin



Pitavastatin



Rosuvastatin

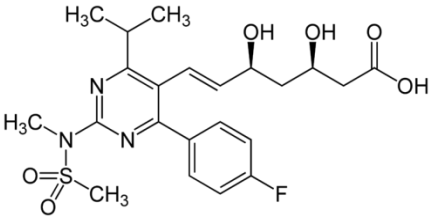


Figure 4: Structures of the statins. Source: Statin 2012

Table 1: Features of the natural and synthetic statins.

Statins	Poly Cyclic	N-containing Heterocyclic Ring	*para*-Fluorobenzene Ring	Fused Cyclohexene Rings (Decene structure)	Fatty Acid with Diol /COOH	Cyclic Ester with -OH	Ester with *sec*-butyl	Isopropyl Groups	Additional Structures
Natural (Fermentation derived)									
Mevastatin	Y	N	N	Y	N	Y	Y	N	
Lovastatin	Y	N	N	Y	N	Y	Y	N	
Pravastatin	Y	N	N	Y	N	Y	Y	N	
Simvastatin	Y	N	N	Y	N	Y	*	N	
Synthetic									
Atorvastatin	Y	Y-pentane	Y	N	Y	N	N	Y	Cyclic Amide
Cerivastatin	Y	Y-cyclohexane	Y	N	Y	N	N	Y (2)	Alkene at C6-C7 of fatty acid chain
Fluvastatin	Y	Y-pentane	Y	N	Y	N	N	Y	Heterocyclic pentane attached to a benzene ring
Pitavastatin	Y	Y-cyclohexane	Y	N	Y	N	N	N	Cyclopropane and fused heterocyclic and non-heterocyclic 6C rings
Rosuvastatin	Y	Y-cyclohexane with 2 N's	Y	N	Y	N	N	Y	Methyl-Sulfur dioxide attached to a secondary amine

METHODS

Review of the literature on statins was done using electronic databases, such as Medline, Science Direct, Proquest Medical Library, and Google Scholar to procure articles on or related to statins using keywords like 'statins,' 'HMG-CoA reductase inhibitors,' and the like.

DISCUSSION

Benefits of Statins:

Reduction of LDL cholesterol and increase of HDL cholesterol

Statins were originally intended for their abilities to reduce LDL cholesterol and increase HDL cholesterol. Some studies have shown that modulations of LDLs and HDLs brought about by statins may result in regression of coronary disease (Nicholls et al. 2011; Nissen et al. 2004; Nissen et al. 2006).In an extended study spanning more than 2 years, 1039 patients with coronary disease were treated with one of two statin drugs, atorvastatin (80 mg daily) or rosuvastatin (40 mg daily), in a randomized clinical trial to determine and compare their individual effects on the progression of atherosclerotic plaques (Nicholls et al. 2011).

Percent atheroma volume and normalized total atheroma volume regression

Before and after the 104-week period, ultrasounds were recorded of a particular artery with stenosis. The external elastic membrane of the vessel and the lumen size were measured (Figure 5), and the following formulas were used to determine the percent atheroma volume (PAV) and the normalized total atheroma volume ($TAV_{normalized}$), that allowed for comparison between participants who had different atheroma sizes:

$$PAV = \frac{\Sigma\,(\text{External Elastic Membrane}_{area} - \text{Lumen}_{area})}{\Sigma\,\text{External Elastic Membrane}_{area}} \times 100$$

$$TAV_{normalized} = \Sigma\,(\text{External Elastic Membrane}_{area} - \text{Lumen}_{area}) \times \text{median no. of no. of images in pullback images in cohort.}$$

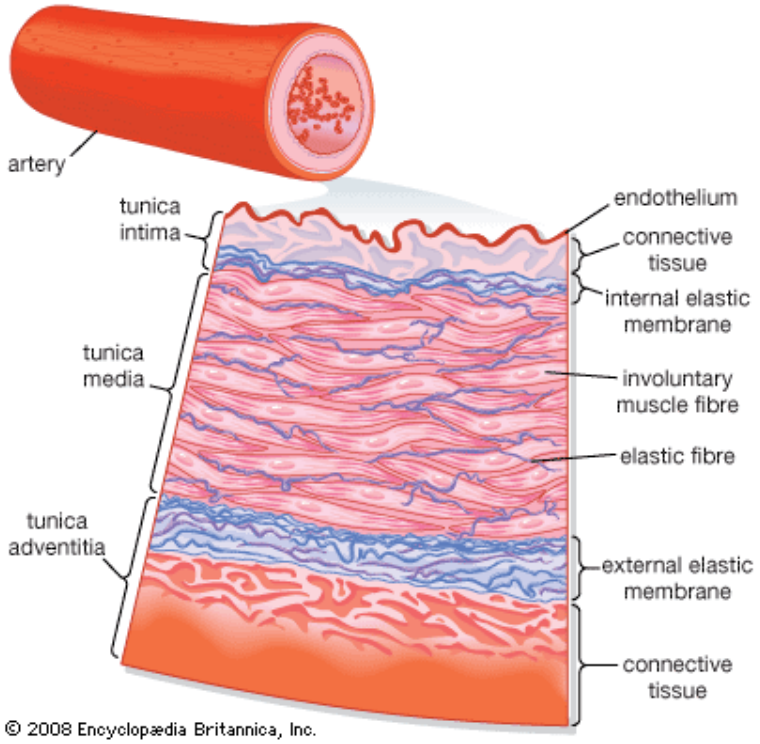


Figure 5: Diagram of an Artery in Cross Section. Source: Diagram of an artery in cross section 2008

The changes in PAV and $TAV_{normalized}$ were calculated as the PAV or $TAV_{normalized}$ at week 104 minus the initial PAV or $TAV_{normalized}$. Interpreting the formulas, an increase in PAV corresponds to a decrease in the opening size of the lumen, or the higher the PAV value, the more closed the coronary artery is. The $TAV_{normalized}$ follows the same methodology as the PAV, with a larger $TAV_{normalized}$ related to more closed arteries in the sample of participants. During the 104 week period, the HDL and LDL cholesterol and triglyceride levels of the participants were measured at 24, 48, 72, and 104 weeks (Nicholls et al. 2011).

The study showed that the two intensive statin regimens lead to statistically significant results. Both atorvastatin and rosuvastatin lowered LDL cholesterol levels and increased HDL cholesterol, yet, rosuvastatin was more effective statistically at achieving an overall lower LDL to HDL ratio, bringing down LDL cholesterol levels below 70 mg/deciliter in many participants, and decreasing the percentage of individuals with LDL cholesterol levels above 100 mg/deciliter compared to atorvastatin (Table 2). The PAV and $TAV_{normalized}$ values decreased significantly corresponding to an increase in the lumen size of the participants' blocked arteries due to shrinkage of the plaques.

Table 2: LDL and HDL Cholesterol Levels After Intensive Statin Regimens. Source: Nicholls et al. 2011

	Statin (mg/deciliter least-squares mean values ±SD)	
	Atorvastatin	Rosuvastatin
LDL cholesterol levels (p < 0.001)		
at baseline	119.9 ±28.9	120.0 ±27.3
at 104 weeks	70.2 ±1.0	62.6 ±1.0
HDL cholesterol levels (p = 0.01)		
at baseline	44.7 ±10.7	45.3 ±11.8
at 104 weeks	48.6 ±0.5	50.4 ±0.5

Although the PAV showed a slightly greater reduction with rosuvastatin than atorvastatin, it was not statistically significant, yielding similar effectiveness in both statins. With respect to the $TAV_{normalized}$, rosuvastatin did significantly reduce the $TAV_{normalized}$ value more than atorvastatin. Rosuvastatin was also more effective in reducing the PAV in women, participants with higher initial HDL cholesterol levels, and in participants with higher initial LDL levels. Two interesting abnormalities found in the participants' lab work were increased levels of a liver enzyme, alanine aminotransferase, in the atorvastatin group and more proteinuria in the rosuvastatin group (Nicholls et al. 2011). Alanine aminotransferase, or alanine transaminase, is an enzyme found in both hepatocytes and myocytes that reversibly converts glutamate to α-ketoglutarate, leading to the formation of pyruvate. Gluconeogenesis converts pyruvate to high-energy glucose; the glucose can then be utilized by the cell. Alanine aminotransferase is used in enzymatic assays and indicates signs of liver damage and/or myopathy (Nelson and Cox 2005; Alanine Transaminase 2012).

This experiment is a clear indicator of the efficiency of statins at yielding mean LDL cholesterol levels below the recommended 70 mg/deciliter for secondary prevention of coronary disease. HDL cholesterol levels also came close to the recommended 50 mg/deciliter, leading the researchers to believe that if given enough time, the statin regimen would meet the desired levels for HDL cholesterol and LDL cholesterol and facilitate the regression, or at least deter the progression, of coronary disease (Nicholls et al. 2011).

Other research has found similar results with pravastatin and atorvastatin (Nissen et al. 2004), and rosuvastatin (Nissen et al. 2006). Still, a major consideration is that although the PAV reflects reduction in the size of a particular atherosclerotic plaque, it does not necessarily translate into preventing an impending cardiovascular episode. Second, $TAV_{normalized}$ regression has not been linked to any clinical significance. Finally, although disease advanced in one third of the participants even with the heavy statin regimen, results indicate the beneficial aspects of statins with regard to cholesterol and plaque regression and demonstrate the general safety of statins even at high doses (Nicholls et al. 2011).

Pleiotropic effects

Statins have been found to aid in a variety of other functions (Liao and Laufs 2005).

Immune responses and inflammation:

Effects on T lymphocytes and KLF-2 gene expression

T cells are important actors in the inflammatory responses of the body. Statins were proven to upregulate the expression of the Kruppel-like factor 2 (KLF-2) gene in activated, or

effector, CD4+ helper T cells and CD8+ cytotoxic T cells, and prevent the downregulation of KLF-2 that normally occurs in recently activated T cells (Bu et al. 2010). The KLF-2 gene is thought to inhibit T cell proliferation that occurs with inflammatory responses and keeps T cells in a resting state as KLF2 mRNA is expressed in naïve and memory T cells (Buckley et al. 2001 and Kuo et al. 1997 cited in Bu et al. 2010).

Effects on immune cells and LFA-1 binding site

A second pleiotropic effect seen with lovastatin in particular is its ability to bind to a novel site on LFA-1, lymphocyte function-related antigen 1, an integrin molecule found on T lymphocytes and macrophages (Weitz-Schmidt 2003). Acting as an allosteric inhibitor, lovastatin changes the conformation of the LFA-1 and decreases its affinity for its substrate intercellular adhesion molecule-1 (ICAM). ICAM, is a molecule found on endothelial cells that binds to integrins (the adhesion molecules found on immune cells). During diapedesis, macrophages and T lymphocytes will roll and attach to selectin molecules expressed on the endothelial surface (Tortora and Derrickson 2012; Watanabe and Fan 1998). Subsequently, the immune cells will strengthen their attachment to the endothelium using β2 integrins on their surface, such as LFA-1 and bind to members of the immunoglobulin family, like ICAM-1, located on the endothelium. Binding at these two sites will lead the immune cell to squeeze between adjacent endothelial cells and reach the site of inflammation. ICAM-1 has been found to be expressed by endothelium where cholesterol-induced plaques are beginning to form. Atherosclerotic plaques are thought to be induced by the sticking of T cells and other immune cells to the endothelium lining blood vessels (Figure 6) (Watanabe and Fan 1998). If statins change the binding site shape of LFA-1 receptors on immune cells, there will be less attachment of the immune cells to the linings of blood vessels, possibly leading to less atherogenesis (Weitz-Schmidt 2003). This may explain the plaque regression seen in clinical trials, however, in previous studies lovastatin was not the statin being studied

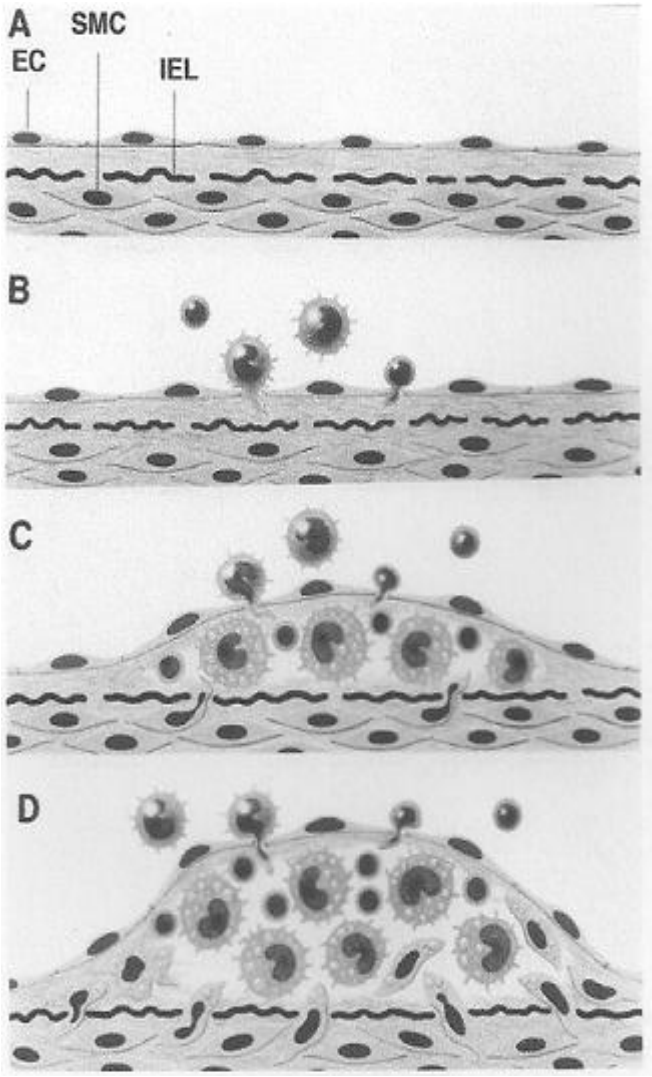


Figure 6: A postulated hypothesis for the pathogenesis of atherosclerosis.
(A) A normal arterial wall. (B, C) Monocytes and T lymphocytes adhere to the endothelial cell surface and subsequently enter subendothelial space. Monocytes are transformed into macrophages and some become foam cells after uptake of lipids. (D) Most macrophages become foam cells and smooth muscles cells in the media start to migrate into the media and proliferate. These cells constitute the typical fatty streak lesion. EC: endothelial cell, SMC: smooth muscle cell, IEL: internal elastic lamina. Source: Watanabe and Fan 1998

(Nicholls et al. 2011; Nissen et al. 2004; Nissen et al. 2006). It is therefore possible that other statins may change additional receptors besides LFA-1on immune cells that are involved in attaching them to the endothelium. Alternatively, it is also possible to conjecture that statins may modulate adhesion molecules on the endothelial surface or that statins other than lovastatin may have another entirely different method for diminishing the size of plaques.

Statins are also involved in increasing the synthesis of nitric oxide gas by stimulation and upregulation of endothelial nitric oxide synthase (Laufs et al. 1998 and Kureishi et al. 2000 cited in Liao and Laufs 2005).

Sepsis prevention

Some research has found evidence of fewer cases of sepsis, including moderate to severe, and even fatal, sepsis cases when patients were taking statins compared to matched controls (Hackam et al. 2006).

Costs of Statins:

Statins have been proven to cause a wide range of negative side effects many of which target muscles, but damage can also occur to nerves innervating skeletal muscles and the kidneys as well.

Damage to skeletal muscle

There are two possible mechanisms for how damage occurs. These will now be discussed.

Targeting the mitochondria

It is necessary to understand how statins lead to skeletal muscle degradation in order to fully grasp the extent of their effects on the body. A study of cerivastatin in male rats implicates its targeting of mitochondria as a plausible cause of muscle toxicity (Obayashi et al. 2011). The researchers studied effects to the soleus muscle, a muscle rich in type I fibers, and the extensor digitorum longus and the tibialis anterior, muscles rich in type II fibers, throughout the course of cerivastatin treatment. While no particular skeletal muscle is purely made up of one type of muscle fiber (i.e. type I, IIA or IIB) (Swenson 2006), a particular muscle may contain a larger percentage of one of the three types of fibers; researchers tend to choose these muscles to study in order to determine trends in muscle fiber types after statin administration While light microscopy did not show any visible signs of damage to any of the three muscles on day 6, electron microscopy of the soleus muscle revealed mitochondria that were swollen, electron dense, deteriorated, and contained inclusion bodies Other abnormalities included autophagic vacuoles, some of which were consuming membrane-bound organelles, activated lysosomes, myeloid structures, and disorderly myofibrils. By day 8, the soleus muscle of the cerivastatin-treated rats showed enlarged mitochondria in addition to vast differences in the diameter of the myofibers and some very darkly staining myofibers.

Overall, the mitochondria in this study showed changes in shape, becoming rounded as opposed to oval, and were sought out and destroyed by lysosomes. Only after the destruction of the mitochondria were myofibrils jumbled and autophagic vacuoles active. These findings led to the logical premise that mitochondria are targeted by cerivastatin (Obayashi et al. 2011). While this and other studies zero in on the damaging effects of statins to mitochondria as the primary targets, this idea has been challenged (Waclawik et al. 1993, Schaefer et al. 2004, and Westwood et al. 2008 cited in Obayashi et al. 2011).

Statins' effects on mitochondria in relation to susceptibility of muscle fiber type to damage

Examination of the mitochondria activity in Hanai et al.'s experiment (2007), discussed below, reinforces a likely conclusion as to why type IIB fibers are more susceptible to statin-

associated muscle damage and why type I fibers are resistant to such damage. Since type IIB fibers lack two protective factors found in type I fibers, specifically more numerous mitochondria and greater expression of a gene that halts tissue atrophy known as PGC-1α, they are likely more vulnerable to damage by statins. Alternatively, if statins target mitochondria in particular, type IIB fibers are at a loss, already having fewer mitochondria with the compounded problem of the statins depleting the few mitochondria that these fibers have left.

Nonetheless, the argument that type IIB fibers are more vulnerable to statins was not seen in Obayashi et al.'s study of cerivastatin in rats (2011). Damage in this experiment was delivered solely to the soleus muscle, a predominantly type I oxidative, slow twitch muscle. Both the tibialis anterior and the extensor digitorum longus muscles, fast twitch, glycolytic type IIB-predominant muscles, did not show the damage seen to the soleus' mitochondria. This contradiction may be reconciled by stressing which statin was used in Obayashi et al.'s study compared to that by Hanai et al. in 2007, namely cerivastatin versus lovastatin. It is possible that these two statins have different methods of inducing skeletal damage. Extrapolating a step further, it seems logical that cerivastatin's potency, leading ultimately to its removal from the public market, may be linked to changes in the mitochondria seen in type I fibers. It may be that rhabdomyolysis of type I myofibers specifically may be more serious overall, both from a physiological and function-related point of view. Anatomically, these muscle cells contain large amounts of myoglobin, which will be released into the blood plasma, to be dealt with by the kidneys if the muscle breaks down. Functionally, type I fibers resist fatigue from long term exercise, maintain sustained contracture for long time periods, act when only weak muscle contracture is needed, and finally, may compromise up to half of the fibers in a particular muscle (Tortora and Derrickson 2012). Conversely, lovastatin and other statins may act in a different manner that causes muscle damage primarily to type IIB fibers (Hanai et al. 2007; Schaefer et al. 2004; Smith et al. 1991 cited in Sidaway et al. 2009; Westwood et al. 2005; Pierno et al. 2006).

Alternatively, mitochondria contain the necessary components of oxidative phosphorylation, one of which is CoQ_{10}. CoQ_{10} is a protein produced by the prenylation pathway that stems from HMG-CoA being converted to mevalonic acid (Figure 3). Since statins inhibit HMG-CoA reductase, less CoQ_{10} is made than may be needed by the mitochondria. When there is abundant PGC-1α expression, it is possible that the more massive mitochondria that are produced by expression of this gene will intrinsically have more CoQ_{10} and will not need to rely on the synthesis of new CoQ_{10} from HMG-CoA reductase activity, inhibitable by statins. As of yet, this idea is still under assessment (Hanai et al. 2007) Again, if CoQ_{10} is the problem, the same logic applies as to why type IIB fibers are more susceptible to statins compared to type I, namely the limited amounts of mitochondria present. If the amount of CoQ_{10} is similarly lessened in all mitochondria, still the overall quantity of CoQ_{10} in type I muscles will likely exceed that found in type IIB muscles just by a greater number of mitochondria. The increased mitochondria, and as a result CoQ_{10}, in the type I fibers will then not be as severely affected by the statin-induced shortage of the CoQ_{10}, a mainstay of the electron transport system.

Potency of cerivastatin

Cerivastatin was determined as the most potent statin since a dose less than 20 mg/kg, of cerivastatin, which does not cause myopathy with other statins, generated myopathy (Sidaway et al. 2009). While cerivastatin does cause severe rhabdomyolysis, it must be remembered that this is a rare side effect that was not found in preliminary testing but seen after release into the mass market. Since it is truly an unusual side effect of statins and linked mainly to the retracted cerivastatin, it would be wont to discontinue use of this entire class of drugs due to fear of this

particular adverse reaction. Therefore, it would not be reasonable to stop prescribing statins to the masses, yet this side effect should be monitored closely in the rare chance that there are signs of myopathy in a particular patient.

Atrogin-1 expression inducing muscle atrophy

Another study discusses a second mechanism for statins' effects on skeletal muscle. In this mechanism, statins are thought to switch on the expression of a gene involved in a pathway leading to atrophy in body tissues (Hanai et al. 2007). The gene, atrogin-1/MAFbx, is part of the ubiquitin proteasome pathway (UPP), a pathway involved in protein breakdown in the body, and codes for an enzyme called ubiquitin-protein ligase that is specific to muscle tissue. Elevated atrogin-1 mRNA levels were found in skeletal muscle biopsies[2] of patients with statin-associated myopathy and in patients with myopathy that were not taking statins compared to healthy controls. Lovastatin was introduced to C2C12 myotubes (skeletal muscle cell precursors) and zebrafish embryos to determine whether there would be a similar abundance of atrogin-1 expression in these organisms after treatment. In the myotubes, increasing amounts of lovastatin resulted in commensurate increases in the amount of atrogin-1 mRNA, its corresponding protein, and muscle proteolysis. Larger amounts of lovastatin lead to markedly shrunken myotubes. The cells progressively deteriorated displaying evidence of vacuoles and extreme distortion ending in the loss of the myotubes after 5 days (concentrations of lovastatin included 0.0, 0.25, 1.0, 2.5, 5.0, and 10.0 μM). Vacuolization of the myotubes may reflect the vacuolization that is seen in skeletal muscle tissues in the T-tubule system reported by Mohaupt et al. (2009). The researchers also proved that the atrogin-1 gene was needed to cause lovastatin-induced morphology changes in the myotubes; myotubes bred lacking the atrogin-1 gene and then dosed with a particular concentration of lovastatin (either 0.0, 0.01, 0.05, 0.25, 1.0, or 2.5 μM) did not show changes to the diameter and morphology of the myotubes unlike matched atrogin-1-containing myotubes at the parallel dose of lovastatin (Hanai et al. 2007). What is interesting to note is a slight dip in myotube diameter when atrogin-1 null myotubes were dosed with 2.5 μM of lovastatin. Whether this would decrease enough to become significant with 5.0 μM or 10 μM concentration will remain unknown as the researchers did not continue to dose the myotubes with these increasing concentration levels. It is also possible that ≥5.0 μM lovastatin concentration greatly exceeds the amount of lovastatin that would be given to a patient in a clinical setting per kilogram. Yet this rationale is hard to justify as wild-type for atrogin-1myotubes were initially dosed at these concentrations to determine the effects to the myotubes.

Depending on the dosage, lovastatin triggered specific morphological changes in the skeletal muscle of zebrafish embryos which were dosed 20 hours post fertilization (Hanai et al. 2007). Changes to zebrafish skeletal muscle morphology were determined by exogenously-prepared antibodies that, upon reacting with skeletal muscle tissue, would latch on to myosin found in the thick filaments. These morphological changes in the muscle were classified based on their severity. Class 1 changes to muscle consistent with 0.025-0.05 μM lovastatin treatment included bowing, gap formation, and disruption of the muscle fibers. Increasing the lovastatin dosage, class 2 morphological changes (0.05-0.5 μM) comprised thin/irregular or diffuse appearance of the myosin strands. Finally, irregular muscle segment boundaries were categorized under class 3 changes due to lovastatin treatment (1.0-5.0 μM).

Confirmation of statins' inhibition of HMG-CoA reductase in zebrafish embryos

The effects of lovastatin were confirmed to be the result of HMG-CoA reductase inhibition. After knocking out the HMG-CoA reductase gene and eradicating any corresponding,

[2] Biopsies were taken from the quadriceps.

loose mRNA in the cell with antisense technology, the skeletal muscle showed similar morphological characteristics to the class 1 'disrupted' muscle fibers of the zebrafish treated with lovastatin (wild-type for HMG-CoA reductase and atrogin-1 genes (Hanai et al. 2007).

Muscle myopathy

The relationship between histological damage of skeletal muscles and painful muscles thought to be caused by statins has been studied (Mohaupt et al. 2009). The vastus lateralis of 83 participants were biopsied. Participants were divided into 5 experimental groups (Table 3). Participants in group 4 (n=29) were presently taking atorvastatin (17%), simvastatin (41%), fluvastatin (7%), pravastatin (31%), or rosuvastatin (3%), and they had previously been prescribed 4 out of the 5 statins currently being used[3]. Group 5 participants (n=19) currently on statins without symptoms of myopathy reported prescriptions for simvastatin (74%) and pravastatin (21%), while in the past, one of the participants had been on simvastatin. As for the participants in group 3 whom had ceased their statin regimens (n=15), the statins previously prescribed included atorvastatin (40%), simvastatin (53%), fluvastatin (7%), pravastatin (53%), and cerivastatin (7%). A careful record of other drugs being used alongside statins were documented including fibrates, immunosuppressants, corticosteroids, blood thinners, macrolid antibiotics, antifungals, and HIV-protease inhibitors. The biopsies were studied for microscopic anatomical variances in the skeletal muscle's structure. For the skeletal muscle damage to be considered significant, the researchers mandated that a minimum of 2% of the myofibers from the biopsy needed to show clear evidence of destruction (Mohaupt et al. 2009).

Table 3: Groups 1-5 for Experiment Relating Statin-Induced Myopathy to Muscle Injury. Source: Mohaupt et al. 2009

Group	Condition	Currently On Statin Regimen Y/N	Pre-existing Statin-Induced Myopathy Y/N	No. of Participants	Gender
1	Healthy	N	N	10	Male
2	Hypercholesterolemia (Unrelated to muscles)	N	N	10 (Age matching to Groups 3 & 4)	7 Males 3 Females
3	Clinically Diagnosed Myopathy	N (At least 3 weeks off treatment regimen)	Y	15	8 Males 7 Females
4	Clinically Diagnosed Myopathy	Y	Y	29	22 Males 7 Females
5	Hypercholesterolemia	Y	N (reported no muscle problems)	19	12 Males 7 Females

[3] Rosuvastatin was not prescribed previously (Mohaupt et. al. 2009).

Patient-reported symptoms of myopathy in groups 3, 4, and 5

About two thirds (67%) of those who discontinued statin use (group 3) and approximately half (48%) of the current statin users (group 4) were presently suffering from myalgia, or muscle pain (Table 4). Another symptom of myopathy expressed by one fifth of past statin users and 38% of current statin users was muscle weakness in the torso and upper arms. A lesser noted symptom of myopathy found was muscle cramping (13% in group 3 participants and 7% in group 4). Finally, 3 out of 15 (20%) participants in group 3 who discontinued statin use mentioned experiencing myalgia, muscle weakness, and/or cramping lasting more than a month after discontinuing statin use (Mohaupt et al. 2009).

Table 4: Results of Experiment Relating Statin-Induced Myopathy to Muscle Injury for Groups 3-5. Source: Mohaupt et al. 2009

Group	**3**	**4**	**5**
Myopathy Symptoms			
Myalgia No. (%)	10 (67)	14 (48)	N/A
Weakness No. (%)	3 (20)	11 (38)	N/A
Cramping No. (%)	2 (13)	2 (7)	N/A
Myopathy Symptoms Lasting More than 1 month after Discontinuing Usage of Statins No. (%)	3 (20)	N/A	N/A
No. of Weeks Since Discontinuing Statin Usage, Median (Range)	12 (3-300)	N/A	N/A
No. of Participants with Significant Muscle Damage	9	16	1
No. of Damaged Myofibers in Participant(s) with Significant Muscle Damage No. (%) {Percentage Range of Damaged Myofibers Having Lesions}	9 (60) {2.8-100%}	9 (60) {3.3-43%}	1 (5)
Percentage of Fibers Injured Median value	9.0%	9.5%	N/A

Experimental results showing specific skeletal muscle damage linked to myopathy

With regard to damaged muscle fibers, participants in group 3 and group 4 showed evidence of significant muscle fiber damage in the form of lesions to their vastus lateralis muscles compared to the control group. Furthermore, of the 25 participants with skeletal muscle injury, 21 (84%) were actively using statins[4]. When viewing the muscles using light and electron microscopy, there was evidence of intact sarcolemmas detaching from the contracting part of the muscle. Other findings specific to statin users with myopathy (and not found in matched controls) included ghost cells (deteriorated cells with hollow T-tubules), inconsistency in muscle cells' sizes, and vacuolization of the T-tubules.

This experiment reveals that many patients presenting with statin-induced myopathy did have structural muscle damage. This is an alarming result as now myopathy may need to be

[4] This article (Mohaupt et al. 2009) is problematic as only 16/25 participants with skeletal muscle injury are current statin users (group 4).

considered as a more serious "red flag", indicating the start of muscle damage. The authors also pinpointed the appearance of myofibers with damage being limited to the T-tubules and the detachment of the sarcolemma. They hypothesized that the vacuoles formed in the T-tubule passageways may lend themselves to making the muscle susceptible to greater damage. Vacuoles in the T-tubule system may prevent the even transmission of an action potential to all myofibers, impeding proper muscle contraction. Further investigation is needed to clearly define how vacuoles in the T-tubules affect muscle fiber function. The other major finding, detachment of the sarcolemma, may also be problematic as it may prevent the proper depolarization of the membrane, leading to inconsistencies in muscle contraction. Further, the researchers suggested that the creatine phosphokinase did not leak into the blood stream, preventing a rise in blood creatine phosphokinase levels, due to the intact nature of the sarcolemma.

Expression of calcium homeostasis genes in myopathy patients' vastus lateralis muscles

In addition, the expression of mRNA for 8 different genes coding for proteins found in the T-tubules and adjoining sarcoplasmic reticulum was studied (Mohaupt et al. 2009). All of the genes chosen by the researchers correspond to proteins that are involved in intracellular calcium ion homeostasis. Calcium's importance lies in the fact that it is essential for muscle contraction (Tortora and Derrickson 2012). Calcium release from the sarcoplasmic reticulum is carefully regulated by proteins in muscle cells to prevent unwanted contractions; the researchers chose to study the expression of these genes to determine fluctuations in their concentrations related to myopathy in patients (Mohaupt et al. 2009).

Of the 8 genes related to calcium homeostasis that were studied, only one of the genes, the ryanodine receptor-3 (RYR3) gene, was expressed in greater quantities in participants with structural muscle injury (n=25). Ryanodine receptor-3 is found in variable amounts in adult skeletal muscle tissue along with ryanodine receptor-1 (RYR1). The high amounts of ryanodine receptor-3 mRNA were thought to be linked to problems with calcium homeostasis; however, the experiment could not prove if the increased amounts of the mRNA were caused by statin-induced muscle damage or by increased expression of the gene before using statins. mRNA for a different gene coding for sarcoplasmic reticulum transporting Ca^{2+} ATPase 3 was also found in greater quantities in participants with muscle injury, however, it was not found statistically significant, which the authors attribute to the diversity in the expression of this gene (Mohaupt et al. 2009).

This study is inherently problematic. Limitations of this study include

a) the small size of the experimental groups,
b) the lack of data indicating an average amount (with a range) of myofibers biopsied from participants in a particular group and,
c) neglecting to mention the average size of the myofibers,
d) ambiguousness and miscalculations mentioned previously,
e) the presumably small amount of myofibers biopsied,
f) determination of the significance of 2% of myofibers being damaged in the biopsy sample, which was thought to be low for the amount of myofibers sampled[5],
g) the variety of statins that the participants were taking, and lastly,
h) failing to follow up with participants.

[5] Participants did not report feeling any pain from this muscle (Mohaupt et al. 2009).

The significance level of 2% of the myofibers displaying damage is most problematic in this study. One myofiber may range from 100 microns to a few centimeters in length once it matures (Skeletal Muscle Fiber Structure 2005; Tortora and Derrickson 2012); in this experiment, a 3 mm x 6 mm biopsy yielded only 15-20 cells, totaling about 2.5-5 cells/mm. This translates to less than a third of one myofiber from those biopsied had to show structural damage to be considered significant. Finally, the researchers discussed that

i) no clear definition was established for what constituted statin-induced myopathy (i.e. certain symptoms etc.) before beginning the study,

a very serious oversight. From this study, numerical data should not be used to support any conclusions due to the ambiguities and inconsistencies in the way in which the article was written. Nonetheless, the electron micrographs are still valid, and anatomical changes to the muscle fibers can be believed as these changes were similar to those seen in other experiments (Obayashi and colleagues in 2011 and Hanai and colleagues in 2007).

Muscle myopathy in relation to statin accumulation in muscle fibers and/or systemic tissues

Studies of statins also fixate on whether the amassing of sta tins has a toxic effect on muscle and systemic tissues. A study performed with rodents with statin-induced myopathy focused on determining how statins buildup in muscle and body tissues over time in order to explain unusual cases of delayed onset of myopathy (Sidaway et al. 2009). Further, a comparison of the accumulation of statins in skeletal muscle with predominantly type I versus type IIB fibers was assessed. In previous studies, slow-twitch, oxidative, type I skeletal muscle fibers were found resistant to necrosis caused by statins, while fast-twitch, glycolytic, type IIB skeletal muscle fibers were more susceptible to cell death due to statin usage (Smith et al. 1991; Schaefer et al. 2004; Westwood et al. 2005 cited by Sidaway et al. 2009).

Experimental methods for testing statin accumulation

Statin-induced myopathy was induced in female rats by treatment with one of the following three statins: cerivastatin, simvastatin, or rosuvastatin. A fourth group of rats was given a smaller dose of rosuvastatin which was not anticipated to cause myopathy. After anywhere from 5-16 days, blood was drawn for creatinine kinase activity testing. Creatinine is a byproduct of metabolized creatine that is found in muscles and is filtered by the kidneys. Creatinine is an indicator of renal functioning, specifically the glomerular filtration rate (Creatinine 2012). Blood samples and skeletal muscle samples (from the soleus muscles and right gastrocnemius) were collected and preserved throughout the 16 day period at scheduled intervals. The muscle samples were inspected under light microscopy for signs of myopathy based on necrosis found in 2 or more muscles or if the plasma creatinine kinase levels exceeded 1000 $IU1^{-1}$.

Additionally, the muscle samples were tested for statins and cholesterol metabolites using the HMG-CoA reductase enzyme inhibition assay. On days 1 and 5, cerivastatin and simvastatin were distributed in doses large enough to cause myopathy, but no myopathy was determined at this time. Further monitoring of the rats during days 5-8 still showed no evidence of myopathy in any of the three statins. At days 10-16, the first signs of myopathy were evident in half of the rats in each of the three experimental statin groups (Sidaway et al. 2009).

Location of statin exposure

While the soleus and gastrocnemius muscles during the 16 day experiment showed very similar statin exposure for the three experimental groups, comparison of the muscles to the blood plasma revealed an unequal distribution of active statin metabolites. Accumulation of statin metabolites favored the skeletal muscles over the blood plasma. Studying the ratio of the active

statin drug in the gastrocnemius muscle compared to the amount of active drug found in the plasma, the three statins differed with the largest ratio calculated for simvastatin[6] > cerivastatin > rosuvastatin. The results denoted a greater amount of simvastatin concentrated in the gastrocnemius muscle relative to the blood plasma compared to the other statin drugs. Similar to the muscle/blood plasma ratio of statin exposure, a ratio of statin buildup in the liver was compared to the blood plasma as well. For cerivastatin, the ratio was very high (96.85) compared to the ratio seen with simvastatin (4.02) (Sidaway et al. 2009).

Conclusions disproving statin accumulation correlated to myopathy in both systemic and skeletal muscle tissues

Important conclusions were deduced from this study. Specifically, the method by which statins generated myopathy was not related to the previously-held notion of statin accumulation, either in the skeletal muscles or the systemic tissues. Accumulation of statins was ruled out as the cause of statin-induced myopathy due to stable levels of statin exposure in the body tissues from the initial dosage to the dosage on day 5. The trend continued in days 5-12 with no significant accumulation of statins in the systemic tissues during this time, yet signs of myopathy were starting to develop. This means that before and during myopathy no differences were seen in statin exposure levels. It is therefore a logical conclusion to attribute delayed onset of statin-induced myopathy to some other mechanism besides prolonged statin exposure in systemic tissues (Sidaway et al. 2009).

Conclusions about whether statin accumulation differs in muscle fiber type

A second important finding was related to the type of muscle fiber affected by the statin treatment. Past studies have isolated the fast-twitch, glycolytic, type IIB skeletal muscle fibers as the most susceptible to necrosis from statin-associated myopathy; in this research study, no difference was found in the amount of statin accumulation between the two types of muscle fibers for any of the three statin therapies. The similarity in statin buildup in the muscles lead to the conclusion that differences in the susceptibility of muscle fibers to necrosis from statins is based on the biochemistry and physiology of the fibers and not their individual statin-accumulating tendencies (Sidaway et al. 2009).

Lipophilicity vs. hydrophilicity in statins and its effect on statin accumulation in muscle fibers

The researchers also brought up the important concept of lipophilic versus hydrophilic tendencies of the statins. While no significant difference was found between muscle fiber types and statin buildup, there was some accumulation of statins in the muscle cells. When creating a ratio between the statins' exposure in muscle compared to the blood plasma, the ratio was tipped more in favor of the muscle cells for cerivastatin and simvastatin. The penetrance of these two statins reflects their characteristic propensity towards being slightly more lipophilic, and the researchers conjecture that the method by which these two statins cross the phospholipid bilayer of the myofiber and enter the cells is based on diffusion and not transporters[7] (Sidaway et al. 2009).

Lipophilic statins and increased myopathy

While lipophilic and hydrophilic statins have led to myopathy in skeletal muscle, another study verified the hypothesis that lipophilic statins in particular increase the risk of myopathy due to their ability to cross the phospholipid bilayer of the cells' plasma membrane. A study was

[6] The ratio for simvastatin was the same for both the 80 mg and 20 mg simvastatin experimental groups (Sidaway et al. 2009).

[7] Conversely, when the researchers studied the liver, transporters were thought to bring statins into the hepatocytes (Sidaway et al. 2009).

conducted on rats that tested two different statins, fluvastatin and atorvastatin. First, the statins were examined for their typical lipid-related results[8]. Then the rodents were euthanized, and the tibialis anterior, soleus muscle, heart, liver, and kidneys were extracted to determine their weight. In the fluvastatin rats taking 20 mg/kg/day, the tibialis anterior showed a significant reduction in weight, however, the soleus muscle did not show this reduction. Furthermore, the heart and kidney of the high dose fluvastatin rats were significantly heavier than the control rodents. This finding was dose-dependent as the rats on 5 mg/kg/day dosage of fluvastatin did not show differences in the sizes of their muscles or organs. Rats on atorvastatin did not show significant differences in organ sizes or muscle except for an increase in muscle size of the tibialis anterior muscle (Pierno et al. 2006).

Alteration to resting chloride membrane potential (gCl). ***gCl reduction***

Pierno et al. in 2006 proved that vast changes occur to the resting membrane chloride conductance (gCl) and the overall ability for sarcolemma excitement when using statins. Resting chloride membrane potential/conductance is an important indicator of sarcolemma functioning in muscle tissue. The gCl stabilizes the membrane after an action potential and assists in repolarization of the membrane for future action potentials (Bryant and Conte Camerino 1991 and Jentsch et al. 2002 cited in Pierno et al. 2006; Aromatans and Rychkov 2006 cited in Pierno et al. 2009). Rats on the 20 mg of fluvastatin showed a significant decrease in myofiber diameter of the extensor digitorum longus muscle and gCl (29% reduction), similar to atrophy seen in the tibialis anterior mentioned previously. Although rats on atorvastatin and 5 mg fluvastatin had larger myofiber diameters than the control rats, the gCl showed the same trend as in the high dose fluvastatin rats with atorvastatin rats having a 24% reduction in gCl and 5 mg fluvastatin rats having a 20% reduction in gCl.

Changes in four key factors related to muscle excitability due to reduced gCl

With the reduced gCl, the muscle fibers after exposure to statins tended to display more excitable behavior that was determined by measuring the following factors:

a) the smallest current needed to procure an action potential,
b) the amount of elapsed time between turning on the current and the first depolarizing spike that indicates the start of an action potential,
c) the maximum number of action potentials that could be elicited by a myofiber when stimulated by a current of a particular value in a 100 millisecond time period, and
d) just how depolarized a myofiber became after an action potential.

The following table reflects the changes to these four factors in each of the experimental groups (Table 5).

[8] An increase in HDL cholesterol was found in the rats on atorvastatin only, but for these rats, the total amount of cholesterol remained the same. Fluvastatin, with both the 5 and 20 mg/kg/day dose, showed modest reduction of total cholesterol of the rats, but no significant increase in HDL cholesterol in particular (Pierno et al. 2009).

Table 5: Effects of atorvastatin and fluvastatin on membrane excitability of extensor digitorum longus muscle fibers in rats. Source: Pierno et al. 2006

Statin Treatment	Muscle Fiber Excitability Changes			
	A	B	C	D
Fluvastatin, 20 mg	Decrease	Increase	Increase	Slight increase
Fluvastatin, 5 mg	No change	Increase	Increase	Slight increase
Atorvastatin, 10 mg	No change	Increase	Increase	No change

A – the smallest current needed to procure an action potential; B – the amount of elapsed time between turning on the current and the first depolarizing spike that indicates the start of an action potential; C – the maximum number of action potentials that could be elicited by a myofiber when stimulated by a current of a particular value in a 100 millisecond time period; D – just how depolarized a myofiber became after an action potential. All information is based on comparison to control.

Changes in voltage threshold for mechanical activation in muscle cells due to statins

Another factor examined was the voltage threshold for mechanical activation in myofibers extracted from the extensor digitorum longus muscles of the 4 rodent experimental groups (Pierno et al. 2006). The voltage threshold for mechanical activation relates to the amount of time needed at a specific current value to cause depolarization of the myofiber, ranging anywhere from 5-500 milliseconds. After subjecting the myofibers of control and statin-treated rats with a particular current for a set amount of time, contraction occurred more readily in the statin-treated rats than the controls. What was more interesting is the fact that the statin-treated rats had more negative resting potentials to begin with compared to the controls, yet they could reach the threshold for depolarization and produce an action potential more easily than control rats with less negative resting potentials when both groups were stimulated by exposure to the same current. In other words, an electrical pulse that could depolarize a statin-treated myofiber of a large negative resting potential would not be able to induce an action potential in a myofiber at the same negative resting potential when not treated prior with statins; to depolarize this non-statin treated myofiber at this negative value, the same size current would need to be applied to the myofiber for a longer duration (Pierno et al. 2006).

Implications of changes to muscle fiber excitability

Signs of serious changes to the excitability of the muscle fiber are evident in this study. Muscle fibers treated with statins will now contract readily when excited by a current whereas before the statin treatment, the same current would elicit an action potential. As a result, this change may lead to cramping or repeated contracting of a muscle in patients on statins. These results proved the original hypothesis that these lipophilic statins show a greater propensity for changing the muscle function evidenced by a decrease in the resting chloride membrane potential (gCl), an increase in the sarcolemma depolarization, leading to more action potentials, and finally, a more negative voltage threshold for mechanical activation (Pierno et al. 2006). Agreeably, the authors also suggest that since no changes were seen morphologically in the myofibers of the statin-treated rats besides for the decrease in fiber diameter, changes to the blood plasma composition may be a better warning sign of impending myopathy, possibly terminating in rhabdomyolysis (Table 6). An interesting finding was the increase in muscle mass in the tibialis anterior of the atorvastatin rats, not necessarily indicating hypertrophy, but may relate to an increase in protein production due to statins (Pierno et al. 2006). This, however, is speculation, but may have some truth.

Table 6: Effects of chronic treatment with atorvastatin and fluvastatin on biochemical parameters in rat plasma. Source: Pierno et al. 2006

Plasma parameter	Control	Atorvastatin $10mg \cdot kg^{-1}$	Fluvastatin $5mg \cdot kg^{-1}$	Fluvastatin $20mgkg^{-1}$
Myoglobin ($ng\ ml^{-1}$)	0.14±0.02	0.23±0.01 *P<0.02	0.16±0.02	0.28±0.05 *P<0.005
LDH ($mU\ ml^{-1}$)	587±76	1255±202 *P<0.005	550±77	915±151
CK ($mU\ ml^{-1}$)	1238±217	2118±202 *P<0.005	1468±40	1795±189 *P<0.05
Creatinine ($mg\ ml^{-1}$)	7±0.3	8±0.5	7±0.8	8±0.6
Potassium ($m\ Eq^{-1}$)	5.9±0.3	6.8±0.7	5.5±0.6	5.3±0.6
Azotemia ($mg\ ml^{-1}$)	0.48±0.02	0.55±0.04	0.45±0.03	0.62±0.05 *P<0.005
N (samples)	10	6	7	7

CK, creatine kinase; LDH, lactate dehydrogenase. Each row shows the mean ± SEM of the plasma parameters measured from the number of samples as indicated.
*Significantly different with respect to control (by Bonferroni's t-test) (Pierno et al. 2006).

Conclusions about atorvastatin potency and the possibility of statin accumulation leading to toxicity

Atorvastatin was found more potent than fluvastatin as only 10 mg of atorvastatin (compared to 20 mg of fluvastatin) resulted in elevated levels of muscle components in the blood plasma (Table 6). Elimination of atorvastatin took longer compared to fluvastatin (Corsini et al. 1995 cited in Pierno et al. 2006). The researchers also conjectured that results with atorvastatin support the view that statins produce myopathy by accumulation, yet accumulation of statins leading to toxicity was disproven in the experiment of Sidaway et al. (2009). The question that now arises is whether the results that Sidaway and colleagues found after this study in 2006 are generalizable findings. A possible reconciliation between the differences in opinion could be that Sidaway and colleagues proved that accumulation leading to toxicity did not occur particularly with simvastatin, rosuvastatin, and cerivastatin, while Pierno and colleagues tested atorvastatin and fluvastatin. While it would be much simpler if the accumulation of the statins could be related to their origin (i.e. naturally-derived statins cause accumulation and synthetic statins do not or vice versa), this does not resolve the issue as Sidaway and colleagues (2009) used both synthetic (rosuvastatin and cerivastatin) and a naturally-derived (simvastatin) statins. Also, the differences in opinion on statin accumulation cannot be attributed to lipophilicity as all five of the statins tested are thought to be more lipophilic by both sets of researchers. More research is needed to determine whether proof exists in the statin buildup theory from more current research studies done within the last few years. Coming back to the potency of atorvastatin in particular, it is most curious that even with muscle proteins in the plasma and myoglobinuria discharged from the kidneys in the atorvastatin-treated rats, no gross, microscopic damage was seen in the myofibers, and no decrease in muscle weight was noted (Pierno et al. 2006).

Electromyography findings of statin treatment on skeletal muscle

Fluvastatin (20 mg/kg/day and 5 mg/kg/day) and atorvastatin (10 mg/kg/day) were tested to determine how statins lower the gCl of muscle fibers, leading to myotoxicity. Biweekly, electromyography using micro electrodes inserted into the rats' gastrocnemius muscles was performed. Recordings of the electrical activity lasted 3-4 minutes in duration. Abnormalities in activity spikes (attributed to myotoxicity) in the muscles of statin-treated rats were determined by comparison to controls. Examination of the electromyographs showed that after 7-8 weeks of statin treatment, 10% of rats on both the high and low doses of fluvastatin and 20% of those on atorvastatin showed additional electrical spikes 500 milliseconds in length, not seen in the control rats, occurring after spikes related to muscle movement (Pierno et al. 2009).

gCl reduction and muscle fiber type

Results showed between 20-35% decrease in the gCl of the extensor digitorum longus muscles of rats on statins compared to the matched controls, yet the soleus muscle did not show any significant change in gCl due to statins (Pierno et al. 2009).

Reversing changes to gCl:

Chelerythrine as a protein kinase c inhibitor

Effects on slow and fast twitch muscles after statin administration

Chelerythrine, a known protein kinase C inhibitor, was added to the extensor digitorum longus muscles to see if it stopped the drop in gCl due to subsequent administration of statins. The effects of chelerythrine were studied both ex vivo and in vitro. Ex vivo administration of 1 µmol/L of chelerythrine to the extensor digitorum longus muscles of the control rats showed a small increase to the gCl. The results were appropriate as a large increase in the gCl was not expected to occur since the extensor digitorum longus muscle is a fast twitch muscle, and the ClC-1 channels are already in an open state[9]. Chelerythrine increased the gCl in all three statin groups compared to statin administration alone. Of the three statin experimental groups, atorvastatin displayed the most significant restoration to the gCl, increasing it by 40% compared to control muscles with statin treatment only and raising it to the gCl level of muscles from the control rats not on statins (Pierno et al. 2009).

Decreased body mass and mobility

Damage to muscle by statins can affect overall health and may limit mobility. Rodents on the 20 mg/kg/day dose (higher dose) of fluvastatin began to eat less by weeks 3 and 4 and showed a decline in their gross weight. Two out of the ten rats in the experimental group on the higher dose of fluvastatin showed difficulty with the righting reflex[10] and demonstrated evidence of paralysis in their lower bodies. The other groups of rats (5 mg/kg/day fluvastatin and 10 mg/kg/day atorvastatin) fared like the control rats and did not show these movement-related issues (Pierno et al. 2006). People taking statins, especially those on high doses should therefore be aware of possible disturbances to their weight, gait, and general ease of manipulation of their muscles.

[9] When the researchers tested the effects of atorvastatin 50 µmol/L dose on the soleus muscle in vitro, no reduction occurred to the gCl reflecting the already lowered gCl that is found in slow twitch muscles. Further study was then focused solely on fast twitch muscles with higher baseline gCls (Pierno et al. 2009).

[10] The righting reflex tests rodents for the speed at which they are able to return to their natural, ventral position (resting on their paws) after being flipped onto their backs.

Effects to the excretory system: Alterations in blood plasma leading to changes in urine composition

The excretory system is also affected by statins. Protein that is released into the blood by dying muscle is filtered by the kidneys. To determine the effects of the statins (fluvastatin and atorvastatin) on both muscle and kidneys, the composition of both the blood plasma and urine were studied (Pierno et al. 2006). Damage isolated to muscle tissue was evident when elevated levels of the following compounds were seen only in the blood plasma (and not urine): myoglobin, lactate dehydrogenase, creatine kinase, potassium, azotemia, or the measure of how much nitrogen originating from urea is found in the blood, and/or creatinine. If these compounds were found in both the plasma and the urine, kidney filtration was also thought to be impaired. The following results were noted (Table 7).

Table 7: Blood plasma and urine composition after statin treatment on rats. Source: Pierno et al. 2006

Statin	20 mg/kg/ day Fluvastatin	5 mg/kg/day Fluvastatin	10 mg/kg/day Atorvastatin
Blood plasma			
Myoglobinemia	Increase	No change	Increase
Lactate Dehydrogenase	No change	No change	Increase
Creatine Kinase	Increase	No change	Increase
Creatinine	No change	No change	No change
K^+	No change	No change	No change
Azotemia	Increase	No change	No change
Urine	*	No change	No change
Myoglobinuria		No change	Increase
Creatinuria		No change	No change
Urinary electrolytes		No change	No change
Na^+		Decrease	Decrease
K^+		Decrease	No change
Cl^-		Decrease	No change
Phosphate		Decrease	Decrease
Proteinuria		Decrease	No change

Changes to the blood (increase, decrease, or no change) are related to the criteria in the control rats not on statins.

*No testing was done on the urine of rats on the 20 mg/kg/day fluvastatin dose.

The different treatments with the statins led to varied compositions of the blood plasma and urine. Fluvastatin strictly at the higher dose led to increased levels of myoglobinemia. Myoglobinemia is the release of the heme-containing pigment called myoglobin into the blood. Normally found in skeletal muscle to aid in delivering additional oxygen to these tissues during vigorous activity, myoglobin that is released into the blood is filtered by the kidneys. Excessive secretion of myoglobin can result in myoglobinuria, urine rich in myoglobin, and ultimately, can damage the kidneys and renal failure may result (Shankar et al. 2002). In this experiment, other increases in substances and chemicals in the blood due to fluvastatin included plasma creatine kinase and azotemia. Atorvastatin led to significant increases in myoglobin, lactate dehydrogenase, and creatine kinase in blood plasma (Pierno et al. 2006).

Motor Neuron Damage: Reports of Amyotrophic Lateral Sclerosis-like symptoms in statin users

A more alarming side effect of statins is the development of lesions on motor neurons. Reports from Vigibase, the database for WHO for International Drug Monitoring, picked up on over 40 profiles of patients on HMG-CoA reductase inhibitors that contained reports of 'upper motor neuron lesion' or 'amyotrophic lateral sclerosis' symptoms. Amyotrophic Lateral Sclerosis (ALS) is a rare, fatal disease in which motor neurons degenerate; the possibility of statins causing these effects is very worrisome. If statins do cause damage to motor neurons, muscles may become atrophied or weak from lack of proper innervation. Many statins were reportedly used that produced ALS-like symptoms by Vigibase including simvastatin, atorvastatin, cerivastatin, lovastatin, and rosuvastatin. Other frequently compounded symptoms experienced by these patients while taking statins included myalgia, myopathy, falling and balance problems, and difficulty with speech and manipulation of the tongue, as well as others patients (Edwards et al. 2007). This information is relevant to the caution that must be exercised when taking statins. More research is needed to further assess the connection between neuromuscular issues and statins.

Prevention of statin damage to muscle

PGC-1α inhibits atrogin-1 expression and its implications

Prevention of lovastatin-induced muscle damage was achieved by the regulation of the PGC-1α gene mentioned previously. PGC-1α was determined in other studies (Sandri et al. 2006 cited in Hanai et al. 2007) to inhibit atrogin-1 expression thereby lessening muscle atrophy. The experimenters tested this premise with zebrafish embryos. After injecting the embryos with cDNA segments containing the PGC-1α gene, expression of the protein coded for by PGC-1α prevented the side effects of lovastatin alone including muscle damage, atrogin-1 expression, and muscle cell shrinkage.

Comparison of mitochondrial activity between embryos given lovastatin with and without added PGC-1α cDNA was also examined. Cells with injected PGC-1α showed more active mitochondria and increased mitochondria activity than cells without the added gene. Similarly, myotubes treated with PGC-1α genes when given lovastatin showed no changes in muscle morphology, atrogin-1 expression ceased, and oxidative phosphorylation genes were turned on, indicating mitochondria activity (Hanai et al. 2007).

This experiment suggests the possibilities for PGC-1α to be used to counter the negative effects that statins have on muscle tissue. The authors speculate that using a drug to trigger the expression of PGC-1α may be a viable option[11] (Hanai et al. 2007). Yet, this study is specific to

[11] The authors mention the drug metformin, used to treat type 2 diabetes, as a specific example of a drug that increases PGC-1α expression (Hanai et al. 2007)

lovastatin and may not allow for generalization to other statins. A second consideration is whether this study can be extended beyond myotubes and zebrafish embryos to clinical practice on humans. It must also be mentioned, however, that increasing PGC-1α expression may be detrimental to other body tissues, and it may reverse the beneficial outcomes that statins have on coronary disease, namely increasing HDL cholesterol and lowering LDL cholesterol. More research is needed to clarify the side effects of abundant PGC-1α expression on systemic tissue and cardiac muscle. Still, the prospect of using a drug that increases PGC-1α expression to prevent muscle damage due to statins is very promising.

Results of knocking out atrogin-1

Interestingly, the absence of the atrogin-1 gene in zebrafish embryos prevented muscle damage due to lovastatin administration or HMG-CoA reductase knock out (Hanai et al. 2007). In order to verify that atrogin-1 is the reason for the changes to zebrafish embryos' skeletal muscle seen after lovastatin administration (as opposed to another gene), the atrogin-1 gene was knocked out allowing for a survey of the lovastatin-treated muscle. The researchers mimicked the procedure used to knock out the HMG-CoA reductase gene (and corresponding mRNA) for the atrogin-1 gene[12]. The results demonstrated a significantly lower degree of lovastatin-induced damage to the zebrafish's skeletal muscle with the knock out atrogin-1 gene compared to the wild-type that was homozygous for atrogin-1. Further, when the zebrafish lacked both the HMG-CoA reductase gene and the atrogin-1 gene, distortions to muscle morphology were significantly less, leading to the conclusion that eliminating the atrogin-1 gene reverses muscle defects that would otherwise be present due to HMG-CoA reductase knock out (Hanai et al. 2007) Targeted knockdown of zebrafish HMG-CoA reductase has a muscle phenotype similar to that with lovastatin treatment and can be rescued by zebrafish atrogin-1 knockdown).

Clinical application viability for atrogin-1

It is questionable as to whether knocking out atrogin-1 can be used clinically to prevent statin-induced muscle damage. This process is likely much simpler in less complex and/or less developed organisms such as zebrafish embryos compared to humans. Additionally, knocking out the atrogin-1 gene may have other repercussions on the body that may far outweigh the benefits seen from eliminating the possibility for tissue atrophy. If atrogin-1 targeting was to be employed to help statin users, more research and testing would be necessary to determine all of the outcomes that result from its expression.

Statins and cardiac muscle

Since statins affect skeletal muscle, concern arises as to whether these same effects will appear in cardiac muscle tissue. Little of the research presented here indicated any effects, positive or negative, to cardiac muscle tissue. However, preliminary findings from the research mentioned here seem to indicate that statins do not target cardiac muscle tissue. In the study of Mohaupt et al. (2009), ryanodine receptor 3 showed increased expression in skeletal muscle, but its analog, ryanodine receptor 1, that is found to a lesser degree in skeletal muscle but principally in cardiac muscle, was not significantly expressed as mRNA more than that seen in the control. Also, atrogin-1 knock out did not generate defects in cardiac muscle tissue so this method may be a possible candidate for negating statins' effects on skeletal muscle (Serrano et al. 2010 cited in Hanai et al. 2007). More examination of the research is needed to determine effects seen to cardiac muscle after statin usage.

[12] Zebrafish embryos without the atrogin-1 gene did not show any significant differences in muscle morphology compared to atrogin-1expressing muscle cells before administering lovastatin, maintaining the integrity of the trial (Hanai et al. 2007).

CONCLUSION

Much research has been done and continues to be performed on statins, which could not all be discussed here. Other findings not discussed included statins effects on Ras and Rho proteins (Liao and Laufs 2005), applications with cancer patients (Demierre et al. 2005), and effects on coagulation and fibrinolysis processes (Krysiak et al. 2003), and much more. Due to the lack of a better option to reduce cholesterol levels, statin usage will continue, but it is advised that statin usage should be limited to patients with a history of coronary heart disease due to high cholesterol levels. It is strongly discouraged for use on patients with heart disease unrelated to cholesterol as well as autoimmune and cancer patients if other treatments are viable that have been known to improve the condition. Use of statins by these patients may not help their original disease and may cause further complications such as muscle breakdown and possible kidney damage. For patients with coronary disease and high cholesterol, it is advised that patients should have their blood and urine regularly tested for changes mentioned here, preferably within 1 month of beginning statin usage and once a month, subsequently. Changes in chemicals, specifically creatine kinase, found in the blood does not necessarily mean that a patient should cease statin usage on the basis of creatine kinase levels. Although participants with myopathy had increased creatine kinase levels, this increase was not limited to the patients taking statins (Mohaupt et al. 2009).

Muscle pain should be reported to the physician immediately, since pain could be a sign of damage to muscles (Mohaupt et al. 2009). Also, difficulty with controlling muscle movements or speech could be a sign of the rare, but serious, motor neuron damage and should be reported. Quality of life and mobility may be reduced with statins if muscle damage is not caught early. In addition, statins may not prevent a cardiovascular episode and do not reverse coronary disease (Nicholls et al. 2011). Yet, overall statins accomplish their lipid-related tasks well and may even produce regression in atheroma size.

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ur=333&sig=116465313956319292164&page=3&tbnh=123&tbnw=126&start=43&ndsp=29&ved=1t:429,r:8,s:43,i:250&tx=49&ty=91.

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ANOREXIA NERVOSA: CURRENT RESEARCH FROM A BIOLOGICAL PERSPECTIVE

Udy Tropp

ABSTRACT

Eating disorders are viewed as serious mental illnesses, carrying significant, life-threatening medical and psychiatric implications, including morbidity and mortality. According to the Academy of Eating Disorders, anorexia nervosa has the highest mortality rate of any psychiatric disorder. The American Psychiatric Association (2004) claims that approximately three percent of the United States female population has a clinically relevant eating disorder. Risk of premature death is 6-12 times higher in women with anorexia as compared to the general population, and it has become the third most common form of chronic illness among adolescent women aged 15 to 19 years. Although the prevalence and seriousness of this problem have gained increasing attention in recent years, relatively little is known about the role that leptin plays in this disorder. Leptin is a starvation hormone as well as a satiety hormone that plays a role in the diagnoses, duration, and recovery of this devastating disease. The review of the research will attempt to define the etiology of the endocrine events and the significant physiological impact on the body's structures relative to this disease. The diagnosis and treatment will address and reflect the physiological effects caused by the semi-starvation state produced by anorexia nervosa. Anorexia is triggered by psychological problems that transform into biological issues. Profound physiological changes brought about by the semi starvation state cause a domino effect. The biological ramifications of the disease should be cured before psychological counseling is attempted.

INTRODUCTION

Diagnosable eating disorders, such as anorexia nervosa, are not uncommon, occurring in approximately three percent of the United States female population (American Psychiatric Association [APA] 2004). According to the Academy for Eating Disorders (2011), eating disorders have become the third most common form of chronic illness among adolescent women aged 15 to 19 years. Anorexia Nervosa (AN) is classified in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) as refusal to maintain a minimally normal body weight (i.e. below 85% medically ideal body weight), profound fear of weight gain, body image disturbance, and amenorrhea (i.e. absence of menses) (American Psychiatric Association 2004). According to the American Psychiatric Association, anorexia nervosa has the highest mortality rate of any psychiatric disorder (Yager, 2000). Even when feeling hungry, afflicted individuals severely limit their food intake based on their irrational fear of gaining weight, due to the distorted perception they have of their body as perpetually being overweight. Despite their dwindling weight, those suffering from anorexia nervosa will always see themselves as fat. It is because of their skewed self-image and irrational fear of obesity that anorexia nervosa is considered a disease that is psychiatric in nature.

HISTORY OF TREATMENT AND PERCEPTION OF ANOREXIA NERVOSA

Anorexia nervosa is not a new phenomenon. Although the disease did not receive its name until recently, its classic symptoms have been around of centuries. Recorded cases of symptoms that appear to be the disorder first appear in 1689. Two case studies by Dr. Richard Morton illustrate that although the symptoms and effects were physical, physicians usually treated the patients as if it was a purely psychological disorder. This often led to disastrous and, at times, fatal results. The first case Morton presents is that of a young woman who exhibited extreme weight loss, distorted self-image, and loss of menarche (three of the four of the current criteria for anorexia nervosa in the DSM-IV (APA 2004). It was recorded that the patient's flesh was flaccid and loose and extremely pale. However, Dr. Morton framed the problem as stemming from "a multitude of cares and passions of her mind." Dr. Morton

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concluded that it was a psychological disorder, that she could be as "different" as she wanted to be, and that this is not a doctor's area of expertise. The young woman died two months later. In another case, a young man displayed similar symptoms. Here too, Dr. Morton concluded that the young man was suffering from "passions of the mind" and prescribed some rest and relaxation in the countryside. The young man died shortly thereafter (Kendall 2011).

These cases constitute just two of many that illustrate how physicians in the past ignored the biological symptoms and effects of the disease. Any psychological condition was dismissed as one having a weak mind or lack of strength, and thus untreatable. No attempts were made to understand the harrowing condition. It was not until the late ninteenth century that anorexia nervosa was to be widely accepted by the medical profession as a recognized condition. In 1873, Sir William Gull, one of Queen Victoria's personal physicians, published a seminal paper which established the term anorexia nervosa and provided a number of detailed case descriptions and treatments. In the same year, French physician Ernest-Charles Lasègue similarly published details of a number of cases in a paper entitled *De l'Anorexie Histerique*. The physicians began to hospitalize and attempt to stabilize the patients physiologically.

During the late twentieth century, the disorder became classified as a purely psychological disease. By the time most patients were diagnosed, they were already close to death. More were forcefully hospitalized and received psychiatric treatment. This method of therapy has been largely ineffective at helping individuals suffering from the disease in recovering long-term. Perhaps the strongest proof of this is that anorexia nervosa has the highest mortality rate of all psychiatric conditions. Long-term data indicate that the mortality rate is 5-10 percent, with only 50 percent of patients regaining their normal weight (Mehler et al. 1999).

This paper will focus on an alternative to a purely psychological approach by concentrating on a biological perspective. The diagnosis and treatment will address and reflect various physiological ramifications caused by the semi-starvation state produced by anorexia nervosa, signaled by hypoleptimia.

METHODS

The materials used in this research paper included the Anatomy and Physiology and Biological Psychology course textbooks. Further, the author utilized the Touro College library search engine and retrieved journal articles from EBSCO as well as Google and Google Scholar to find basic information and peer reviewed articles on the topic.

DISCUSSION

There are four criteria that must be satisfied to be considered anorexia by the DSM-IV (APA 2004). Two of these criteria are psychiatric conditions: distorted body image and the irrational fear of gaining weight and/or becoming fat. The other two are biological concerns brought about by the first two factors. Body Mass Index, calculated from a person's weight and height, provides a reliable indicator of body fat. For most people, it is used to screen for weight categories that may lead to health problems (Centers for Disease Control and Prevention 2011). The third criterion is refusal to maintain a weight of at least 85 percent of that expected for height. This can be translated into the fifth-tenth body mass index percentile. Figuring for body mass index, as opposed to merely weight, is vital because weight alone does not tell the full picture. If someone is five feet one inch and 105 pounds, the individual is not underweight. If someone is five feet eleven inches, the individual is severely underweight at 105 pounds. "Adults should normally have a body mass index between 18.5 and 24.9… a body mass index of under 17.5 is indicative of anorexia nervosa" (Blows 2011). A body mass index below 17.5 means the body is now in a semi-starvation mode. It is scrambling to survive and will use and conserve whatever physiological reserves it possesses in order to remain alive and functioning. There is a cascading effect of short-term and long-term physical effects in the body. One of the effects is amenorrhea. Amenorrhea is the absence of a menstrual period in a woman of reproductive age. The

presence of amenorrhea is the fourth criterion, and it is purely physical and biological. The fact that a state that can only exist in women is one of the four criteria for anorexia is telling. Psychologically, there are many reasons why, according to the National Association of Anorexia Nervosa and Associated Disorders (2013), approximately 90-95 percent of anorexia patients are women. Because their fat distribution is higher, women's systems rely on leptin levels more heavily than men. Women, having higher leptin levels than men, are thus more susceptible, because when leptin levels are lowered, the effects are more rapidly and radically felt by women.

The point at which the disorder transforms from a purely psychiatric issue into a biological one is complex. Acute calorie deprivation for 2-3 days results in a significant decrease in leptin concentration before major changes in bodyweight or fat mass go up 20-30 percent (Chan et al. 2005). Thus, the body's leptin levels are significant. It begins when the caloric intake is decreased so much as to decrease the resting energy expenditure, also known as resting metabolic rate. This represents the amount of calories required in a 24-hour period by the body during a non-active period. At what point is the body considered to have entered the semi starvation state? The consensus seems to be that when a person decreases the total caloric intake of less than 50 percent of what the body requires, a semi starvation mode ensues. The metabolic rate is significantly lowered and begins to make significant changes in the body's functions. These adaptations are controlled processes involving apoptotic shutdown and organ hypotrophy that can delay death. In the early stages of malnutrition, there is scope for tightening the efficiency of various metabolic functions and for closing down some that are non-essential in the short term (e.g. reproduction). As the malnutrition worsens, long-term decisions must be made by the body to keep the individual alive. Before all those changes can be made, the resting energy expenditure must drop. What tells the metabolic rate that there is not enough caloric intake to survive and it must begin to make changes? What acts as the peripheral signal to change the body actually into the semi starvation mode and lower the resting energy expenditure?

One such signal is leptin, which has two functions. One function is as a pleiotropic hormone that relays information about peripheral energy storage and availability to the brain (Kumar et al. 2010). Its complete role as both a starvation and satiety hormone is illustrated in Figure 1. Leptin is a lipoprotein, which can pass the blood-brain barrier. It is made by adipose cells and inhibits the production of fat and increases the individual's physical activity potential. Leptin is twice as high in human beings that are obese, and this is related to their fat mass and high rate of leptin production by the body.

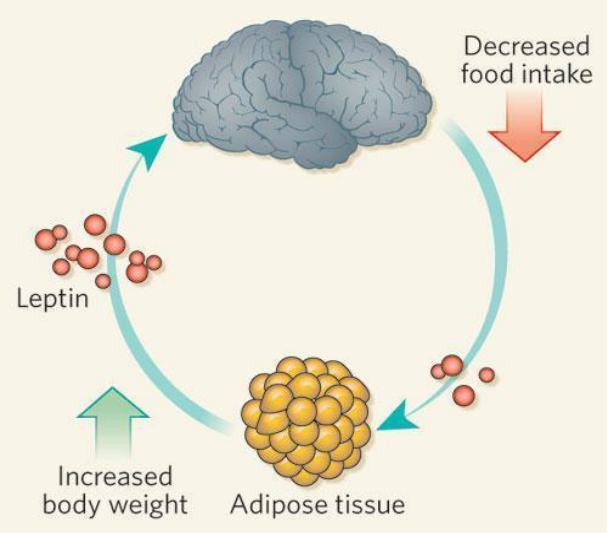
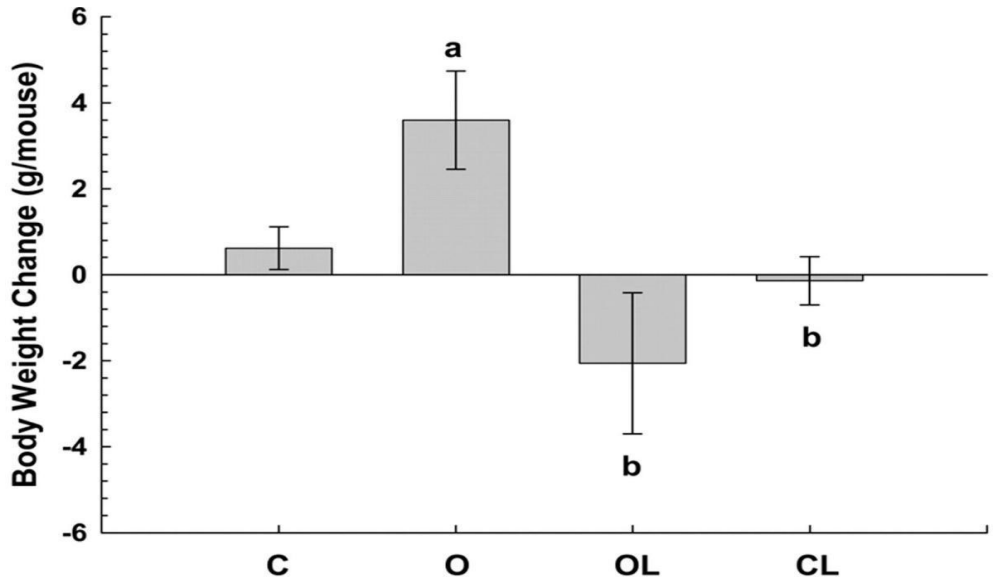


Figure 1: Cycle of Leptin production in the body. Source: Friedman 2009

This figure shows that an increase in body weight also increases the amount of leptin produced. Leptin then crosses over the blood brain barrier and inhibits the brain, which leads to a decrease in food intake of the body. Once synthesized, leptin is secreted through a constitutive pathway and not stored in the cell. Chan and his colleagues (2005) describe leptin as a 167 amino acid protein product of the ob gene that was discovered in 1994 through positional cloning in the ob/ob obsese mouse, a model of morbid obesity resulting from the absence of leptin due to a gene mutation. It is secreted in a pulsatile fasion and has a substantial diurnal variation with an increase of about 50 percent in the late evening and early morning hours that might be related to an intrinsic circadian component, meal timing, and the sleep-wake cycle. The original study and research was conducted using mice as illustrated in Figure 2. Severely obese mice were observed and discovered to have a mutation that did not allow them to produce the leptin hormone. When artificial leptin was administered, the mice began to slim down.

Figure 2: The Effect of leptin on body weight changes in C57BL/6J (control) and ob/ob (leptin-deficient) mice. Source: Claycombe et al. 2008



C, control; O, obese; OL, obese with leptin treatment; CL, control with leptin treatment

Schematic illustrations show of the effect of leptin on body weight changes *ob*/*ob* (leptin-deficient) mice. Mice were weighed at day zero before the start of leptin and saline injection. Body weight measurements were gathered daily for the next seven days, and the amount of leptin injected was adjusted accordingly. The mean values were calculated by averaging daily body weight differences for each mouse for all groups. Leptin treatment occurred over the span of seven days. As shown, the bar with letter *a* is significantly different from the control group. The bars with letter *b* are significantly different from the obese group. The hypothesis was that this satiety hormone could be a possible treatment of individuals suffering from obesity. Because individuals with anorexia do not eat proper meals and are on an incorrect cycle, leptin levels can be severely affected as well. However, researchers realized how complex, not only obesity is, but leptin as well. When studying obese individuals, they found that many had enough leptin. The theory was that although these obese individuals had enough leptin to signal their hypothalamus that they are not hungry, the body did not perceive the proper amounts of the hormone due to a default in their receptors. Therefore, leptin is not

the key to unlocking the mystery of causation and treatment of obesity. Although this theory proved to have some faulty hypothesis, the discovery of leptin is vital in the study of weight and anorexia as a disorder and its role as a satiety and starvation hormone. It was realized that leptin might be more important at the other end of the energy homeostasis spectrum, i.e. energy deprivation. In this context, studies in mice, as well as people, have shown that leptin has a role in the neuroendocrine adaptations to starvation which includes changes in hormone concentration that have a protective effect for patients with anorexia. Energy deficient conditions such as anorexia reflect low leptin levels, which could play a vital part in their pathophysiology ad potentially their treatment.

Leptin is initially produced by the adipose tissue and is secreted into circulation. It crosses the blood-brain barrier with the help of a transporter protein to bind to leptin receptors in the arcuate nucleus of the hypothalamus (Blows 2011). The nervous and endocrine systems act together to coordinate functions of all body systems as illustrated in Figure 3. The endocrine system helps regulate virtually all types of body cells. For many years, the pituitary gland, or hypophysis, was thought to be the master endocrine gland because it secretes several hormones that control other endocrine glands. We now know that the pituitary gland has a grand master as well: the hypothalamus. This small region of the brain below the thalamus is the major link between the nervous and endocrine systems.

At the point when the body mass index hits below 17.5, leptin signals the hypothalamus to go into semi-starvation mode. Three main brain axes are affected by the semi-starvation mode: the hypothalamus-pituitary-gonadal, hypothalamus-pituitary adrenal, and hypothalamus-pituitary thyroid axis. The metabolism slows down, and the first system that is shut down is the reproductive system.

Figure 3: Energy balance is regulated through complex interactions of factors in the body and the brain among which the hormone leptin plays an essential role. Source: Hofbauer & Huppertz 2002

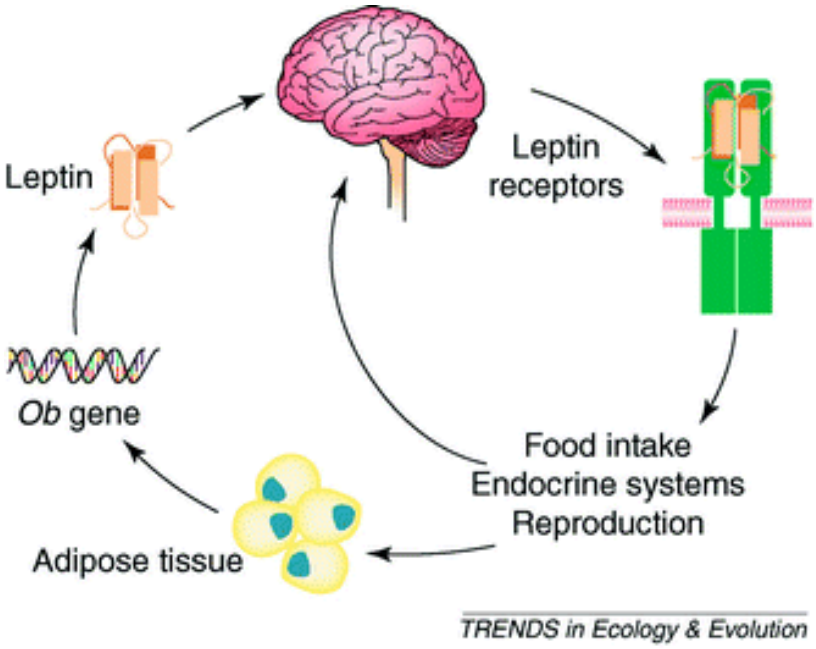


Figure 3 illustrates leptin and energy balance. Leptin is encoded by the Ob gene, which is expressed in white adipose tissue. Leptin is similar in structure to cytokines, which are heterogeneous group of endogenous, bioactive peptides released mainly from inflammatory tissue and cells of the immune system. It is secreted into the blood and crosses the blood-brain barrier, probably via a carrier system which has a limited maximum transport system. In the hypothalamus, leptin acts on specific receptors of the class I cytokine receptor subtype. Through stimulatory or inhibitory effects on downstream effector systems, leptin reduces appetite. However, in its semi starvation mode, it also influences various endocrine systems, such as adrenocortical and gonadal hormones. In the long term, decreased food intake leads to a reduction in adipose tissue and consequently a diminished production of leptin, which then increases food intake. The fourth criterion of the DSM-IV is met when the body enters amenorrhea. Like a domino effect, energy becomes more and more scarce throughout the body, triggering numerous hormonal cascades. Each cascade hits a specific axis. However, each cascade works along the same series of steps. The initial trigger is hypoleptimia, which will signal the hypothalamus to secrete either a releasing or inhibitory hormone. Each axis will react differently and, therefore, need a different type of hormone. At this point, the pituitary gland will release a specific hormone. Although the hormone will differ at each axis, its ultimate result will remain the same. This releasing hormone will trigger a specific endocrine gland to secrete yet another hormone, which will directly affect one of the critical homeostatic ranges of the body. This is turn will disrupt one of the vital metabolic processes that keep the organism alive. The same initial trigger, leptin, and ultimately the same devastating results (having affected homeostatic ranges) are observed.

The first axis cascade, of the hypothalamic-pituitary gonadal axis, begins by a state of hypoleptimia, and this causes a reduction in gonadotropic-releasing hormones (the inhibitory hormone secreted by the hypothalamus). At the same time, there is sequential reduction in the follicle stimulating hormone (FSH) and luteinizing hormone (LH), which is normally secreted by the anterior pituitary. The luteinizing hormone is responsible for the ovarian-follicle growth that leads to ovulation. With the reduction of the ovarian function, ovulation and reproductive functions cease, including menstruation (Usdan et al. 2008). Estrogen is a key factor in ovulation because the amount must reach a certain level

for ovulation to be triggered. Without enough estrogen, ovulation will not occur. Estrogen levels decrease dramatically in women with anorexia nervosa because one of the components of estrogen is cholesterol, which is derived from fat. With little to no fat being consumed in one's diet, there are simply not enough raw materials to create the necessary estrogen.

Any factor causing an organism's condition to waver away from homoeostasis can be interpreted as stress. In such instances an organism's fight or flight response recruits the body's energy stores and focuses attention to overcome the challenge at hand; thus, stress throws off nearly all the homeostatic ranges in the body. Cortisol is one of the stress hormones produced in high levels as a result of severe caloric restriction, and it helps the body manage stress when a person is in semi-starvation mode. Cortisol, which is produced by the adrenal gland, metabolizes fat and sugar into energy as its primary function. Anorexia catapults the body into starvation mode, and the body interprets a shift of homeostatic ranges as extreme stress. Cortisol levels spike, and the body shifts to fight or flight mode to survive. This leads into the second hormonal cascade.

The hypothalamic-pituitary-adrenal axis is affected next. As glucose becomes scarcer with the continued severe restriction of carbohydrates, the hypothalamus triggers a rise in corticotropin-releasing hormone (CRH) which, in turn, triggers the pituitary to increase adrenocorticotropic hormone (ACTH) secretion. This raises the cortisol levels of the adrenal gland. At this point, the autonomic nervous system takes over, particularly the sympathetic portion, shifting from voluntary to involuntary, and the fight or flight response becomes activated. As this is occurring, the rising level of cortisol triggers lipolosis and gluconeogenesis. Any other available raw materials that are not specifically carbohydrates are converted into glucose to provide energy from the body to survive. The first step the body takes is moderate, using raw materials other than carbohydrates to maintain the energy required. As the disease progresses and there is less raw material available, the body begins to consume itself (i.e. muscles) in a further attempt to keep homoeostatic ranges and the essential body systems running.

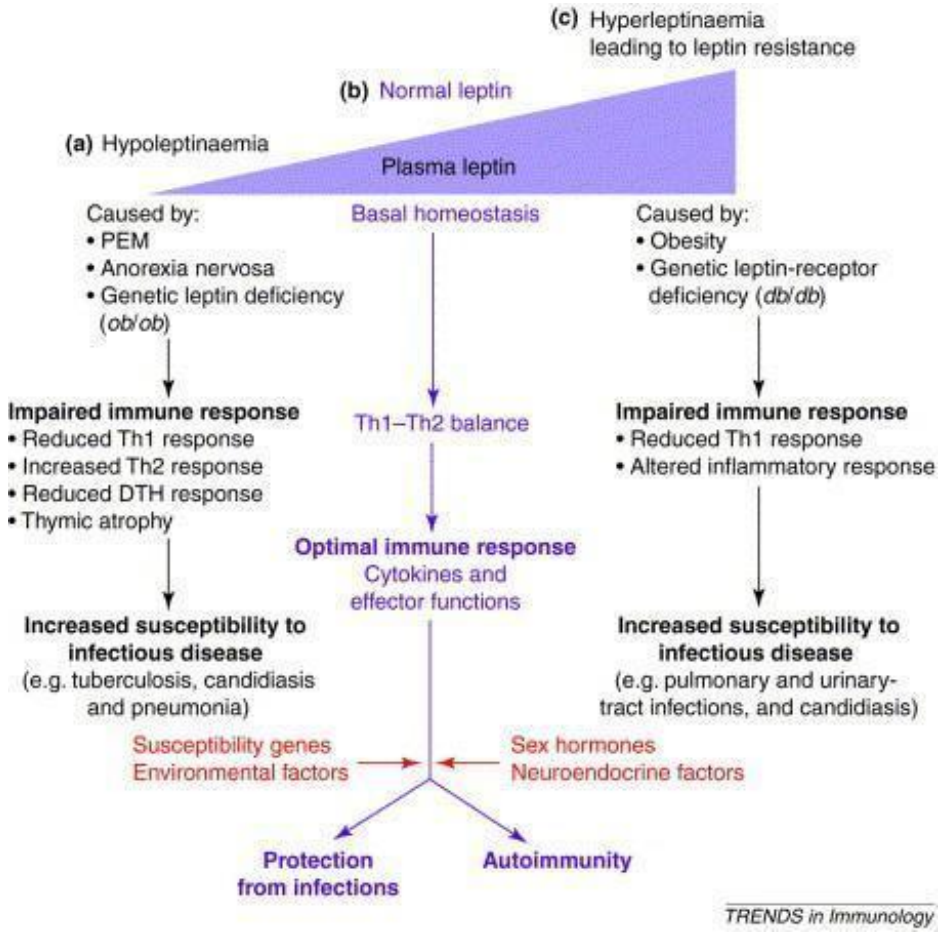
The rising cortisol level and the semi-starvation state elicit a response from the hypothalamic-pituitary-thyroid axis, the third main brain axis. The thyrotropin-releasing hormone (TRH) from the hypothalamus is decreased, prompting a decrease in the thyroid-stimulating hormone (TSH) from the pituitary gland. This, in turn, decreases the release of T3 and T4 and the overall function of the thyroid. This is significant because while most hormones require specific receptors within cells to affect them, the thyroid gland produces hormones that can interact and affect almost every cell in the body. While thyroid irregularity is not fatal, it does throw the body into disarray. The patient cannot sustain health because thyroid hormones contribute to so many important physiological processes, such as development, growth, and metabolism. Because of this hypothyroidism, the whole metabolic rate is lowered and can go into hypothermia. Since the appetite cannot be appropriately controlled, glucose will not be absorbed properly, as hypothyroidism mimics the exact initial symptoms of anorexia (i.e. lack of food intake). At this point, hypothyroidism is a symptom of a progression of the disease.

As discussed, the body initially attempts moderate measures to continue its upkeep, regardless of the severe decrease of nutrition, and eventually moves on to more radical measures. Reproduction activity ceases and the body begins inhibiting production of hormones responsible for growth stimulation, focusing on increasing hormones that help maintain bone, tissue, and muscle already present. The body attempts to preserve the raw materials it already possesses. An example of this is that the body decreases the production of insulin-like growth factors which are responsible for the promotion of cell proliferation. Insulin-like growth factor is thought to be a primary growth factor and is required to achieving maximal growth. In contrast, there is a significant increase in growth hormone, which is a peptide hormone that stimulates regeneration in humans .One of the functions of the growth hormone is to help maintain muscle and bone mass of the adult body. It promotes healing of injuries and tissue repair. It also helps convert all reserves other than glucose, such as fats and amino acids into energy the body can use to survive.

Bone mass is lost early on in the course of the disorder. The body's desperate attempt for survival causes it to shut down bone formation, in order to conserve its small energy reserve. The body's desperate attempt for survival causes it to shut down bone formation, in order to conserve its small energy reserve. "Recently bone has been recognized as a highly metabolic tissue requiring energy such that new bone formation is appropriately suppressed with inadequate nutrition" (Sum et al. 2011). Until the point of decreased bone mass, with proper refeeding, the negative cascade of biological disarray wreaked on the body by starvation is reversible. Decreased bone mass, however, is a permanent loss, as bone cannot replenish itself. Bone regeneration is an active area of research. This impact of cessation of bone formation has an especially devastating effect on adolescents with anorexia. Anorexia patients, regardless of age, often have a very early onset of osteopenia, and as high as 25% of the patients develop osteoporosis. The body is now focused on trying to maintain a homeostatic range of calcium to keep the normal metabolic processes operating. Bone health in a normal, healthy adult is maintained through bone remodeling, which occurs in two phases: bone formation and bone resorption. Both are necessary to maintain health calcium homeostasis. Bone formation causes calcium to be extracted from the blood and uses it to form new bone. Bone resorption is when the body breaks down bone to extract the calcium needed for other body functions, such as neurotransmission. "Women with anorexia have increases in markers of bone resorption with decreases in markers of bone formation" (Sum et al. 2011). Not only is new formation not occurring, but bone is being broken down constantly for the needed calcium. This leads to extremely porous bones. Any slight tap can lead to fractures, so much so that it can be a cause of "sevenfold increases incidence of spontaneous fractures" (Hebebrand et al. 2007). Estrogen is also essential to bone formation, and with the drastically reduced levels, as previously mentioned, it maintains the state of bone resorption and cannot begin bone formation. This point is illustrated in menopausal women. They have a very high rate of osteoporosis due to their natural drastic decrease of estrogen.

As calcium levels drop significantly in anorexics, neurotransmitter releases drop tremendously as well. Serotonin (a key neurotransmitter) production drops significantly. Levels also fall because serotonin is synthesized from carbohydrates that we intake through our diets. Since there are not enough raw materials to create the hormone, it is not present. As a result of serotonin deficiency, the individual feels satiated without eating the proper amount of food. This is a transformation in which anorexics no longer control their food intake purely by choice. It becomes a physical condition in which, as opposed to ignoring messages and feelings of hunger their bodies send them, they really are not hungry. As the disease progresses, the amygdala and hypocampus of the limbic system atrophy. Emotions are now affected and biology takes over even the emotional aspect of food and eating. Memory and feelings about food, which effect how much and what a person chooses to eat, are distorted and they no longer can make proper choices. A complex and life-threatening physical problem has taken over.

Figure 4: Body Moving from Hypoleptinaemia to Hyperleptinaemia. Source: Matarese et al.



In traditional treatment, the first step is hospitalization and refeeding, usually force-feeding. An important initial step, however, is a blood test to check leptin levels. This is important because the degree of hypoleptinaemia in acute anorexia nervosa is an indicator of the severity of the disorder; thus, pronounced hypoleptinaemia is not only indicative of an exceedingly low fat mass, but also reveals that the neuroendocrine adaptation to semi-starvation has maximally progressed in such critically ill patients (Hebebrand et al. 2007). As the refeeding process progresses, the leptin levels are going to move from hypoleptinaemia (starvation hormone) to hyperleptinaemia (satiety hormone) as depicted in Figure 4.

During the course of a normal pattern of life in a person without anorexia, leptin levels constantly fluctuate, but within certain limits. The danger of refeeding is that the levels move from zero to 60 in a very short period. The leptin in the anorexic patients have become a satiety hormone, and biologically, the patients cannot eat because they feel full. This is yet another example of the disease and treatment being biologically based. In spite of all the therapy patients receive, if they are not hungry, they cannot force down the food. To prevent this issue, doctors should begin refeeding extremely slowly, i.e. moving from 600 up to 700 calories a day, as opposed to the normal 1800 calories. Another danger of refeeding too quickly is throwing the person's electrolytes out of balance because of drastic fluid shifts, which can cause cardiac arrhythmia. Furthermore, introducing too many

carbohydrates very quickly is problematic because it creates a large influx of insulin and "the introduction of insulin to the starved system poses a variety of severe hemodynamic and electrolyte consequences…. The sodium retention caused by increased insulin levels can lead to hypervolemia and resultant cardiac and respiratory failure" (Usdan et al. 2008).

The leptin levels should be monitored several times a day, particularly after meals, to ensure that they are increasing but not dramatically into a state of hyper (which causes its own set of biological problems). The leptin levels will also tell the doctors how soon they can introduce more calories as well as how much they should be increasing the caloric intake at each interval, i.e. if moving from 600 to 750 calories produced a dramatic increase in leptin, it should perhaps be reduced to 700.

Moreover, small amounts of leptin should also be artificially given to patients with anorexia to slowly bring up their levels. The purpose is twofold. The first reason is to stop the cascade; it will fool the body into thinking that it is not in a semi-starvation state, and the other reactions, i.e. bone loss and amenorrhea, will not be triggered. If leptin levels are monitored properly, i.e. given artificially when necessary and used to dictate refeeding amounts and schedules, it can take about six months or more to normalize and stabilize their leptin levels. That will allow the patient's biological condition to improve dramatically by stopping many of the processes that began because of extremely low levels, i.e. hypothyroidism. If the leptin is introduced prior to the domino-like effects produced by the semi-starvation and before resting energy expenditure levels drop, the underlying psychological causes of anorexia can be addressed, as opposed to dealing with the near death physical conditions and complications of the disease. Once the refeeding process is near completion and normal body mass index and body fat ranges are attained, leptin levels must still be monitored closely. This is important to ensure that leptin transitions from the energy expenditure regulator role caused by anorexia and semi-starvation back into the satiating hormone of a healthy body. Additionally, research has shown that for the first six months, leptin levels in anorexia patients that have achieved a proper and healthy weight are often higher than people with normal, healthy weights and eating habits. Consequently, the leptin, in the satiety position, can hinder their weight maintenance because of this false feeling of fullness. (Hebebrand et al. 2007)

Due to the importance of leptin and the information that it provides in diagnosis, treatment, and prevention, it can be argued that determination of leptin levels should be included in the DSM-IV as part of the criteria for anorexia nervosa (American Psychiatric Association 2000). Hypoleptinaemia is integral in diagnosing how far the disease has progressed and what form of treatment is needed. It is believed that if doctors use leptin levels properly to diagnose and treat patients with anorexia, mortality rates can and should decrease.

CONCLUSION

All body systems are affected by anorexia nervosa in a nearly domino-like effect. Severe malnutrition can ultimately lead to multi-organ failure and death. The psychiatric causes of the disease are likely to be impossible to address while a patient is extremely physically ill and in danger of losing his or her life. Physical reactions from increased leptin and serotonin levels in the body create a situation in which the patient no longer feels hungry. Thus, a truly effective cure for anorexia nervosa must take far more expertise that can be provided by a psychiatrist alone. Various medical professionals, in addition to mental health professionals, must work together to provide care in their individualized specialties in order to bring about an effective and lasting cure for this destructive and multifaceted disease. Where damage has been caused by the onset of anorexia nervosa, doctors must work together to ensure that these conditions do not continue to deteriorate, as well as work to improve them. Doctors must have a coordinated plan in order to truly cure the disease and ensure that the patient does not relapse into a state of malnutrition and disease. The false assumption of anorexia as a purely psychiatric condition has contributed to a fairly high mortality rate. Although anorexia is rooted in mental illness, its profound physiological ramifications must be dealt with first in a bottom up

approach. It is only after a biological cure is effected can the road be paved to successful psychological counseling. It is hypothesized that with more research on the biological basis of the disorder and treatment, particularly the role of leptin in diagnosing and treating this harrowing disorder, the mortality rates can be significantly reduced, and a greater understanding of anorexia nervosa can be achieved.

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