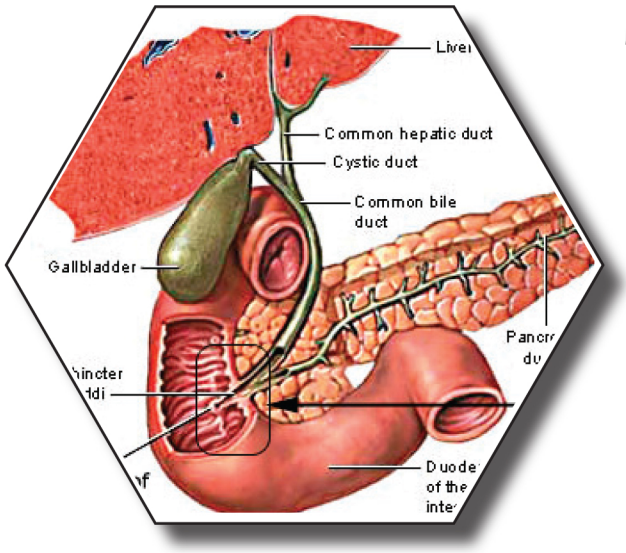

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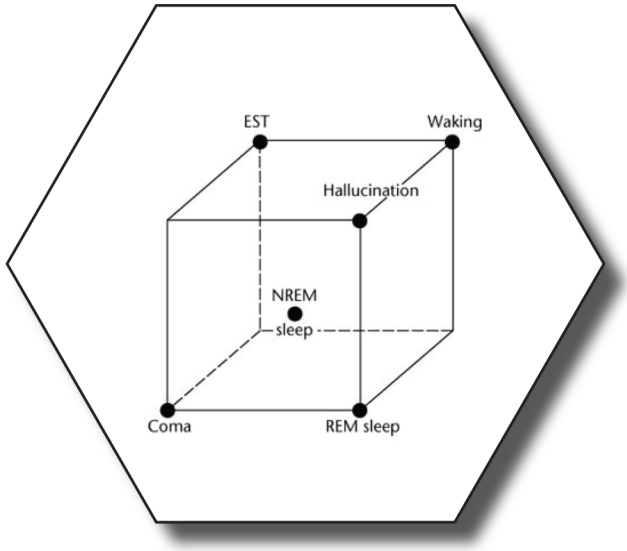
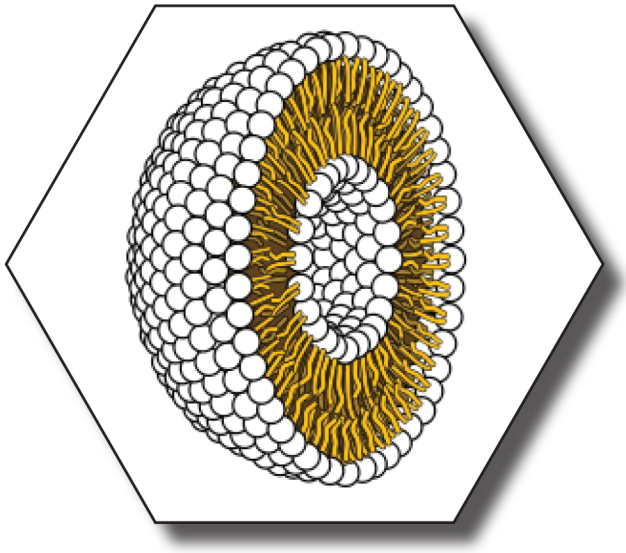


Volume 4, Issue 2



Spring 2011

The Science Journal of the Lander College of Arts and Sciences
Volume 4, Issue 2 Spring 2011

TABLE OF CONTENTS

Cover Pictures

Should Benzodiazepines Be Prescribed To Treat Insomnia And Anxiety Related Disorders?

JUDITH NICOLE MARGARETEN

INTRODUCTION

Benzodiazepines are commonly prescribed drugs used to treat insomnia and anxiety. They are often found in forms such as Xanax (alprazolam) and Valium (diazepam). For many, these drugs have proven essential for ensuring a restful night's sleep, but for others they are the cause of sleepless ones. Negative effects of benzodiazepines such as addiction, dependence, and impaired cognition plague many patients. While doctors are prone to prescribe these medications readily due to their high level of effectiveness, this practice can pose a great risk to certain populations .

Structure of Benzodiazepines

Benzodiazepines consist of a benzene ring and diazepine ring (see fig 1). Varying side chains which attach to this basic structure account for the various drugs formed from this compound. Xanax (alprazolam), Librium (chlordiazepoxide), Klonopin (clonazepam), Tranxene (clorazepate), Valium (diazepam), Paxipam (halazepam), and Ativan (lorzepam) are prescribed for long term use while ProSom (estzolam), Dalmane (flurazepam), Doral (quazepam), Restoril (temazepam), and Halcion (triazolam) are prescribed specifically for short term use.

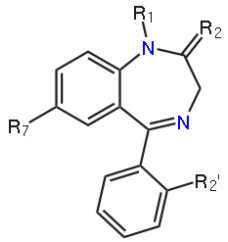


Figure 1. Structure of a Benzodiazepine Ring

Mechanism of Action

GABA is the primary inhibitory neurotransmitter of the central nervous system. GABA receptors exist in two forms, GABAA and GABAB. GABAA receptors (GABAAR) are present on neuron membranes and work as ligand-gated ion channels. When GABA binds to GABAA receptors, the flow of chloride ions into the cell increases. The influx of negatively charged chloride ions causes the cell to undergo hyperpolarization. In turn, the transmission of action potentials is less likely to occur, and neurotransmission is inhibited.

GABAA receptors are found as part of a protein complex. This complex also houses different allosteric binding sites which can indirectly affect GABAA when activated. Benzodiazepine can bind to one of the allosteric sites on this complex. When benzodiazepine binds to its receptor on the protein complex, it causes the GABAA receptor to experience a conformational change. This change locks the GABAA receptor in a form to which GABA has a higher level of affinity. This results in an even greater influx of chloride ions than would normally be triggered by the binding of GABA. In this way, benzodiazepines work as an agonist of GABAA and slow down the brain and nervous system .

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Uses of Benzodiazepines

Benzodiazepines are most often associated with the treatment of insomnia and anxiety. Insomnia is a sleeping disorder causing difficulty in sleeping or falling asleep. Anxiety often accompanies depression or other psychological disorders and is characterized by excessive stress, worry, or fear. The ability of benzodiazepines to inhibit brain activity and neurotransmission helps to promote sleep and relaxation, and induces a calming effect. Benzodiazepines are also used to treat muscle spasms, convulsive disorders, and to induce sedation prior to surgery. Further uses of benzodiazepines are listed in the tables below.

Clinical Uses of Benzodiazepines

- Anxiety disorders
  - Acute anxiety
  - Generalized anxiety disorder
  - Panic disorder
  - Phobias (social, simple)
  - Post-traumatic stress disorder
  - Obsessive-compulsive disorder
- Insomnia
- Anxiety associated with medical illness
  - Cardiovascular
    - Gastrointestinal
    - Somatoform disorder
- Convulsive disorders
- Acute status epilepticus
  - Neonatal seizures or febrile convulsions
    - Preeclampsia
    - Tetanus
- Adjunct to other anticonvulsants
- Amnestic (before surgery or procedure)
- Spastic disorders and other types of acute muscle spasm
  - Cerebral palsy
    - Multiple sclerosis
    - Paraplegia secondary to spinal trauma
- Involuntary movement disorders
  - Restless leg syndrome
    - Akathisia associated with neuroleptic use
    - Choreiform disorders
    - Myoclonus
- Detoxification from alcohol and other substances
- Agitation or anxiety associated with other psychiatric conditions
  - Acute mania
    - Psychotic illness
    - Anxiety associated with depression
    - Impulse control disorders
    - Catatonia or mutism
- Other adjunctive uses
  - Surgery
    - Dentistry
    - Diagnostic studies, such as computed tomography, magnetic resonance imaging and endoscopy
    - Cardioversion
    - Chemotherapy

Information from Hollister L, Muller-Oerlinghausen B, Rickels K, Shader R. Clinical uses of benzodiazepines. J Clin Psychopharmacol 1993;13(suppl 1):1-169.

Table 1. Benzodiazepines are used to treat a myriad of ailments.

The Effectiveness of Benzodiazepines in the Treatment of Insomnia

Benzodiazepines have been established as an effective treatment for insomnia. In an analysis of twenty-two studies of benzodiazepines and benzodiazepine-receptor agonists, significant improvements were found in total sleep time, number of awakenings, and sleep quality of the treated patients. However, only nine of those studies contained information complete enough to allow for accurate calculation of effectiveness. The nine studies analyzed involved a total sample of 343 patients who were treated with benzodiazepines and 337 who were members of control groups. All participants fell between the ages of 18 and 65. Participants who received the medication fell asleep sooner than 71% of the control group slept longer than 76% of the control group, woke up less often than 74% of the control group, and had better sleep quality than 73% of the control group. All of the studies examined in this meta-analysis used a "placebo –control group, parallel-group design, randomized patient assignment, and double-blind assessment of outcomes."(Nowell, et al., 1997)

An additional study was conducted on 54 patients who were prescribed benzodiazepines. The participants of the study were of sixty-five years of age or older and were at the hospital during the three day study period. Thirty one of the patients had not used benzodiazepines previously.

Half of these patients reported shorter sleep onset while taking the medication at the hospital. In other areas of sleep assessment, such as duration of sleep, number of awakenings, and sleep quality, many had an improved sleep experience in the hospital compared with their sleep at home. However, little difference in sleep improvement was observed between those who were newly introduced to benzodiazepines and those who were already using them prior to entering the hospital. This indicates that the improvement in sleep which the patients experienced could be attributed to the hospital environment or other factors rather than to the prescription of benzodiazepine medications. While the outcome of this study does not support the effectiveness of benzodiazepines, it was conducted on a relatively small group and for a short period of time. These factors limit the value of this study and its conclusions .

Risks Associated With Benzodiazepine Use

While benzodiazepines are considered to be one of the safest sedatives, their use is sometimes accompanied by side effects which can put a patient at risk. Patients most commonly found to be at risk are those who take benzodiazepines for extended periods of time, who abuse other substances, and those who are elderly.

Dependency & Withdrawal

According to the FDA fast-onset, short acting benzodiazepines should be prescribed for only five consecutive weeks in order to avoid the risk of dependency. In reality, however, they are often used for longer periods of time which can lead to complications. Millions of Americans have inadvertently become dependent on benzodiazepines as a result of receiving repeated prescriptions over time. Of the estimated 1 million users in the United Kingdom and 4 million users in the United States, 50% are thought to be dependent on benzodiazepines.

Tolerance as a result of prolonged exposure

Tolerance is defined as "a need for markedly increased amounts of the substance to achieve the clinical effect, or markedly diminished effect with continued use of the same amount of the substance." For those who use benzodiazepines to promote sleep, tolerance can develop relatively quickly. Dependency can increase within a few short days or weeks. Many of those who use benzodiazepines to induce sleep tend to gradually increase their doses beyond recommended levels. Patients may pressure their doctors to increase their prescriptions or perhaps visit multiple doctors or clinics to obtain numerous prescriptions simultaneously. Sometimes patients may even turn to street drug dealers to obtain the doses they crave. Such practices put users at further risk of becoming dependant on the drug.

Withdrawal

Withdrawal symptoms associated with discontinued use of benzodiazepines include "restlessness, irritability, insomnia, muscle tension, weakness, aches and pains, blurred vision, and heart racing." Many continue to take benzodiazepines in order to avoid going through a withdrawal stage. However, continuing to take the drug may cause patients to become increasingly dependent and may dull the drug's ability to provide relief.

In cases where users are not using benzodiazepines for recreational use, the negative effects experienced upon discontinuation are usually a result of relatively low-dose benzodiazepine dependence. However, even when administered at low dosages, long term exposure can result in

dependence and negative withdrawal symptoms. With longer-acting drugs, one or two months of exposure can put a patient at risk. With shorter-acting drugs, taking the drug daily for a week can affect the patient. Short-acting benzodiazepines have also been proven to cause rebound insomnia which negatively affects the user's sleep the night after the drug is used.

Drugs which have slower elimination such as Xanax and Valium are more likely to cause side effects because metabolites accumulate in the body if the drug is used repeatedly. However, users of these drugs are less likely to suffer from withdrawal. On the other hand, drugs such as Doral and ProSom, which have shorter half-lives and elimination time, are more likely to cause withdrawal symptoms.

Long-term or high-dose exposure to benzodiazepines results in a decrease in the effectiveness of the body's natural GABAA. It is thought that continuous administration of the drug causes the body to adapt to presence of the drug. When the drug is removed, the forces to which it is normally opposed are left with no adversary. In the case of benzodiazepines which are used to treat anxiety and induce sleep, abrupt discontinuation can cause a surge in nervous activity.

While the exact mechanism responsible for benzodiazepine dependence has not been found, theories have been proposed and studies have been done which may provide an answer, at least in part. Benzodiazepines serve as an agonist of GABAA. When a drug which works as an agonist is administered chronically, it causes a decrease in the number of receptors found in the body. This is known as down regulation. The receptors are decreased by means of internalization, degradation, or recycling of the receptor. A decrease in receptors would then cause the drug to become less effective as well as decrease the body's ability to respond to its natural GABA. However, some studies have found there to be no significant decrease in the number of receptors in those chronically exposed to benzodiazepines. Therefore, other theories attribute the ineffectiveness of benzodiazepines to modification of the GABAA receptors rather than to a loss of those receptors.

Abuse By Abusers

The Abuse of Benzodiazepines by Those Who Abuse Other Substances

According to the APA, the prevalence of those individuals dependent on benzodiazepines among substance-abusers was found to be significantly higher than among the general population. Benzodiazepines are often used in conjunction with other drugs by those who seek to use drugs for recreational purposes. Approximately 80% of those who abuse benzodiazepines are polydrug abusers. In a study conducted among Australian heroin users, 91% of the two hundred and twenty-two participants had used benzodiazepines. Of those who had used benzodiazepines, 67% were currently using the drug on a regular basis. In a study by the National Institute of Drug Abuse, 73% of heroin users used benzodiazepines weekly for more than one year. Those who abuse alcohol are also at risk of becoming dependent on other drugs such as benzodiazepines.

In response to an article published in The American Journal of Addictions regarding the safety of prescribing benzodiazepines for the treatment of insomnia and anxiety in substance abuse patients, Edgar P. Nace of Texas Southwestern Medical School published his view in The Brown Digest of Addiction Theory and Application. Dr. Nace argues that substance abuse patients are at a greater risk of abusing benzodiazepines than the general population and, therefore, doctors should use caution when prescribing the drug. He cites that in samples of known substance abusers, 10% of the sample population were dependant on benzodiazepines at the time and over 50% were abusers of the drug.

Benzodiazepines have varied uses for those who are addicted to other drugs. While few use benzodiazepines alone for recreational purposes, drug abusers may take benzodiazepines to boost the effects of opioids or to alleviate withdrawal symptoms. For example, some may take benzodiazepines between heroin "fixes" to dull the negative feelings such as anxiety which they may feel. In cases where other drugs an abuser takes become unavailable, he may take benzodiazepines alone in high dosages to placate his cravings. Many abusers also state that they use benzodiazepines to prolong the "high" they receive from taking recreational drugs such as heroin and cocaine. Alcoholics use benzodiazepines to alleviate anxiety which results from chronic alcohol intake as well as to produce the pleasurable effect induced by mixing the drug with alcohol.

Short-acting benzodiazepines are more commonly used by substance abusers who benefit from the drugs' rapid effects. However, clonazepam, which has a long-half life, is also a known benzodiazepine "street drug," so it would seem that the delayed effects characteristic of the drug do not deter abusers. In addition, lipophilic benzodiazepines, which have the ability to cross the blood-brain barrier, and other benzodiazepines which have shorter half-lives and are highly potent are usually preferred by substance abusers. The Center for Substance Abuse Research notes that benzodiazepines are likely to be abused due to the sedative state which they induce. As all benzodiazepines are sedatives, every variety of the drug has some degree of potential for abuse.

Toxicity and Substance Abuse

The different varieties of benzodiazepines available vary in potency. Alprazolam is approximately twenty times more potent than diazepam. Therefore, those who are prescribed 6mg of alprazolam daily, a common dosage in the United States, are taking the equivalent of 120mg of diazepam, which is considered a very high dose. Doctors are made aware of these differences as administering a drug with too high a potency can prove very harmful. However, those who take the benzodiazepines for recreational purposes are unaware of the different posed by different benzodiazepines. Generic drugs sold on the street are often unmarked or inappropriately labeled and an overdose of flurazepam or temazepam can result in fatality (Ashton, 2002a).

Benzodiazepines have additive effects with other drugs with sedative actions including other hypnotics, some antidepressants (e.g. amitriptyline [Elavil], doxepin [Adapin, Sinequan]), major tranquillisers or neuroleptics (e.g. prochlorperazine [Compazine], trifluoperazine [Stelazine]), anticonvulsants (e.g. phenobarbital, phenytoin [Dilantin], carbamazepine [Atretol, Tegretol]), sedative antihistamines (e.g. diphenhydramine [Benadryl], promethazine [Phenergan]), opiates (heroin, morphine, meperidine), and, importantly, alcohol. Patients taking benzodiazepines should be warned of these interactions. If sedative drugs are taken in overdose, benzodiazepines may add to the risk of fatality (Ashton, 2002b).

Adverse Effects in the Elderly

The risks normally posed by benzodiazepines are amplified in elderly populations. The negative effects which result from combining benzodiazepines with other drugs are more likely to surface in elderly patients who commonly take other medications regularly. In addition, elderly patients are more sensitive to dosage and, therefore, can more easily fall prey to the dangers associated with taking benzodiazepines which are too potent. Psychomotor skills are affected by benzodiazepines, thus slowing the body's responses. This can be very detrimental in elderly patient who may have slower responses even without taking the drug. An increased number of hip fractures and falls in elderly patients have been attributed to benzodiazepine use.

The effectiveness of benzodiazepines is found to be lower in elderly patients, while the negative effects are increased. Therefore, when it comes to the elderly, the drug is recommended for short term use only. In practice, however, benzodiazepines are usually prescribed to the elderly long-term. Despite the guidelines which have been instated as a result of much research, 80% of elderly benzodiazepine patients receive treatments for over two years. (Note: Long-term use is defined as treatment lasting more than two weeks.) Benzodiazepines can offer much needed relief for sleep disorders, so it is not surprising that those receiving the treatment and benefitting from the drug would not want to stop taking it. In addition, many elderly patients would prefer to continue taking the medication rather than suffer from the withdrawal symptoms which they would experience if the drug's administration were discontinued.

Falls and Fractures

While many associate benzodiazepine use with hip fractures and injurious falls, the studies conducted have not provided consistent conclusions. A study conducted in France hoped to resolve this conflict by following a very large population of those over sixty-five years of age for up to ten years.

In this study, the individuals who qualified had experienced an injurious fall. An injurious fall was defined as one which resulted in hospitalization, fractures, head trauma, or fatality. Controls were selected at random from the numerous eligible subjects available. The mean age of the sample was seventy-eight. For the purposes of analyzing results, the group was divided between those above eighty years of age and those below the age of eighty. A very significant correlation was found between benzodiazepine use and injurious falls in those above the age of eighty. "The population attributed risk of benzodiazepine use on injurious falls in subjects aged >80 years was estimated using an aged-stratified model as 28.1%." Those conducting the study estimated that should the rate of injurious falls in the general population of France mirror the statistics found in their study, 71,000 injurious falls could be expected every year in those >80 years . Of those falls 20,000 would be related to benzodiazepine use. Of those 20,000 falls, 1,800 would be fatal. In those below the age of eighty a strong correlation could not be defined (Pariente, et al., 2008).

In a study conducted by Dr. Bula, of Cntre Hospitalier Universitaire, Lausanne, Switzerland, no correlation was found between injury and those using benzodiazepines chronically as opposed to those who were non-chronic users. Dr. Bula's team followed 304 patients above the age of seventy-five for a six month period. However, chronic users of benzodiazepines were noted more likely to become delirious during their stay in the hospital. As mental impairment is one of the negative side effects associated with benzodiazepine use, it is possible that the chronic users also experienced other negative side effects which went unobserved by Dr. Bula's team.

"Driving Under the Influence"

As benzodiazepines are known to affect cognition, especially in elderly populations, driving under the influence of benzodiazepines has become an issue. The rate of car accidents per mile involving elderly people is double than of those who are middle aged. Slowed reaction time, impaired joints, and vision problems are often to blame. A study conducted by McGill University in Montreal brought attention to a new culprit. The study examined the driving records and related documents of 225,000 people between the ages of 67 and 84. A rate increase of 45% was found in the number of car accidents resulting in injury involving those who had started taking benzodiazepines within the week prior to the accident. It is thought that during this time the patients were

still adjusting to the side effects of the drug such as daytime drowsiness and overall slower motor coordination. However, even among those who had assumedly already adjusted to the medication, the rate of accident was 26% higher among those taking benzodiazepines for as long as one year than those not receiving the medication. In addition, long-lasting benzodiazepines which linger in the blood stream for longer periods of time were related to higher rates of accidents ("Driving Under the Influence," 1997).

Of Doctors and Dependence

Doctors' disregard for the guidelines of benzodiazepine administration can lead to many negative side effects including toxicity, difficulty sleeping, falls, and impaired motor skills. Unfortunately, doctors commonly continue to prescribe benzodiazepines to their elderly patients although they are only recommended for short-term use.

In a unique study conducted by a collaboration of doctors and professors from various parts of the United States, doctors were questioned about their seemingly irresponsible practice. Their reasoning was usually centered on the fact that their patients were old and in need of peace or sleep, and the doctors were sought to provide whatever relief they could. They were not very concerned with the patients becoming dependent or addicted, as many doctors felt that little harm would come if their patients could continue to take safe dosages of benzodiazepines for the rest of their lives. Others feared that stopping a patient's benzodiazepine supply would cause them to lose the patient. They believed that a patient who is accustomed to taking a certain benzodiazepine would simply switch to another doctor who would provide the desired prescription. Below are some of the more memorable responses provided by the doctors as to why they continually prescribe benzodiazepines despite the risks:

"You'd like to say, well, I can just put a great big brick wall in front of me and the patient with benzodiazepines but it doesn't work that way,,. You feel like you've gotta give the patient something to help."

"It's literally like taking candy from a baby and people that have enjoyed the effects of that class of drugs don't wanna give it up. I can't lose patients over this."

"It's just so much easier to just prescribe something and just walk away."

"I'm not worried about fifteen or twenty years down the road I'm gonna still be giving her Ativan. She's not gonna be alive in twenty years down the road and I'm sort of surprised she's alive now... She really needs it. If it helps her get through her days better, great."

All the doctors interviewed were aware that there were additional risks involved in prescribing benzodiazepines to elderly patients. Many of the doctors interviewed seemed to be making their decisions on moral grounds rather than on scientific ones. While some find fault in the practices of these doctors, many actually agree with their practices believing that if monitored, benzodiazepine dependence can benefit the patient while doing minimal harm (Cook, et al., 2007).

CONCLUSION

In order to prevent the negative side effects associated with long-term exposure, benzodiazepines should not be administered or taken for extended periods of time. Patients should adhere to the dosages prescribed by their doctors and not seek to increase their doses or obtain additional dosages from other sources. In the event that a patient does become dependent on benzodiazepines, dosages should be reduced gradually. Long-acting drugs may be replaced by short-acting

ones to aid the gradual withdrawal process. Anti-convulsants are also used to curb anxiety which may arise as a result of withdrawal. These methods of weaning a patient off of benzodiazepines can minimize the backlash of symptoms which usually accompany withdrawal.

While most authorities claim that benzodiazepines are rarely abused by the general population, those who abuse other substances are highly susceptible to abusing benzodiazepines. Doctors are warned to take care when prescribing benzodiazepines to substance abusers; some even suggest that doctors avoid prescribingit to that demographic altogether. If a patient who is a substance abuser must be prescribed benzodiazepines, his intake should be carefully monitored.

Elderly patients are believed to be more susceptible to the side-effects associated with taking benzodiazepines. Therefore, additional care should be taken to ensure they receive proper dosages. The effects the drug may have on the patient should be observed in order to evaluate if the patient is able to drive safely and perform other daily tasks unimpeded.

As benzodiazepines are highly beneficial and an effective treatment for the treatment of insomnia and anxiety, they should be prescribed for the general public. However, extra care should be taken when prescribing the drug to those demographics which are additionally susceptible to the negative side-effects and risks posed by the use of benzodiazepines.

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Effects of Calcium Supplementation on Cardiovascular Disease in Postmenopausal Women

RAIZEL ROBINSON

INTRODUCTION

In the United States, 53% of the population use dietary supplements. Of the people using supplements, 43% use calcium and 37% use vitamin D. However, 63% of women older than 60 years of age reported using dietary supplements (Park et al., 2009). Calcium supplementation, without co-administered vitamin D, increases the risk of myocardial infarction and cardiovascular-related diseases among postmenopausal women. On the contrary, calcium supplementation with accompanied vitamin D may not produce similar effects.

Postmenopausal women take calcium supplements in an attempt to prevent osteoporosis. This is because postmenopausal women require a larger amount of calcium intake. Throughout a person's life, blood calcium levels continuously decrease due to greater loss of calcium via renal clearance (Tfelt-Hansen and Torring, 2004), reduced intestinal absorption and perhaps other mechanisms. If the amount of calcium lost by the body consistently exceeds the amount absorbed, than a loss of bone results (Hsia et al., 2007). A low calcium intake has been associated with an increased risk of osteoporosis, bone fractures, and falls (Bailey et al., 2010). To compensate, postmenopausal women need up to 20% more calcium per day than their younger counterparts (Prince et al., 2006). If the amount of calcium lost by the body consistently exceeds the amount absorbed, than a loss of bone results (Hsia et al., 2007). Postmenopausal women take calcium supplements in an attempt to prevent osteoporosis. Calcium is a major mineral component of the skeletal system. Bone structure is maintained by the constant remolding of bone as it responds to the normal physiological and pathological skeletal stresses of daily living (Sahota, 2000). A low calcium intake has been associated with an increased risk of osteoporosis, bone fractures, and falls (Bailey et al., 2010).

Since vitamin D plays a role in absorption, these women also require excess vitamin D. Many authoritative doctors and researchers recommend calcium supplements without considering the detrimental side effects that they may cause. Calcium and vitamin D supplements seem ideal. They are pharmacologically active and cost-effective for the treatment of osteoporosis. In addition, they have synergistic effects with antiabsortive agents on bone mass density in relation to fracture prevention (Nieves, 2004). Based on these findings, doctors believed that these supplements can be administered indefinitely without detrimental side effects.

Currently, although researchers have found that calcium and supplements marginally reduce the risk of fractures in elderly women who are deficient in calcium and vitamin D, calcium supplementation increases the risk of myocardial infarctions and heart disease (Bolland et al.,

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2010). Considering the detrimental effects that calcium may have on the cardiovascular system, it is questionable whether calcium supplementation, in an effort to reduce osteoporosis, justified.

Mechanisms for Calcification in the Cardiovascular Vessels

Many mechanisms for calcium absorption have been suggested to explain its effects on the risk of cardiovascular disease and myocardial infarctions. Calcium supplements can acutely elevate serum calcium levels; possibly accelerating calcification in the walls of vascular structures. Vascular calcification lends itself to the occurrence of vascular events such as myocardial infarction and coronary heart disease (Pentti et al., 2009). High calcium uptake has been associated with vascular calcification and mortality in patients with end-stage renal disease (Giachelli, 2004). However, this data may not be representative because of the age of the participants and reduced renal function.

Calcium phosphate deposition, in the form of bioapatite, is the essential characteristic of vascular calcification. This deposition can occur in the blood vessels, myocardium, and cardiac valves. In the coronary arteries, calcification is positively correlated with an increased risk of myocardial infarction and atherosclerotic plaque burden. It is possible that vascular calcification initiates or progresses cardiovascular disease. Vascular calcification, especially calcification in the tunica media of large arteries, leads to increased stiffening, and therefore decreased compliance of the vessels. The resulting loss of important cushioning function of these arteries is a factor that leads to impaired arterial distensibility, increased afterload favoring left ventricular hypertrophy, compromised coronary perfusion, and hypermedial arterial calcification (Giachelli, 2004). Giachelli has suggested four different mechanisms for vascular calcification (refer to figure 1.). First, blood vessel tissues normally express inhibitors of mineralization, such as pyrophosphate and matrix gla protein. The lack of these molecules leads to spontaneous vascular calcification and

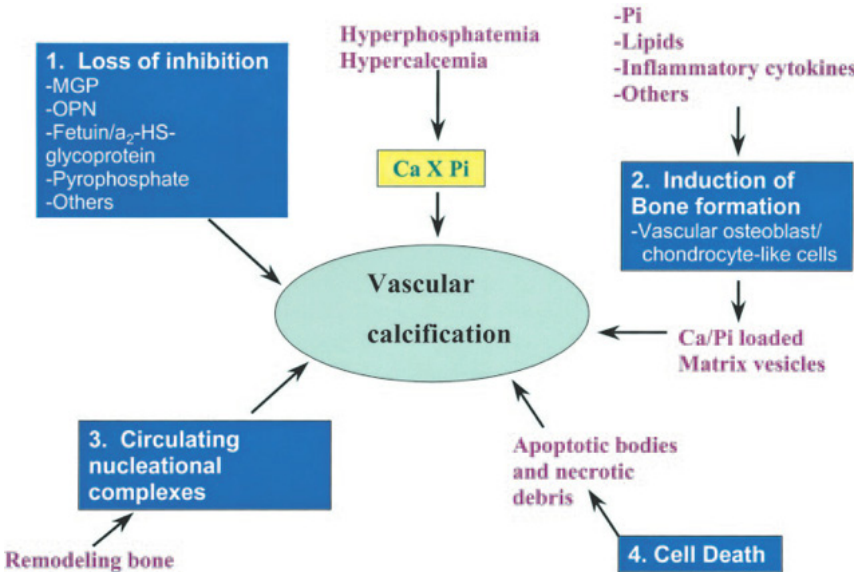

Figure 1. Schematic illustrating four, non–mutually exclusive theories for vascular calcification: (1) loss of inhibition as a result of deficiency of constitutively expressed tissue-derived and circulating mineralization inhibitors leads to default apatite deposition, (2) induction of bone formation resulting from altered differentiation of vascular smooth muscle or stem cells (3), circulating nucleational complexes released from actively remodeling bone, and (4) cell death leading to release of apoptotic bodies and/or necrotic debris that may serve to nucleate apatite at sites of injury.

(Reprinted from Giachelli CM, 2004)

increased mortality. Second, osteogenic mechanisms may play a role in vascular calcification. It has been observed that cells derived from the vascular media undergo a bone and cartilage-like phenotypic change and calcification in vitro under various conditions (Giachelli, 2004, Allison et al., 2006). Third, bone turnover leads to the release of circulation nucleational complexes and crystal growth. Lastly, cell death can provide phospholipid rich membranous debris and apoptotic bodies that may nucleate apatite, especially in patients with atherosclerosis, or other diseases where necrosis and apoptosis occur. Elevated calcium and phosphorous levels promote apatite nucleation and crystal growth to a point that would exacerbate vascular calcification initiated by any of the other four previously mentioned conditions.

Effects of Calcium Supplementation

The link between vascular calcification and osteoporosis in postmenopausal women has been associated with the third condition cited by Giachelli (2004), which is bone turnover. Elevated serum calcium levels accelerate vascular calcification and increase mortality in patients (Bolland et al., 2010). Yang et al. (2004) experimented with smooth muscle cell culture systems. He increased the calcium concentration of the media to hypercalcemic levels. The hypercalcemic mixture led to enhanced mineralization and phenotypic transition of vascular smooth muscle cells. The muscular cells underwent a phenotypic change in which they stopped expressing smooth muscle specific genes and simultaneously expressed genes commonly associated with bone differentiation, including osteocalcin, osteopontin and Runx2 (cited in Giachelli 2004). In the a study of Proudfoot et al. (2004) elevated calcium levels (1.8-to 5.0 mM) stimulated human smooth muscle cell calcification in vitro. Calcification is also present in atherosclerotic lesions, thus raising the possibility that increased calcium consumption may increase the risk of cardiovascular disease (Bostick et al., 1999). When women consume calcium supplements, an increase in circulating calcium levels may occur. This increase may possibly lead to myocardial infarctions and cardiovascular disease through the aforementioned mechanisms.

It has been suggested that calcium supplementation may lead to increased aortic valve and coronary artery calcification (Messika-Zeitoun et al., 2006). Aortic valve and coronary artery calcification are associated with cardiovascular risk factors. However, Bhakta et al. (2009) did not find evidence that calcium supplementation would increase calcification in their independent assessments of aortic valve calcification and coronary artery calcification with calcium supplementation among patients older than sixty years old. Their study attempted to confirm whether calcium supplementation increased the rate of aortic valve calcification and coronary artery calcification. In their study of 257 patients, 144 were women. Twenty-five participants, all of them women, reported using calcium supplements. Bhakta et al. (2009) did not observe a statistical increase in aortic valve calcification or coronary artery calcification in the subgroup of women using calcium supplementation. Additionally, there were no significant differences between the two groups regarding baseline serum calcium, renal function, diabetes, hypertension, and cholesterol or body mass index. The presence of oral calcium supplementations was not associated with a statistically accelerated aortic valve calcification or coronary artery calcification progression. However, the results of this study are limited. First, since this study was not a clinical trial, beneficial or harmful effects of therapeutic intervention cannot be ruled out. Second, due to the small sampling size of 25 women using calcium, it is impossible to generalize the study results to the larger population. Yet, one advantage in this study is the use of Electron Beam Computed Tomography (EBCT), which yields more exact results than an echocardiogram.

In contrast, calcium has a lowering effect on the risk of cardiovascular disease and myocardial infarction. Firstly, calcium absorption has a beneficial effect on the high-density lipoprotein cholesterol to low-density lipoprotein ratio in the body (Moyad 2003). The use of calcium supplements have been shown to increase that ratio of by almost 20% in healthy postmenopausal women. Cholesterol changes of this magnitude may be associated with 20-30% reductions in vascular event rates, such as myocardial infarction (Bolland et al., 2007). This increase of high lipoprotein cholesterol results from calcium binding to fatty acids and bile acids in the gut, and leading to a malabsorption of fat, as shown from studies of humans and animals. Calcium, if consumed in quantities greater than the amount needed to maintain homeostasis, binds bile acids in the gut and leads to their excretion. Calcium supplementation is therefore associated with an increased excretion of fecal bile acids. Bile acids-binding resins, such as cholestyramine, lower blood levels of cholesterol, and is found to reduce the risk of ischemic heart disease (Bostick et al., 1999). Supplemental calcium was found to lower serum cholesterol in rats, rabbits, and goats as well, but not young pigs. It may be suggested that that calcium supplementation would have similar effects on humans.

Another positive reason to supplement with calcium, despite the consequences is that people living in calcium rich water areas have lower cardiovascular disease mortality than people utilizing soft water. Additionally, evidence suggests that calcium supplementation causes reduction in blood pressure, although these reductions are small and transient. This may suggest that calcium, through other mechanisms, generates a positive effect on the human cardiovascular system (Bolland et al., 2007).

Effect of Calcium Supplementation on the Risk of Cardiovascular Disease

Several large clinical studies were conducted to verify the effects of calcium supplements on the cardiovascular system. The Women's Health Initiative did the first and largest clinical experiment (LaCroix et al., 2009). They conducted a randomized control study of 36,282 postmenopausal women. Eligible postmenopausal women, ages 51-82, were randomly assigned to calcium and vitamin D supplementation (calcium carbonate, 500 mg, with vitamin D3 200 IU, twice daily, for a total of 1,000 mg calcium and 400 IU vitamin D3 per day) or to a placebo (Wactawski-Wende et al., 2006). Concurrent calcium and vitamin D supplementation were permitted. The Women's Health Initiative concluded that amongst women younger than 70 years, calcium and vitamin D supplementation appears to reduce risks of cardiovascular disease. However, the detection of the benefits of calcium with vitamin D in this experiment is distorted. Sixty four percent of the women in the placebo group had an intake of least 800 mg calcium per day and 42% had a daily vitamin D intake of at least 400 IU from concurrent diet and supplementation allowed outside the study. The low dose of 400 IU vitamin D per day may not be large enough to affect a change. Trials that have previously demonstrated benefits of calcium with vitamin D supplementation have administered at least 700 IU per day to the patients (Hsia et al., 2007). Furthermore, more than half of the participants in both groups were receiving hormone-replacement therapy (Finkelstein, 2006).

Adherence to the study drug was not complete, as only 60% of the study participants took at least 80% of their study medication for the duration of an average of six years. Death caused by myocardial infarction or coronary heart disease was confirmed in 499 women assigned to active calcium/vitamin D and 475 assigned to a placebo (refer to table 1. on page 15). According to this study, the risk of coronary heart disease, because of the use of active calcium/calcium and vita-

min D, is inversely related to bone mass index (BMI) (LaCroix et al,. 2009). Women with higher BMI are at lower risk for coronary heart disease with active calcium/vitamin D supplementation, whereas those with lower BMI were at higher risk. This aspect of the Women's Health Initiative observation is in line with that of The Kuopio Osteoporosis Risk Factor and Prevention Study (2009), who also found that women with a higher BMI are at lower risk for cardiovascular disease.

However, after evaluating the risk of coronary cerebrovascular events in the Women's Health Initiative randomized trial of calcium plus vitamin D supplementation, Hsia et al. (2007), did not find significant correlation between the risk of cardiovascular heart disease and calcium supplementation. Temporal trends do not indicate a significant increase in the risk of coronary heart disease over seven year's time with calcium supplementation (Hsia et al., 2007).

There are several explanations to clarify the basis for the lack of difference observed between the calcium-supplemented group and the placebo group in Women's Health Initiative study on the risk of coronary heart disease. First, the use of personal supplements, as well as the poor adherence to the study medication may have blurred the researchers from identifying the effect of the study treatment. Second, the Women's Health Initiative protocol led to only a small increase in median plasma 25-hydroxyviamin D levels from 42.3 nmol/liter to 54.1nmol/liter. This is because the dose of vitamin D was inadequate to affect substantial change. In order to prove whether vitamin D supplements affect cardiovascular disease, the women would require supplementation of at least 1,000 IU vitamin D, thereby achieving the recommended level of 75 nmol/liter. By extrapolating this data, it can be inferred that Vitamin D supplementation at moderate to high doses may have beneficial effects in reducing the risk for coronary heart disease (Hsia et al., 2007). In contrast, calcium supplementation seems to have no apparent affect on the risk of cardiovascular disease. A third explanation as to why the data of the Women's Health Initiative is not valid is that concurrent postmenopausal hormone therapy interfered with treatment effects. Most of all, cardiovascular heart disease was not the primary design of the study; the primary goal of the Women's Health Initiative trial was to determine the calcium and vitamin D component in causing hip fractures (McGowan and Pottern, 2000).

	Calcium/Vitamin D (N=18, 176), n (Annualized %)	Placebo (N=18,106) n (Annualized %)	Hazard Ratio (95% CI)	P=
Myocardial infarction or CHD death	499(0.39)	475(0.37)	1.04(0.92-1.18)	0.50
Myocardial infarction	411(0.32)	390(0.31)	1.05((0.91-1.2)	0.52
CHD Death	130 (0.10)	128(0.10)	1.01 (0.79-1.29)	0.92

Table 1. Cardiovascular Events by Treatment Group Assignment (cardiovascular event includes coronary heart disease (CHD), and myocardial infarction.)
(Taken from Hsia, et al., 2007)

A second study, the Iowa Women's Health Study, was a cohort study of cancer incidence in a sample of Iowa women. In 1986, 99,826 women aged 55-69 were invited to participate in an observational trial. A secondary study was conducted to determine the result of calcium supplementation. These women were mailed a questionnaire about sociodemographics, body measurements,

physical activity level, smoking and alcohol consumption, gynecologic history, dietary intake and supplement use. A 127-Food-Item Harvard Food Frequency questionnaire was utilized to assess these women's usual food and nutrient intake. Forty-two percent of the women completed the baseline questionnaire. The average age of the participants at baseline was 60.6 years, and 78.6 at follow up. The Iowa Women's Health Study confirms that many more women use calcium supplementation alone than calcium and vitamin D simultaneously (Refer to Table 2).

	Baseline (1984)		Follow-up (2004)	
	% of women using supplements	Number of women using supplements	% of women using supplements	Number of women using supplements
Calcium	46.2	8,474	60.4	11,078
Vitamin D	10.9	1,995	11.9	2.185

Table 2. This table shows the increase in the use of calcium/vitamin D supplements over the twenty years and that many more women use calcium, than Vitamin D.

One disadvantage of this study is that the questions on the follow-up questionnaire varied from the initial assessment. For example, on the baseline questionnaire, vitamin D intake was inquired as an individual question. In contrast, the follow-up questioned the use of vitamin D supplements in a combined question of miscellaneous supplements. Although there were questions about use ofvitamin supplements , the questionnaire did not ask about over-the-counter-medications or herbal use. Certain non-food products, for example, Tums, contain nutrients that were not taken into account.

The evidence of Park et al. (2009) demonstrates that women within the highest quartile of calcium intake can reduce their risk of myocardial infarction by approximately 35%. They found that calcium intake would reduce the risk, regardless of whether it is of dietary or supplementary source or if taken together with vitamin D (reviewed in Bostick et al., 1999).

In contrast, the researchers of the Kupio Osteoporosis Risk Factor and Prevention Study observed that calcium or calcium with vitamin D supplementation increases the risk of coronary heart disease. Within a cohort of 10,555 women ages 52-62, over 25% reported use of calcium or calcium plus vitamin D supplementation. Eight thousand, four hundred and twenty seven (8,427) women, without a preexisting condition of coronary heart disease, were categorized as postmenopausal (Pentti et al., 2009). The criteria for coronary heart disease included: obvious symptoms of angina pectoris, myocardial infarction, coronary artery bypass graft surgery, percutaneous balloon angioplasty, chest pain, and significant coronary artery stenosis on a coronary angiography. During the course of seven follow-up years, 513 cases of coronary heart disease were reported in the cohort of 10,555 women. Of the 513 cases, 424 were from the postmenopausal subgroup (n=8,424). Three hundred and two cases of coronary heart disease occurred in postmenopausal women not using calcium or vitamin D supplements; only 122 women who were using supplements developed a condition. Many more nonusers of calcium supplements were diagnosed with coronary heart disease than users of calcium or calcium and vitamin D. Interestingly, calcium-supplement users in this study who had a lower BMI, with a mean of 26.3 kg/m^2; were non-smokers or former smokers than calcium supplement non-users; and had fewer childbirths than nonusers had. These differences provide an alternate explanation as to why more nonusers of calcium supplements developed coronary heart disease, rather than concluding that it was specifically calcium/calcium plus vita-

min D that triggered these results. After considering all the variables that can affect the risk of heart disease, the relative risk of developing coronary heart disease was 1.0 vs. 1.26, nonusers of supplements to users of supplements respectively. This statistical evidence demonstrates that the use of calcium or calcium with vitamin D increased the risk of coronary heart disease in the subgroup of postmenopausal women. It should be noted, however, that for women slightly after menopause, calcium supplements did have a reducing affect on the risk of coronary heart disease. However, as the years past menopause increased, so did the risk of a heart condition. The risk of cardiovascular heart disease increased in women ages 60-62 by 61% with the use of calcium supplements (Pentti et al., 2009). In addition to other drawbacks, this study could not determine coronary heart disease morbidity, to the varied doses of calcium supplementation as the participants used products that contained a wide range of doses of calcium. Again, this makes it impossible to verify whether the increased coronary heart disease risk was solely related to calcium, or calcium and vitamin D (Pentti et al., 2009).

Bolland et al. (2007) conducted a five-year randomized placebo-controlled study in New Zealand to determine the effect of calcium supplementation on myocardial infarction, stroke, and sudden death in healthy postmenopausal women. One thousand, four hundred and seventy one (1,471) postmenopausal women (mean age 74) participated. Of those women 732 received calcium supplementation and 739 received placebos. Myocardial infarction occurred more often in the calcium group than in the placebo group (45 events in 31 women vs. 19 events in 14 women). Study participants were aged 55 or more, had been postmenopausal for a minimum of five years, and had a life expectance of at least five more years. This study excluded women who were receiving treatment for osteoporosis, those with any major disease and women with serum 25-hydroxyvitamin D levels less than 25 nmol/l. Participants received 1,000 mg of elemental calcium daily as citrate or an identical placebo. Ninety-percent of the women had a follow-up every six months for five years. Cardiovascular events were self reported or reported by family members and then verified.

3a. Number of women (number of events) self reported by patients or family members.

Vascular Event	Calcium Group	Placebo Group
Myocardial Infarction	31(45)	14(19)

3b. Number of women (number of events) self reported or reported by family members and verified.

Vascular Event	Calcium Group	Placebo Group
Myocardial Infarction	21(24)	10(10)

3c. Number of women (number of events) self reported, reported by family members and verified or not reported but confirmed by the national database of hospitals admissions in New Zealand.

Vascular Event	Calcium Group	Placebo Group
Myocardial Infarction	31(36)	21(22)

Table 3. This table depicts the difference between the number of women who experienced myocardial infarctions and the number of total events between the calcium-supplemented group and the placebo group (taken from Bolland et al., 2007).

Initially, there seems to be a statistically significant increased number of myocardial infarctions in the calcium group compared to the number of infarctions in the placebo group. However, after adjusting the data to include cardiovascular events not reported by participants or family members, the increase was not substantial (refer to Table 3c.). When the data was evaluated as event rates, the rate ratios for myocardial infarction were minimally significant. A notable difference was detected when the data for myocardial infarction was plotted over time. The numbers of recorded cardiovascular events between the two groups began to diverge after about twenty-four months, and thereafter continued to separate, although statistical significance was not attained.

In Bolland et al.'s 2007 study, the postmenopausal women had serum 25-hydroxyvitamin D levels greater than 25 nmol/l. Participants received either 400 mg of elemental calcium as a citrate, or a placebo. Results of this experiment show that although cardiovascular events, such as angina and chest pain, did not differentiate greatly between groups, myocardial infarction and transient ischemic attacks did have a significant statistical increase (refer to Table 3.c). There were 36 events of myocardial infarction in the calcium group, in contrast to 22 events of myocardial infarction from women non-using calcium supplements.

Three years later, in 2010, Bolland et al. conducted a meta-analysis to reassess whether calcium supplements increase the risk of cardiovascular events in all patients; not specifically women. After analyzing approximately 12,000 participants from 11 trials, seven of which were comprised solely of females, the researchers concluded once again that calcium supplements, without co-administered vitamin D, increases the chances of cardiovascular events. Their research excluded studies that incorporated vitamin D as a variable between the control group and calcium- supplemented group. Bolland et al. (2010) detected a 31% increase in the risk of incident myocardial infarction in the calcium-supplemented group. Calcium treatment was associated with an increased risk of myocardial infarction in people with dietary calcium intake above the median of 805 mg/day, but no increase was associated with participants with dietary intakes lower than the median. Additionally, recurrent cardiovascular events were more common in the calcium-supplemented group; 19 versus 13 cases of calcium supplemented women to non-supplemented women respectively had more than one cardiovascular event.

However, the trials considered in the study of Bolland et al. (2010) excluded trials in which calcium and vitamin D were administered together against a placebo comparator, trials were only eligible if vitamin D was given to both intervention and control groups, or to neither. Furthermore, any trial in which calcium was administered in the form of dietary modification or a complex nutritional supplement was excluded from the study (Bolland et al., 2007). Since vitamin D supplementation has been associated with decreased mortality, the research of Bolland et al. (2007) does not necessarily reflect the risk of cardiovascular disease among postmenopausal women taking calcium supplementation alongside vitamin D.

The experimental data of Bolland et al. (2010) shows that if 69 people are administered calcium, without co-administered vitamin D, one myocardial infarction will result. The risk of myocardial infarction tended to be greater in those with dietary calcium intake above the median, but independent of age, sex, and type of supplement. The finding of this study is consistent with the conclusion of most of the trials analyzed by Bolland et al. (2010).

Bolland et al.'s (2007 and 2010) study results and conclusions differ from those of the Women's Health Initiative. Despite the great improvements observed in the ratio of high-density lipoprotein cholesterol to low-density lipoprotein cholesterol, Bolland et al.'s studies (2007 and 2010) do not show any evidence that calcium supplementation may decrease the incidences of

vascular events. On the contrary, an increased risk for myocardial infarction is most noticeable in those with high compliance with the study drug. Although this study does not completely prove that calcium supplements can be detrimental to the cardiovascular system, it does suggest that administering calcium supplements is not a matter to be taken lightly.

There are several ways to explain the differing results of Bolland et al. and the Women's Health Initiative. Of the 1,471 participants in the study of Bolland et al. (2007), 105 women were older than 80 years of age at baseline. Many other factors may have caused a myocardial infarction in an elderly population other than calcium supplementation. Women participating in the Women's Health Initiative study were younger, with a mean age of 62, were heavier and had higher calcium intakes on average. More importantly, the women in the Women's Health Initiative took vitamin D with calcium, and a majority of the women were undergoing hormone replacement therapy. The women in Bolland et al.'s (2007) study did not use vitamin D supplements or oestrogen. Additionally, Bolland et al. (2007) conducted a randomized control study by dispensing large dose of calcium citrate, a highly bioavailable calcium supplement that has already proven affects on bone turnover and bone density. In contrast, the Women's Health Initiative that used a low bioavailable drug, calcium carbonate, had a lower compliance with the study drug, and their test results had to compete with the effects of the use of oestrogen and non-trial supplements (Hsia et al., 2007).

Vitamin D and its Effect on Cardiovascular Disease

Oestrogen levels of postmenopausal women are constantly decreasing. Consequently, these women are predisposed to osteoporosis. Vitamin D enhances intestinal absorption of calcium and influences bone metabolism. Because Vitamin D supplementation is helpful in increasing bone mineral density, it is beneficial to ingest vitamin D along with calcium. Therefore, postmenopausal women are a primary concern for vitamin D deficiency (Gaugris et al., 2005). The question now is whether co-administered vitamin D with calcium supplementation affects the risk of myocardial infarction or heart disease. Various researchers have studied this subject and come to different conclusions.

Stages of vitamin D status	25(OH)D concentrations (nmol/l)	Biochemical/clinical symptoms
Deficiency	0-25	Severe hyperparathyroidism
Insufficiency	>25-50	Elevated PTH levels, low intestinal calcium absorption rates, reduced bone mineral density, subclinical sympathy
Hypovitaminosis D	>50-70 to 100	Low body stores of Vitamin D, slightly elevated PTH
Adequacy	70-100 to 250	No disturbance of vitamin D-dependent functions
Toxicity	>250	Intestinal calcium hyperabsorption, hypercalcemia

Table 4. This table demonstrates that a deficiency of vitamin D leads to elevated parathyroid hormone (PTH) levels and low intestinal calcium absorption (Taken from Zittermann. 2006)

Parathyroid hormone maintains the short-term homeostasis of extracellular ionized plasma calcium (ECF-Ca 2+) by increasing calcium re-absorption and mobilizing calcium from the labile bone pool. The major control of ECF-Ca 2+ is through the renal production of 25(OH)2D. The homeostasis of calcium ions is complex due to the influence of the gastrointestinal tract, the bones

and the kidneys. Once vitamin D is absorbed from one's diet or produced by the skin from the presence of sun exposure, it is metabolized in the liver to 25-hydroxyvitamin D. The kidney then serves as the endocrine gland to produce the biologically active form of vitamin D. This active form of vitamin D, 1-α,25-dihydroxyvitamin D3, is synthesized in the proximal tubule by the renal cytochrome P450 enzyme 25- hydroxyvitamin D3-1-α-hydroxylase. Additionally, the kidney regulates the concentration of vitamin D through determining the final excretion of calcium ions in the urine.

Homeostatic levels of calcium ions are necessary in order to insure normal bone mineralization (Hoenderop and Bindels, 2005). According to Hoenderop and Bindels (2005), The vitamin D endocrine system is crucial for the proper development and maintenance of this Ca2+ homeostatic system. The homeostasis of extracellular ionized plasma calcium (ECF-Ca 2+) is tightly regulated by a number of hormones, including Vitamin D. Vitamin D is transported to the liver, binding to a specific α-globulin and to a small extent, albumin and lipoproteins. The vitamin is then hydroxylated to calcidiol, 25-hydorxylated vitamin D- 25(OH)D. Afterwards, the vitamin D passes to the kidney where it is further hydroxylated to 1,25-dihydorxylated vitamin D- 1,25(OH)2D. This active metabolite increases ECFCa2+ by increasing calcium and phosphate absorption from the gut and mobilizing calcium from the bone (Sahota 2000).

Because 25(OH) D is the substrate for the renal enzyme that produces 1,25(OH)2D3, it may have a direct and indirect effect on calcium absorption. A deficiency of vitamin D will reduce the intestinal absorption of calcium (Diaz-Lopez and Cannata-Andia, 2006). Inadequate vitamin D levels contribute to high levels of parathyroid hormone which lead to excessive bone remodeling and ultimately to bone weakening (Wang et al., 2010). Moreover, excess parathyroid hormone levels increase blood pressure and cardiac contractility and leads to cardiomyocyte hypertrophy and interstitial fibrosis of the heart. Thus, excess parathyroid hormone contributes to cardiovascular disease. According to Jean et al. (2008), 25 (OH) D deficiencies may lead to mineralization and bone formation defects. Vitamin D inhibits thrombosis and arterial calcification. It is therefore essential for calcium homeostasis and cardiovascular health.

It has been shown that women who use vitamin D supplements have a lower mortality from cardiovascular disease than those that do not. For example, in a large cohort of 51,037 U.S. patients receiving hemodialysis, those who received activated injectable vitamin D had lower cardiovascular disease mortality (7.6 per hundred-person years) than those who did not receive the therapy (14.6 per hundred person-years) (Wang et al., 2010). The investigation of Bostick, et al. (1999) on the Iowa Women's Health Study showed similar results. However, although Bostick, et al.'s (1999) study assessed nutrient intake by using questionnaires, and assessed the risk of cardiovascular disease by using predetermined endpoints, their study did not evaluate participants' sun exposure and duration of supplement use. Additionally, an Australian study of elderly women that compared the risk of ischemic heart disease between women assigned vitamin D placebo plus calcium supplementation to those assigned to both vitamin D and calcium supplementation found similar results (Wang et al. 2010).

One way to explain the lowered risk of cardiovascular disease among women using vitamin D supplements in addition to calcium is that 1,25-vitamin D directly inhibits vascular calcification (Zittermann, 2006). 1,25-vitamin D levels are inversely correlated with the extent of vascular calcification; the higher the intake of vitamin D, the less vascular calcification there will be (Watson et al, 1997). Without sufficient vitamin D, women may not be able to absorb ample amounts of calcium. Heaney et al. (2003) observed in their study of 34 postmenopausal women that the mean

calcium absorption under vitamin D repletion was 35.3% (+/-11.8) of the load and without supplemental vitamin D, 22.5% (+/-12.0) of the load. It is clear from their study that without sufficient vitamin D, women will absorb a minimal amount of calcium.

Inadequate vitamin D intake seems to be associated with an increased risk for cardiovascular diseases. Should the hypothesis that vitamin D deficency contributes to cardiovascular disease be true, perhaps cardiovascular mortality would be higher in countries of higher geographic latitude and vice versa.

CONCLUSION

Many researchers, such as those who conducted the Women's Health Initiative and the Iowa Women's Health study, have concluded that calcium supplementation reduces the risk of coronary heart disease, myocardial infarction and other cardiovascular related conditions. Others did not observe a statistically important difference between the placebo and supplemented groups. Finally, other researchers observed detrimental effects of calcium supplements on myocardial infarction rates, such as the study of Bolland et al. (2007 and 2010). However, even in the case study of Bolland et al. (2010), the increased risk for myocardial infarction, in the calcium-supplemented group was not statistically significant, once the values of myocardial infarction events were verified from the national database. Additionally, this study did not consider the effect of vitamin D as a variable.

It may be suggested that calcium supplementation with co-administered vitamin D can reduce the risk of cardiovascular disease. On the contrary, calcium supplementation, without co-administered vitamin D appears to have deleterious effects on cardiovascular disease. Further research is required to understand better the mechanisms of calcification and the role of vitamin D in calcium absorption, as it relates to lowering the risk of cardiovascular disease.

In conclusion, when women are treated with calcium, more instances of myocardial infarction will be caused than the number of symptomatic fractures that will be prevented (Bolland et al., 2007). This statistical data brings to the forefront the idea that calcium supplementation in effort to prevent osteoporosis, may not be an appropriate choice.

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Cardiac Jelly and Its Roles in Heart Development

SARA ZUCKER

INTRODUCTION

The complexity of the developing and functioning heart has always intrigued scientists. One of the many ambiguous areas in the understanding of cardiac development is the role of a gelatinous substance commonly referred to as cardiac jelly, (Davis, 1924) and more recently the myocardial basement membrane (Little and Rongish, 1995). Researchers have been studying this material to determine its roles. Their studies led them to believe that this jelly-like substance may be involved in vital embryological roles including the actual morphogenesis of the heart, such as heart valve formation and pumping. When cardiac jelly was enzymatically removed, the morphology of the heart changed because cardiac jelly exerted a force on the cardiac tissues which influenced morphogenesis (Mironov et al. 2005). The components of the cardiac jelly are thought to play roles in regulating cell shape, migration, proliferation, and differentiation. They are involved in the binding of growth factors that control cell behavior and cell-to-cell communication and it is targeted in controlling gene expression and maintenance of tissue related functions. With congenital heart malformation leading human birth defects- it occurs in 1% of all births-the analysis of cardiac jelly can lead to a better understanding of the way the heart develops and runs and can ultimately lead to a technique that mends those deformities (Little and Rongish, 1995; Eisenberg and Markwald, 1995).

Development of Cardiac Jelly

Cells commit themselves to become cardiogenic during gastrulation. They arrange themselves into bilateral tubes composed of an inner endocardium and an outer epimyocardium that will later become the muscular outer tube of the heart. Between these two layers lies a primitive extracellular matrix, which will later contribute to the cardiac jelly. In stage 7 or 8 of cardiac development, this matrix contains basement membrane proteins among other components. At stage 9, the bilateral tubes fuse and two concentric layers, the myocardium and endocardium, are apparent. (Little and Rongish, 1995; Moore, 2008). There are two distinct regions present as well; cranial, which contains the primordium of the right ventricle, and caudal, which contains the trabeculated portion of the left ventricle. At stage 10 this tube begins to beat. During the loop stage, stage 12, the primordia of the right ventricle and aorta are present. This stage is characterized by a looping process that changes the shape of the heart drastically. It is at this stage that the extracellular matrix is referred to as cardiac jelly. In stages 14-17, heart chambers and valves are forming. Swelling can be found in the matrix while the endocardium of the atrioventricular canal and outflow tract are transformed into mesenchymal cells which invade the cardiac jelly and are now called cardiac

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cushions. Many of the components found in cardiac jelly aid this transformation (Little and Rongish, 1995).

Composition and Location of Cardiac Jelly

Cardiac jelly is composed of three main substances; collagens (I, III, IV), glycoproteins, like fibronectin, laminin, and fibrillin, and mucopolysoaccharides (glycosaminoglycans) such as hyularonate, chondroitin, and heparin (Hurle et al. 1980; Little and Rongish, 1995). Both the shape of the cardiac jelly and the internal pressure of the heart may come from the glycosaminoglycans and proteins found in the cardiac jelly (Nakamura and Manasek, 1981).

There are also fiber components of cardiac jelly which are arranged in a radial orientation (Taber et al. 1995). It consists of a myocardial basement membrane that has a lamina densa and an extended lamina reticularis and an endocardial basement membrane with only a lamina densa. Together, the matrix is distributed unevenly with thin amounts of jelly located at the ventral and dorsal midlines of the heart and thicker amounts at the original left and right sides of the heart. This arrangement will be extremely important later on in the role of pumping which is aided by cardiac jelly. Because of this uneven distribution, the endocardial tube does not exhibit a circular profile. (Manner et al. 2008). The difference in thickness between the bulbus cordis (embryonic right ventricle and embryonic outflow tract) and the ventricular segment (embryonic left ventricle) of the heart loop are a result of differences in the thickness of the cardiac jelly itself (Manner et al. 2006).

The cardiac jelly also has a distinct shape that reflects the shape of the heart. This allows the outer myocardium to be removed without affecting the shape of the cardiac jelly (Nakamura and Manasek, 1978).

In terms of location, the primary heart tube consists of an outer myocardium and inner endocardium (Eisenberg and Markwald, 1995) separated by a layer of cardiac jelly (Armstrong and Bischoff, 2004). Cardiac jelly is located along the entire length of the primitive heart; however, researchers separated cardiac jelly based on its location. Either cardiac jelly is found between the myocardium and the endocardium (MECJ), or it fills up the dorsal mesocardium between the endoderm and endocardium (EECJ). The latter is only present as long as the dorsal mesocardium is around. There are differences in the compositions of MECJ and EECJ which thereby gives these two areas different functions (Hurle et al. 1980). For this paper, we will refer to them synonymously.

Role of Cardiac Jelly in Cardiac Looping

One of the proposed roles of cardiac jelly is its involvement in cardiac, or dextral, looping. This process involves transformation of the "straight bilateral precardiac splanchnic mesoderm" heart tube into the c-shaped heart loop (Nakamura and Manasek, 1981). At this stage, the first signs of left-right asymmetry in the heart can be detected and two distinct regions are apparent; the primitive ventricular region and the primitive outflow tract, or the conus separated by the conoventricular sulcus. The primitive ventricular region will start bending ventrally and toward the right in a C-shape or dextral bend (Manner, 2000). Manasek believed that this bend was a result of the internal pressure of the expanding cardiac jelly (Nakamura and Manasek, 1981). They hypothesized that the dorsal mesocardium acts as a "stiffener" that compels the tube to bend as it inflates due to the pressure of cardiac jelly. They even used rubber models with similar tension and pressure and observed the same results (Taber et al. 1995). This was supported by the observation of increased

mucopolysaccharides in the cardiac jelly specifically at this time (Hurle et al. 1980). However, this was disproved when subsequent scientists degraded cardiac jelly with hyaluronidase with no disturbance of the dextral looping (Taber et al. 1995). Other components of the cardiac jelly besides hyaluronan may be involved in the looping process, so to rule out cardiac jelly as the driving force behind cardiac looping may be premature.

Later on, studies of another event in dextral looping began to indicate a possible role for cardiac jelly in this phase, although slightly indirectly. At the dextral stage, the atrioventricular canal develops between the primitive ventricles and primitive atria (Manner, 2000).

Cardiac jelly within the atrioventricular canal swells and it is now considered the primordia of cardiac cushions (Eisenberg and Markwald, 1995). In other words, this jelly is the precursor of the atrioventricular endocardial cushions. These cushions will eventually be responsible for the division of the primitive atrioventricular canal into the left and right atrioventricular canals. These cushions are also found in the ventricular segments, the outflow tracts, or conus, and the truncus. Although not much is understood about these cushions, they may be related to cardiac looping and deviations in the formation of the loop may have been a result of improper cushion positioning (Manner, 2000).

Temporary Heart Valve Formation- Cardiac Jelly-Endocardial Cushions

In the primitive heart, no valves exist to prevent backflow, allowing a "backlash" or a retrogression of blood. As the embryo grows older, the progression of blood becomes smoother. This was attributed to "mounds" of endocardium piling up within the atrioventricular canal blocking the cardiac lumen. These mounds completely prevented backflow. The expanding cardiac jelly formed the endocardial mounds. Not only was this action found at the atrioventricular canal, but by the ventricular conus and truncus arteriosus-the places where the semilunar valves of the aorta and pulmonary trunk will form-as well. The atrioventricular canal alternates with the truncus so that when one mound is opened the other is closed. This enables cardiac jelly to aid in the pumping of blood (Patten et al. 1948).

Because of this role of cardiac jelly, researchers proposed that the blood passing through the cardiac jelly molds it. In turn, this will influence the mounds and ridges needed to form the valves and septa of the heart (Patten et al. 1948). Here, the two roles allotted to cardiac jelly were its ability to act as a pump while changing its shape and position as the heart develops and as a medium through which the blood flow will shape the heart (Overman and Beaudoin, 2005).

Role of Cardiac Jelly in Endothelial-Mesenchymal Transdifferentiation

Endothelial-Mesenchymal Transdifferentiation or EMT is the process by which endocardial endothelial cells migrate into the cardiac jelly and transform into mesenchymal cells. The cushion cells will come from endocardial endothelial cells that are transformed into mesenchyme by this process (Armstrong and Bischoff, 2004). This process only occurs in the atrioventricular canal and ventricular outflow tract. Small particles develop and deposit themselves within the cardiac jelly, forming larger complexes called adherons. The components of the adherons include the ES antigens, which are multiprotein complexes that may provide the signal for EMT (Eisenberg and Markwald, 1995). The ES antigens join fibronectin and other components of the cardiac jelly to regulate this transformation (Little and Rongish, 1995). Therefore, cardiac jelly containing the transdifferentiated cells is the precursor of cardiac cushions which in turn are responsible for heart valve and septum formation.

The early heart has four different segments: the ventricular outflow tract, ventricle, atrioventricular canal and atrium. After dextral looping, the cardiac jelly associated with the atrioven tricular canal and outflow tract regions begins to expand. In the outflow tract region, the cardiac jelly forms "parietal and septal ridges" (Nakajima et al. 1997). The expansion is accompanied by an invasion of mesenchymal cells known as EMT (Eisenberg and Markwald, 1995). At stage 39, the first invasion of mesenchymal cells occurs in the atrioventricular region while the outflow tract is only invaded beginning from stage 40 and becomes populated with cells at stage 41 (Lee and Jeannet, 2009). The cardiac jelly cells, or cushion primordia, grow until these cushions are ready to undergo valvuloseptal formation where the cushions become valves and septa (Eisenberg, 1995).

The important role that cardiac jelly plays in the process of cushion formation is the signal it provides to initiate the endocardial differentiation, EMT. These signals are soluble factors found in the jelly and can cause EMT to begin. It has been found that the extracellular matrix regulates the growth of these cells.

All three of the glycosaminoglycans found in cardiac jelly have been shown to play a role in cardiac development (Armstrong and Bischoff, 2004). When chondroitin sulfate was not expressed in the heart, no atrioventricular formation occurred and cell migration was impaired (Peal et al. 2009).

Hyaluronic acid (HA) has been targeted as a major component for the process of EMT. It has been linked to chamber septation and heart valve formation (Camenisch et al. 2002). Hyaluronic acid is one of the glycosaminoglycans found in cardiac jelly. It is a hydrated gel that allows the extracellular space to expand in addition to regulating its ligand availability. Experiments were performed that removed the enzymes responsible for forming hyaluronic acid. The cardiac jelly did not form and in consequence, neither did the endocardial cushions.

When early researchers used hyaluronidase, they discovered that the overall shape of the heart was not changed, but the heart shrunk and became flaccid. They concluded from here that the myocardium, although it can retain its shape without cardiac jelly, needs the jelly to retain its size (Nakamura and Manasek, 1981).

EMT and migration of endocardial cells were greatly reduced in the presence of hyaluronidase (Nakamura and manasek, 1981). It has been shown that both the migration as well as the transformation of cells requires hyaluronic acid. Additionally, hyaluronic acid promotes transformation into prevalvular mesenchyme and provides a medium for the cells that are entering. Scientists believed that components within the cardiac jelly, such as hyaluronan, might be responsible for the initiation of EMT. However, hyaluronic acid does not act alone in regulating these tasks. They are regulated by the ErbB family of receptor tyrosine kinases. Cells undergoing EMT express ErbB3 in the membrane of endocardial and mesenchymal cushion cells. Hyaluronic acid allows for endothelial cells to become mesenchymal cells by activating the ErbB family during EMT. Hyaluronic acid activates the ErbB receptor complex on the atrioventricular canal endocardium in order to induce EMT. These cells become mixed into the cardiac jelly and will remodel the cushions into cardiac valves. There is currently insufficient research to say if hyaluronic acid is involved directly in transformation or if it is responsible for forcing endothelial cells to become mesenchymal. However, the research providing evidence that connects hyaluronic acid and ErbB and the roles they play may be of critical importance in understanding cardiac formation and repair. Mice without ErbB3 died with hypoplastic cardiac cushions and decreased mesenchyme. (Armstrong and Bischoff, 2004; Camenisch et al. 2002)

Heparin, which is also found in cardiac jelly, has been found to play a role in proper cardiac valve formation (Camenisch et al. 2002).

Role of Cardiac Jelly in Heart Pumping

Pumping is another vital function of the heart attributed to cardiac jelly. As early as the 1940s, studies showed that had the endocardial and myocardial portions of the cardiac tube been in direct contact with each other without the cardiac jelly layer in between, no heart pumping would have been possible. They showed that the width of the cardiac jelly layer must be 45% of the radius of the lumen in diastole for pumping to take place (Barry, 1948; Manner et al. 2008). Others found that cardiac jelly provides the physical size necessary for the heart to pump blood and acts like a valve to ensure that blood is only flowing in the forward direction (Gessner et al. 1965). The heart begins to pump blood by waves of contractions known as peristaltoid. The details of these contractions are beyond the scope of this research paper. During the systole phase of heart pumping, the myocardial tube contracts concentrically while the endocardial tube narrows eccentrically. As was mentioned earlier, the eccentric formation of the endocardial tube is a consequence of the uneven distribution of the cardiac jelly. At the ventral and dorsal midlines of the heart, the cardiac jelly is extremely thin. This elliptical form of the endocardial tubes enables blood to be pumped more efficiently compared to the circular shape and minimizes the mechanical stress caused by contractions. Both the thickness that cardiac jelly provides as well as its uneven shape enables the heart to perform one of its most vital functions, pump blood (Manner et al. 2008).

Clinical Significance

Although many roles have been attributed to cardiac jelly, most researchers are still hypothesizing what this substance really does. Therefore, the ramifications of an altered or absent jelly cannot be directly linked to any specific malformation or deformity of the heart. However, several ideas have been presented.

Decreased mucopolysaccharides have been linked to congenital malformations. Sulfate mucopolysaccharides in the limbs of a chick embryo were found to cause skeletal defects. These glycosaminoglycans decrease as the embryo gets older while cardiac jelly naturally diminishes after day 14. The greatest effect of decreased sulfate occurs on day 13 which is coincidentally the day that cardiac jelly is most actively involved (Overman and Beaudoin, 2005).

Mitral Valve Prolapse is a condition where the heart valve exhibits changes in its shape and rigidity. Data shows an increase of chondroitin sulfate, also one of the glycosaminoglycans found in cardiac jelly, in these valves. However, chondroitin sulfate has not yet been conclusively targeted as the cause of this condition. (Peal et al. 2009).

When EMT and cells began to invade the cardiac jelly, many genes, such as Sox9, were activated. When this gene was not present, other genes, such as ErbB, were unable to be activated. A human disease linked with this is campomelic dysplasia whose symptoms include “endochondral bones, XY sex reversal and occasional kidney and pancreas deformities (Akiyama et al. 2004)”.

Altering the collagen composition in the embryo by blocking certain receptors may prevent cardiac cushions from forming, and as noted before, various defects are associated with cardiac cushion flaws (Lamparter et al. 1999).

In Cardiac Lethal Mutant Axolotl, the myocardium is unable to assemble myofibrils. The cardiac jelly is affected here because it doesn’t allow sufficient hyaluronate or proteoglycan

production. Once cardiac jelly is altered, mesenchymal cells won't migrate and cushion tissue does not form (Lemanski and Fitzharris, 1989).

CONCLUSION

Although there has been an increase in the understanding of the primitive heart and its components, further research is necessary to determine the roles of this magnificent organ. Cardiac jelly found in the extracellular matrix of the heart has been linked to many vital morphogenic processes integral for proper heart formation and function such as heart valve formation and pumping, among others. How and why this unique jelly-like substance operates within the heart is not yet clearly defined but with further research, scientists may grasp its significance and use their knowledge to treat the various malfunctions that arise.

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Dextromethorphan

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INTRODUCTION

Dextromethorphan (DXM) is a cough suppressant. It works to raise the coughing threshold in the brain by affecting the cough center in the medulla. As of July 2010, it is the only over-the-counter, oral, nonprescription antitussive widely available in the United States. It is also a very popular drugs used for abuse because it is cheap and readily accessible in large quantities.

Also called demorphan hydrobromide, dextromethorphan's molecular formula is C18H25NO. HBr.H20 (Figure 1). It is a 3-methoxy-17-methylmorphinan monohydrate, the dextro isomer of levophenol, and a derivative of morphine. Included in its various action mechanisms, it is a NMDA receptor antagonist, a sigma-1 receptor agonist, and a serotonin reuptake inhibitor. It has many similar chemical properties to opiates, but it is generally not addictive, nor is it a pain reliever. It is similar to codeine because of its cough suppressant properties; however, dextromethorphan is not an expectorant. (Magarey, 1996)

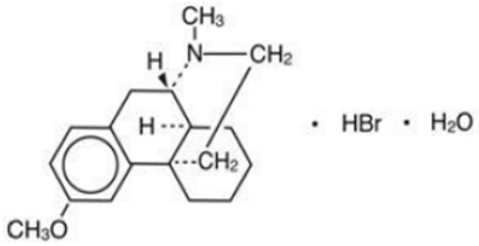

Figure 1. The molecular formula of dextromethorphan hydrobromide monohydrate is C18H25NO•HBr•H2O, and its molecular weight is 370.33 amu. (Magarey, 1996)

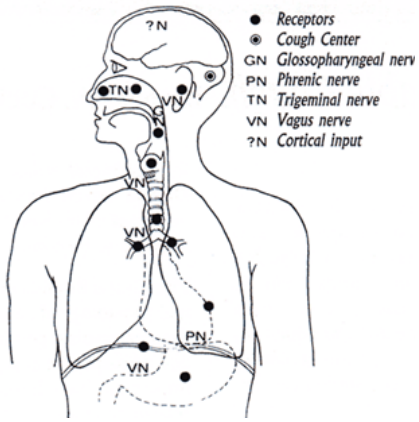

Figure 2. The cough center in the brain is at the pons and the medulla. The phrenic nerve exits the brain to provide motor function to the diaphragm. (Chung, 2007)

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Over 100 products use dextromethorphan as an active ingredient. Some of the more commonly used cough medicines that contain DXM include Alka-Seltzer Plus, Robitussin Cough products, Sudafed products, Tylenol Cold and Cough, Vicks 44 Cough Relief, and Vicks DayQuil and NyQuil.

Dextromethorphan as a Cough Suppressant

The general action of the cough occurs as a result of a large assortment of stimuli. These irritants affect the rapid- adapting receptors, influenced by stimulants such as cigarette smoke or dust, and the slowly-adapting receptors, which initiate expiration when the lungs have not com-

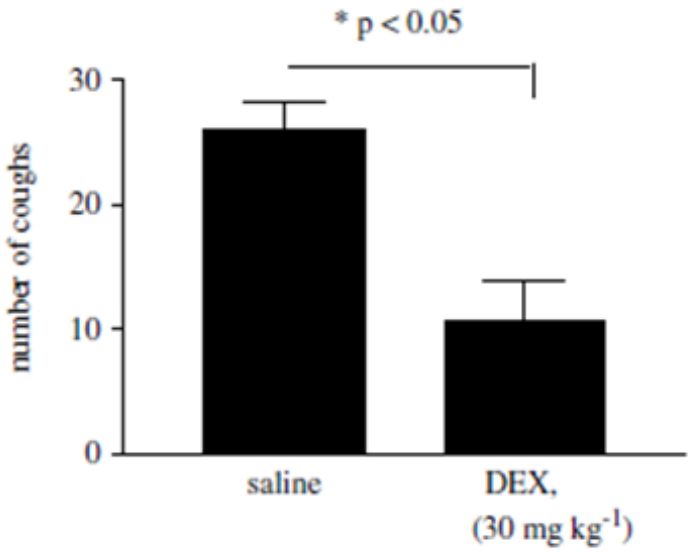

Figure 3. The animals that received an injection of dextromethorphan had 60% less coughs than the animals that were injected with a saline solution. (Brown, et al, 2004)

pleted inflation. These receptors and sensory neurons are situated at the larynx and tracheobronchial tree, especially at the carina and the bronchiol branches (Figure 2). The irritants cause the airways to forcefully expel the air, pushing against the closed glottis. This is what is known as coughing. (Chung, 2007)

Dextromethorphan is used primarily as a cough suppressant. Glutamate receptors and sigma receptors in the caudal portion of the nucleus tractus solitarus (NTS) may be the active site of antitussive drugs. To prove this hypothesis, groups of rabbits were injected with antitussive drugs and receptor agonists. The injections were given at the caudal portion of the NTS. The control group was given the same drugs, but they were injected in the nucleus cuneatus. Then, coughing was induced in the rabbits by inserting a nylon fiber into the trachea and lowering it until the carina. The fiber was moved around the airway, touching the bronchial walls and the carina. Coughing was measured by calculating the activity of the phrenic nerve and the oblique abdominal muscles, using electromyography to detect the electric potential of the muscle cells. The rabbits that had received the antitussive drug in the NTS had a significantly decreased the number of coughs in comparison to the control group. The receptor agonists, including DAMGO and baclofan, also reduced

the number of coughs. Within forty to seventy minutes, the rabbits experienced a complete recovery and did not cough at all, even when physically stimulated with the nylon fibers. The experiment demonstrated that the receptors in the caudal portion of the NTS are the main site of antitussive drugs. Receptor agonists suppress the cough reflex, and can eliminate coughing completely if administered at higher concentrations. (Mutolo et al, 2008)

Dextromethorphan acts as an agonist at the sigma-1 receptors. Sigma receptors are most commonly found in the central nervous system, primarily at the nucleus tractus solitarus. The NTS is located near the cough center in the brainstem and it contains the first synapse of the nerves leading to the airways and lungs.

Therefore, it has been hypothesized that dextromethorphan can prevent coughing activities in the NTS using the sigma factors before the cough reflex stimulus reaches the brain. There was a study done on guinea pigs to

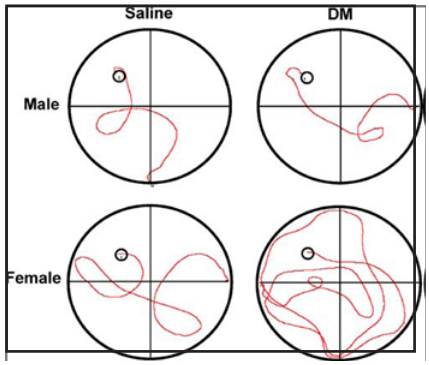

Figure 4. The dextromethorphan had no apparent affect on the male rats. However, the female rats with dextromethorphan took a longer, more circuitous route to find the escape platform than the female rats with saline. This showed the affect of DXM on memory retention. (Zhang, et al, 2007)

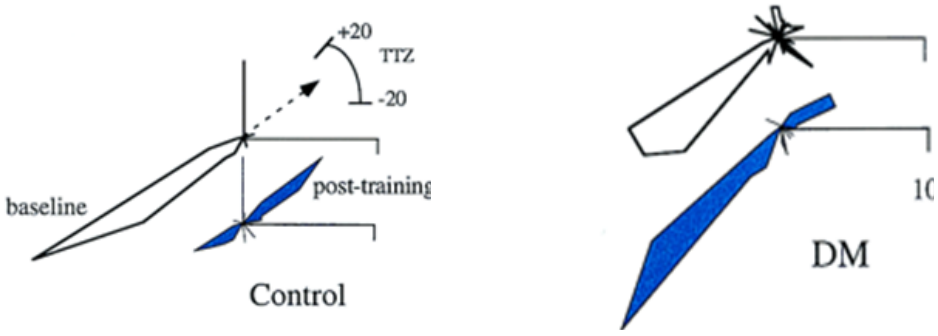

Figure 5. In the control group, the direction of the thumb post-training moved in the same direction as it had been trained. However, DXM blocked the plasticity of the motor cortex; therefore the thumb moved in the original direction that it had during the first electrical stimulation. (Butefisch et al, 2000)

determine whether dextromethorphan, as a sigma factor agonist, can inhibit excessive coughing. The animals were first treated with citric acid through a nebulizer to induce coughing. Some of the pigs were injected with dextromethorphan; the others were injected with saline. Coughing in the animals with dextromethorphan was reduced by 60% in comparison to the animals in the control group (Figure 3). (Brown et al, 2004)

The Potential Dangers of Dextromethorphan Abuse

Dextromethorphan is not addictive physically, but it can become addictive psychologically. In response to the cessation of abuse, there will not be any neurological hyperactivity that causes the brain's dopamine system to crave the drug, and there will usually not be any negative with-

drawal symptoms. (In cases of long-term, extreme abuse, there may be physical dependency and mild withdrawal symptoms, including insomnia and depression.) However, people who use DXM often may develop a habit of abuse. They can become psychologically dependant on the substance. There was a case study done with 53 volunteers, all with varied histories of DXM use. They were brought together to determine the side effects of dextromethorphan abuse. The results showed that 46.5% of the people repeated the DXM abuse because of psychological dependency, while 32.6% did it only for recreational purposes. (Ziaee et al, 2005)

More than 7% of teenagers, according to a study in 2008, take large amounts of over-the-counter dextromethorphan at one time in order to put themselves into an altered state of consciousness. The teenagers enjoy putting themselves in a state of euphoria. However, the affects may also include dizziness, nausea, loss of coordination, sweating, blurred vision, headaches, numbness in the extremities, vomiting, slurred speech, and stomach pain. Eventually, a person may have psychological affects too, including hallucinations, confusion, and dysphoria. Research has also shown that DXM has long-term affects as well. It may cause memory loss and learning disabilities. As the doses increase, the affect gets increasingly worse. It may even be fatal. (Etingoff, 2008)
According to the experiment conducted by Ziaee et al (2005), during the first day after the ingesting the abusing doses of DXM, there were many different kinds of side effects experienced by the subjects. Among the autonomic side effects, 54.7% of people were sweating, 45.3% had tachycardia (increased heart rate), 41.5% felt fatigue, and 22.6% feel tachypnea (increased respiration rate). Another kind of symptom experienced was the gastrointestinal side effects. 30.2% of the people felt nausea, 24.5% were vomiting, and 7.5% had diarrhea. Approximately 30% of the people had neurological side effects, including dyskinesia (diminished voluntary movements), speech disorder, dizziness, while 20% felt imbalance and dysaphia (impairment in the sense of touch). During the first week after taking the abusing dose, 15-20% of the people reported insomnia, nightmares, and anhedonia (inability to experience pleasant emotions from normally pleasant experiences).

Criteria
1. Changes in metabolism when patients are treated with CYP2D6 inhibitors
2. Differences between metabolism of healthy subjects and patients with liver disease
3. Correlation of CYP2D6-mediated metabolite concentration with CYP2D6 activity and content in human liver microsomes
4. Proven in vitro specificity of the metabolic step used
5. High contribution of the metabolic step to overall drug metabolism
6. Good availability of the drug (i.e. registration as a therapeutic drug

Figure 6. Criteria for a probe drug to use in determining CYP2D6 enzyme activity. (Frank et al, 2007)

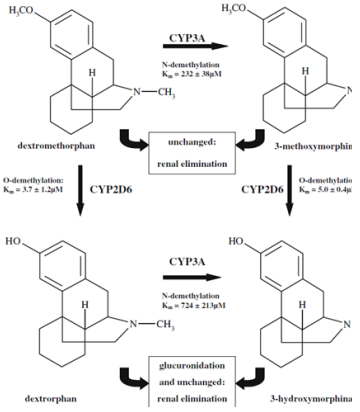

Figure 7. The metabolic process of dextromethorphan. (Frank et al, 2007)

Additional Side Effects of Dextromethorphan

A major affect of dextromethorphan is its impact on memory retention. Not only is it an agonist at sigma-1 receptors, but it is also a noncompetitive antagonist of N-methyl-D-aspartate

receptors (NMDA). Lab tests showed that high doses of DXM during adolescence will increase the expression of NMDA1, a functional subunit of the NMDA receptor, which can have an effect on learning capability and memory. The laboratory study began by injecting a group of newborn rats with a DXM solution, while the control group of newborn rats was injected with saline. The rats were then trained in the Morris water maze; eventually they learned how to find an escape platform situated in the center of the maze. Then the rats learned to find the escape platform when moved to a new location. Finally, the scientists set the escape platform in the original position to examine the rats' ability to retain memory. This reversal training had no apparent effect on the male rats, but the female rats with dextromethorphan took much longer to find the escape platform in comparison to the female control group (Figure 4). At 18-20 months, the rats were killed and the brains of the rats were removed. The male rats had increased NMDAR1 expression in the hippocampus, and females in the hippocampus and the prefrontal cortex. The hippocampus is responsible for memory retention, and the prefrontal cortex controls cognitive behaviors and decision-making. The results show that dextromethorphan administered at the adolescent stage can have a significant affect in these parts of the brain later on in life. Also, females tend to be more vulnerable to DXM at adolescence. Therefore, chronic abuse of dextromethorphan at a young age will result in long-term memory reduction. (Zhang et al, 2007)

Another affect of dextromethorphan is its ability to influence the plasticity of the motor cortex in humans. Plasticity is the capability of the brain to adjust to new experiences and newly learned information. There was a study done to test the hypothesis that DXM would obstruct synaptic plasticity. A subject was strapped tightly to a chair; only his thumb was able to move freely. There was a stimulating coil attached to his head. First, a transcranial magnetic stimulation was applied through the coil to the motor cortex of the brain. The thumb's direction of movement was measured, and the angle of activity between the flexor pollicis brevis and the extensor pollicis brevis muscles was measured. That movement was documented as the thumb's baseline. In most subjects, the thumb moved in the extension/abduction direction. Next, the subject voluntarily moved his thumb repeatedly in the opposite direction of the baseline, in the flexion/adduction direction. The purpose of this was to train the thumb and embed the new movement in the subject's memory. Then the stimulation was electrically applied again to see which direction the thumb would move. This entire process was done first as a controlled experiment without any drugs, but the second time the subject was given dextromethorphan orally. In the control state, the thumb moved in the direction in which it was trained. However, DXM blocked the thumb from following its previous training session; instead, it caused the thumb to revert back to shifting towards the baseline direction. The study was repeated on more subjects with the same results (Figure 5). The conclusion of the study was that dextromethorphan has an effect on the plasticity in the motor cortex. The NMDA receptor is required for long term potentiation, the strengthening of the association between nerve cell synapses. Because DXM is an inhibitor of the NMDA receptor, the nerve cells are not as strongly connected and therefore it reduced the plasticity of the motor cortex. (Butefisch et al, 2000)

Functional Use of Dextromethorphan as a Probe Drug

A functional use of dextromethorphan is as a probe drug. Probe drugs are used in many pharmacological studies to assess the activity of a drug metabolizing enzyme. To determine the amount of enzyme activity of a specific cytochrome, bodily fluids such as urine or saliva are taken and examined. The activity of CYP2D6, mainly located in the liver, cannot be determined by

genotyping, because its actions are determined by environmental factors. Its genotype polymorphisms cause it to have a large range in its amount of substrate drug clearance. Therefore, adequate probe drugs are required before measuring compounds in bodily fluids. This is known as "phenotyping." Normally, eliminating a substance from the body would help to determine enzyme activity. However, the elimination of a drug is conducted by several different enzymes in addition to renal excretion. Therefore, partial clearance of a compound metabolized by CYP2D6 is necessary to properly determine enzyme activity.

Dextromethorphan meets all of the requirements for an adequate probe drug (Figure 6). Thirty milligrams of dextromethorphan hydrobromide was established as most useful for phenotyping. The low dose will do what is necessary, but will not cause harmful side effects in adults. The DXM metabolic pathway begins with CYP2D6 or CYP3A. CYP2D6 will cause O-demethylation and convert DXM to dextrorphan. CYP3A will convert dextromethorphan into 3-methoxymorphinan and dextrorphan into 3-hydroxymorphinan by causing N-demethylation to occur. CYP2D6 will convert 3-methoxymorphinan into 3-hydroxymorphinan by causing O-demethylation (Figure 7). At any point during the metabolic progression, the molecules can be processed by glucuronidation and then eliminated via renal excretion. The urine is then examined for the derivatives of DXM to determine the amount of CYP2D6 enzymatic activity. Because CYP2D6 metabolizes over 90% of DXM in humans, DXM is the most appropriate probe drug to use for this procedure. (Frank et al, 2007)

Dextromethorphan as a Commercial Drug

Dextromethorphan is considered to be a safe drug when used properly, but can be extremely dangerous if used beyond the suggested dosages. Normal therapeutic dosages for adults are 10 to 20mg every four hours. Children between the ages of six and twelve should take 5 to 10 mg every four hours, and children between the ages of two and six should take 2.5 to 5 mg every four hours. DXM is not recommended for children under the age of two unless administered under strict medical supervision (Magarey, 1996).

Dextromethorphan is also a serotonin reuptake inhibitor. Serotonin is a neurotransmitter that is involved in regulating a human's appetite, sleep, muscle contractions, and emotions. Inadvertent interactions of dextromethorphan with other serotonin reuptake inhibitors can cause excess serotonin in the synapses of the neurons thereby causing serotonin toxicity syndrome. Some of the symptoms are mild, such as anxiety, agitation, tachycardia, and tachypnea. Other, more serious symptoms include diarrhea, fever, and coma. However, sometimes the symptoms can be chronic or even fatal. Therefore, DXM should not be administered if the patient is already taking another serotonin reuptake inhibitor drug.

The Food and Drug Administration (FDA) had determined in 1976 that dextromethorphan is a "nonnarcotic antitussive agent…and has no significant abuse liability."(USFDA, 2010) It was classified as a Category I ingredient, which meant it was recognized as safe and effective. In 1987, the FDA published the Final Monograph for Antitussive Drug Products, which allowed the use of dextromethorphan for anyone over the age of 2. It also established to maximum daily dosages. Adults and children over twelve years old should not take over 120 mg in twenty-four hours, children from six to twelve years old should not take more than 60 mg, and children between two and six years old should not take more than 30 mg. (United States Food and Drug Administration, 2010).

Starting in 1990, many cases of teenage abuse of dextromethorphan were reported. Abuse dosages range from approximately 100 mg to 2,000 mg taken at one time. Since 2005, at least five teenagers have died as a result of overdosing dextromethorphan. Carl Fetko, a teenage boy from California, was one of the many tragic fatalities as a result of dextromethorphan abuse. He began taking DXM products for pleasure; he enjoyed the feeling of euphoria. His mother, Misty Fetko, found in her son's journal that he "enjoyed the hallucinations achieved upon intentionally abusing cough and cold products." She said that her son described the "pull that he felt towards the dissociative effects of abusing the cough medicine and seemed to crave them."(Fetko, 2010). According to the journal, as he felt more drawn to abuse, he gradually increased the dosages until finally it caused his death (Fetko, 2010).

During the 111th Congress, spanning 2009 through 2010, a bill was presented to prevent the widespread abuse of dextromethorphan. The Dextromethorphan Abuse Reduction Act states that it is illegal to sell dextromethorphan products to an individual under 18 years old, unless a doctor has issued a prescription. This includes sales in stores and over the internet. If an individual is caught not abiding to the law, there will be a penalty of a heavy fine (Library of Congress, 2010). The Dextromethorphan Abuse Reduction Act has been introduced to the Senate, and may become a permanent law in the future.

In September 2010, the FDA met again to discuss a way to address the growing abuse of dextromethorphan. They concluded that it should not be a controlled substance. Today, the Consumer Health Products Association is recommending that sales of dextromethorphan should be banned for teenagers under 18. They may also want the sales of dextromethorphan to require a prescription (Consumer Healthcare Products Association, 2010). The Drug Enforcement Administration asked the FDA to reconsider the status of dextromethorphan, since the number of visits to hospital emergency rooms due to dextromethorphan abuse has drastically increased since 2004. In 2004, there were 4634 cases of abuse, while this number rose to 7988 cases in 2008. However, at the FDA meeting it was argued that controlling dextromethorphan would have a bad effect on public health, because required prescriptions would force people to visit doctors more frequently, and therefore increasing health care costs. (Enderle, 2010).

CONCLUSION

Dextromethorphan is an over-the-counter antitussive and is found in many cough and cold medicines. It effectively reduces the cough reflex by blocking the activity of the neurological receptors. However, it also has been illicitly abused. Overdose of DXM has many harmful side effects, and may even be fatal. Today, health organizations and the United States government are working to regulate the sales of DXM to ensure that the citizens of our country are safe and healthy.

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Epigenetics as an explanation for phenotypic variation in monozygotic twins

MARINA POMERANTSEVA

INTRODUCTION

Researchers often use twins as natural samples to test hypotheses regarding the contribution of genetic factors to different phenotypes, especially diseases. The classical method is comparing traits in identical, or monozygotic (MZ) twins to those of dizygotic (DZ) twins. This method has had a significant impact on our current understanding of etiologic factors in many diseases which do not follow simple Mendelian law (i.e. complex diseases), including schizophrenia, bipolar disease, and major depression.

Twin Studies

Monozygotic twins originate from a single fertilized egg which separates and becomes two separate embryos. Dizygotic twins originate from two separate eggs fertilized by different sperm. Monozygotic twins are often described as physically and genetically identical. Most twin studies rely on the assumption that monozygotic twins share 100% of the same genes, whereas dizygotic twins share approximately 50% of the same genes. Accordingly, it is reasonable to assume that all monozygotic twin pairs should share the same heritable diseases, and half of dizygotic twin pairs should share such diseases. Much of the time, however, this is not the case.

The chief purpose of twin studies is to detect the genes responsible for a given disease. Over the past two decades, there have been a series of discoveries of genes that contribute to human diseases. Most of such disease genes, however, are those that cause simple Mendelian diseases like sickle cell anemia, hemophilia, cystic fibrosis and Duchene muscular dystrophy. On the contrary, the genes underlying complex diseases that exhibit a heritable component but do not follow Mendelian laws have for the most part remained unidentified. The origins of the heritable component in diabetes, schizophrenia, asthma, inflammatory bowel disease, and the overwhelming majority of cancers, for example, have not been found.

In twin studies of complex diseases, one of the difficulties is the rate of discordance (phenotypic dissimilarity) of monozygotic twins. Discordance of monozygotic twins reaches 30% for idiopathic epilepsy, 30-50% for diabetes, 50% for schizophrenia, 70% for multiple sclerosis and rheumatoid arthritis and 80% for breast cancer (Boomsung et al., 2002).

Environment as an Explanation of Discorance

This significant discordance in disease between MZ twins cannot be explained purely based on chromosomal DNA sequence (Chak Ravarti, and Little 2003). The difference in disease concordance in MZ twins is an example of a more general phenomenon, that of phenotypic differences between genetically identical organisms. These differences have usually been attributed to

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the effects of environment, as a cause for differences that remain after genetics are accounted for (Plomin and Daniels, 1987). Differences due to environmental factors, however, are difficult to measure. An example of a significant environmental effect on disease risk is the effect of smoking on lung cancer (Alberg and Samet, 2003), but direct evidence of other measured environmental effects on phenotype is rare. There is more and more evidence that the assumption that non-genetic differences have to be due to the environment is not so accurate after all.

There were a series of studies conducted on over 100 MZ pairs of twins which test the importance of environment as a cause for twin dissimilarity (Bouchard et al, 1990; Marcon et al., 2002). The data of MZ pairs raised together (MZT) was compared to the data of twins raised apart (MZA). The degree of dissimilarity between the MZT and the MZA was assumed to be the result of different environments (Bouchard et al, 1981). A series of tests were administered to each pair of MZA and MZT twins, and the correlations of their scores on each scale were calculated. Surprisingly, the correlations within MZT and MZA twin pairs on personality measurements were almost identical. Both intra-class correlations are less than 1, and the difference between them is not significant.

Other studies have yielded similar results. Svensson, et al (2003) studied the etiology of migraine headaches, gathering data from 80 pairs of twins. The authors found that susceptibility to migraine was mostly inherited and that twins separated earlier had even greater similarity in migraine headaches. Migraine reports were almost similar in MZA compared to the MZT group; rMZA=.58 and rMZT =.46.

The results of the studies cited above show that intra-class correlations factors rMZT and rMZA are always far less than 1. Therefore, there is always a substantial non-genetic component of heritability. At the same time, rMZT and rMZA are not significantly different. Thus, different environments cannot explain the fact that the rMZT is less than 1. Therefore, the remaining variation in phenotype is not due to environmental factors. In many other recent studies, researchers also came to the conclusion that the pure heritage-environment approach is not enough to explain the twins data described above (Wong, 2005; Haque, 2008).

Similar experiments were done with animal strains that were inbred for many generations which consequently have almost identical chromosomal DNA sequence. MZ and DZ twins can be generated by artificial fertilization and artificial twinning procedure (Keith and Machin, 1997). In these procedures, the environment can be strictly controlled, which is not possible with humans.

In a series of experiments designed to find the relative contributions of genes, environment and other factors to laboratory animal phenotype, Gartner (1990) was able to demonstrate that the majority of random non-genetic variability in mice was not due to the environment. Genetic causes of differences were reduced by using inbred animals, but reduction of genetic variation did not substantially reduce the amount of observed variation in phenotypes such as body weight or kidney size. Strict standardization of the environment within a laboratory did not have a major effect; only 20-30% of the variability could be attributed to environmental factors, with the remaining 70-80% of non-genetic variation due to some other factors.

The results of the experiments showed that despite the complete genetic similarity of all the mice and identical pre- and post-natal environments, the MZ twin pairs exhibited a greater degree of phenotypic similarity among co-twins than did the DZ twin pairs. A possible conclusion from these facts is that there exists some mechanism of heritability that is neither genetic nor environmental. This mechanism would be generated before the twinning stage and would influence the

zygote. Gartner and Baunack (1981) referred to this non-genetic influence as the "third component", after genes and environment.

Multiple species of animals have recently been cloned. Experiments using cloned animals also allow separating the effects of chromosomal DNA sequence from other factors that can influence phenotype. Although the offspring in these cloning experiments have the same genome as the donor animals, they exhibit a variety of phenotypic abnormalities that obviously cannot be attributed to genetic causes (Edwards et al, 2003). The differences found include diseases as well as other, more subtle, differences. The most famous of these cloning experiments was performed on sheep, but along with seemingly healthy lambs, many clone siblings died prenatally as a result of overgrowth, pulmonary hypertension and body wall defects (Rhind et al, 2003). Clones in other species also show considerable variation in lifespan and disease phenotypes in comparison to normal members of that species (Carter et al, 2002; Wellsatal, 2004).

This demonstrates that significant phenotypic variation, including fatal disease, can emerge from animals that have an identical, cloned genetic background. These early examples of cloned animals were subjected to intense scrutiny in highly supervised and controlled environments, yet they still exhibited disease in an inconsistent fashion. If environmental factors were the source of this phenotypic variation, then one would expect the same emergence of disease among non-cloned members of these species. Thus, in the case of cloned animals, it is also impossible to explain phenotypic differences by genetics and environment only.

Epigenetics and DNA methylation

Many researchers consider epigenetics as a not-accounted-for mechanism that explains heritability of complex non-Mendelian diseases. By definition, epigenetics refers to modification in gene expression that is controlled by heritable but potentially reversible changes in DNA methylation and/or chromatin structure (Heinkoff and Matzker, 1997).

DNA methylation is a subject of extensive research because this process plays a unique role in organism development. DNA methylation involves the addition of a methyl group to the 5 position of the cytosine pyrimidine ring or the number 6 nitrogen of the adenine purine ring This modification can be inherited through cell division. DNA methylation is typically removed during zygote formation and, in some cases, reestablished through cell division during development. DNA methylation is a crucial part of cellular differentiation. DNA methylation stably alters the gene expression pattern in cells in such a way that cells can "remember where they have been." In other words, cells programmed to be pancreatic islets during embryonic development remain pancreatic islets throughout the life of the organism without continuous signals telling them that they need to remain islets. In addition, DNA methylation suppresses the expression of viral genes and other deleterious elements that have been incorporated into the genome of the host over time. DNA methylation also forms the basis of chromatin structure, which enables cells to form the myriad characteristics necessary for multi-cellular life from a single, immutable sequence of DNA.

The addition of a methyl group to DNA usually takes place in the region of CpG islands. CpG islands are clusters of cytosine and guanine repeats, commonly found near a gene-coding DNA region. Methylation of CpG islands adjacent to a gene often leads to its inactivation (Cedar, 1988). As a result, the expression of a sequence of DNA can be modified by becoming "silenced" or "switched off".

The epigenetic approach may help explain the results of the studies discussed above. The fact that the differences found in MZT are similar differences in MZA, for a large number of traits,

suggests that in such twins stochastic events may be a more important cause of phenotypic differences than specific environmental effects. If the emphasis is shifted from environment to stochasticity, it may become clear why MZ twins reared apart are not more different from each other than MZ twins reared together. It is possible that MZ twins are different in some traits not because they are exposed to different environments but because those traits are determined by meta-stable epigenetic regulation on which environmental factors have only a modest impact. From this point of view, MZ twin discordance for complex, chronic, non-Mendelian disorders such as schizophrenia, multiple sclerosis or asthma could arise as a result of a chain of epigenetic events in one of the twins. During embryogenesis, childhood and adolescence, there is ample opportunity for tissue differentiation and stochastic factors to accumulate in only one of the two identical twins (Petronis, 2004; Petronis et al, 2003). In addition, some external environmental aspects, such as nutrition, medication, addictions, and other factors can influence certain phenotypes by changing epigenetic profiles (Jaenish and Bird, 2003).

The mechanism of methylation, "silencing" of genes, is not yet entirely clear. There are several approaches to explain the phenomenon. First, the methylation of the DNA itself may physically impede the binding of transcriptional protein to the gene by occupying the place near the gene, which is otherwise suitable for proteins to stay close to the gene. Second, the electric field of methyl may diminish electric forces nearby due to polarization and consequently diminish electrical interactions between a gene and its proteins. Many researchers, however, give more complex explanations for the molecular mechanism of "silencing". For such mechanisms, methylation of the cytosine residue in the DNA molecule, and acetylation and other modifications of histones have been described (Machin, 1996).

Epigenetic studies may help in the identification of the molecular effects of the environmental factors. There are many environmental events that result in epigenetic changes (Hursting et al, 2003; Ross, 2003; Waterland and Tirtle, 2003). The advantage of the epigenetic studies is that identification of molecular epigenetic effects of environmental factors might be easier and more efficient than direct epidemiological studies.

To evaluate the influence of a given environmental factor on the epigenetic status of one of a pair of MZ twins, researchers seek genes or regions of DNA that are methylated differently in cells of two twins, if one of them experienced an environmental influence. In most cases, researchers try to evaluate epigenetic status by applying one of two methods. In the first method, special enzymes are applied to the sample, representing the DNA. These enzymes are chemical compounds which react differently to methylated and non-methylated regions of DNA. In this case, the intensity of the chemical reaction between an enzyme and a region of DNA investigated is proportional to the number of methylated sites. To separate a genomic region of interest, special chemicals are applied to the genome which multiply the yield of the DNA region investigated to the total effect of the chemical reaction. The second method of investigating the epigenetic status of a given sample involves studying the emission of the sample which is characteristic to methylated parts of the DNA. The intensity of this emission under some standard conditions is proportional to the number of methylated sites (Petronis, 2002).

Kaminski, et al (2009) investigated DNA methylation profiles in 114 monozygotic and 80 dizygotic twins. The purpose of the research was to identify not only phenotypic, but also epigenetic differences in monozygotic and dizygotic twins. As epigenetic factors can contribute to phenotypic outcomes, a DNA methylation analysis of several different tissues was conducted. Epigenetic signals of 6,000 unique genomic regions in MZ twins were registered. The results of

the experiments showed that DZ co-twins exhibit higher epigenetic differences in cells than MZ co-twins. In general, this epigenetic difference may result from DNA sequence difference between co-twins. In the case discussed, however, the authors conducted special experiments to show that difference in DNA sequence cannot be greater than .05%, which is why the authors consider the observed difference to be a real epigenetic difference.

The results of the study suggest that in addition to identical DNA, epigenetic similarity may also contribute to phenotypic similarities in MZ co-twins. DZ co-twins are more different from each other than MZ co-twins not because they possess some DNA sequence differences, but because they originated from epigenomically different zygotes. This leads to the conclusion that epigenetic factors themselves may be inherited. This means that molecular mechanisms of heritability may not be limited to DNA sequence differences.

Epigenetic twin studies are providing insight into molecular causes of differential susceptibility to a disease in genetically identical organisms. If this approach is successful, the results may be generalized to singletons.

The results of many years of research in the field of twin studies give reason to think that some phenotypic changes may be inherited not only through direct genetics, but also through epigenetic changes. It is easier to change the epigenetic code through the environment than through DNA sequence, and it may be passed on to the next generation. From this point of view it is possible that some unhealthy habits, such as drug abuse or smoking, pose a danger to future generations.

CONCLUSION

The field of epigenetics as a new direction in twin studies is of great interest to me personally. As a monozygotic twin, my sister and I have always been wondering what the cause of the differences between us is. We also notice that the differences increase drastically as we get older. Epigenetics has provided an explanation to satisfy our curiosity.

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Is the Gallbladder Really Unnecessary? An Evaluation of Gallstone Treatment

DANIELLE WEINBERG

INTRODUCTION

The red numbers on your alarm read two o'clock AM, but you're wide awake. A dull ache below your ribs has tossed you from the blissful ignorance of sleep into the painful reality of another nocturnal attack. As the pale streaks of daylight appear, you grit your teeth in agony while the ambulance goes over yet another bump in its rush to get you to the emergency room. A blur of white coats, lab tests, and CT scans, and then you're staring into the bright lights above the operating table as the nurses prepare you for emergency gallbladder surgery. "Does it have to be this way?" you wonder, "Is there no other option?" The purpose of this paper is to answer just such a question. After clarifying gallbladder anatomy and the etiology of gallstone disease, it will evaluate surgical and nonsurgical options for the treatment of gallstones. In the course of the discussion, it will examine the mechanism of action, efficacy, and safety of each treatment option. Once the analysis is complete, it will be possible to conclude if there are nonsurgical therapies that may be preferred over surgical removal of the gallbladder for relief of gallstone disease.

Gallbladder Anatomy And Physiology

The gallbladder is a pear-shaped organ that can be found underneath the right lobe of the liver. Its function is to store the bile secreted by the liver and then, in turn, secrete this bile into the small intestine to aid in digestion of fats. Bile is composed of over 95% water with bile acids, phospholipids, cholesterol, bile pigments, and bicarbonate ions dissolved within (Portincasa et al, 2009). The general physiological purpose of bile acids is to emulsify dietary fats in the small intestine, thus aiding in the digestion process. This paper, though, will focus on the role of bile acids and phospholipids in solubilizing cholesterol in bile. While cholesterol is the building block of bile acids, it is also secreted unchanged into bile in order to maintain cholesterol homeostasis in the blood. The synthesis of bile acids and the danger of excessive amounts of cholesterol will be discussed in subsequent sections. Bile pigments are composed

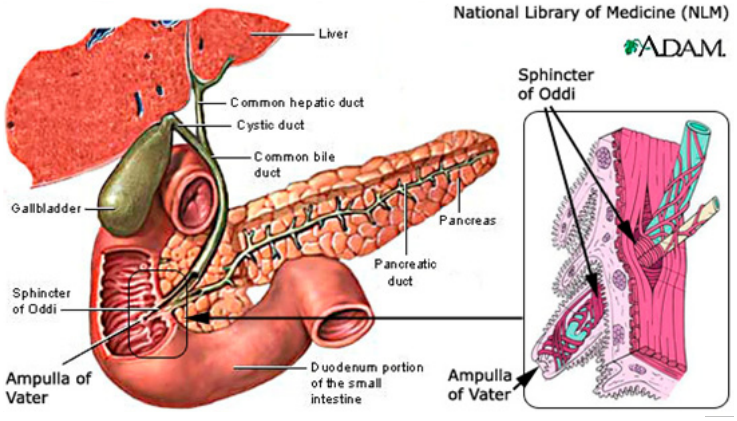

Figure. 1 Gallbladder anatomy

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primarily of bilirubin, which is a breakdown product of hemoglobin. By secreting bilirubin into bile, the liver ensures that toxic levels do not build up in the body and are instead eliminated in the feces. Finally, bicarbonate ions are secreted by the pancreas into the bile to neutralize the acid entering the small intestine from the stomach (Widmaier et al, 2008).

While the liver is always in the process of secreting bile, not all this bile travels directly to the small intestine. At the point where the common bile duct joins the pancreatic duct and empties into the duodenum, a ring of smooth muscle surrounds the duct and prevents the release of bile. As long as this ring of muscle, the sphincter of Oddi, remains contracted, bile secreted from the liver is shunted into the gallbladder for storage. In the gallbladder, the dilute hepatic bile is concentrated many times over as salts and water are reabsorbed into the blood. Bile secretion from the gallbladder is controlled by a hormone called cholecystokinin, or CCK. After a fatty meal, the presence of fatty acids and amino acids in the small intestine stimulate the release of CCK into the blood. From there, the hormone travels to the gallbladder to stimulate its contraction, and to the sphincter of Oddi to stimulate its relaxation. This dual action allows the gallbladder to release its store of bile into the cystic duct, and the bile continues through the common bile duct and sphincter of Oddi to its final destination in the duodenum (Widmaier et al, 2008). It is only when problems arise in this chain of events that normal physiology is interrupted and gallstone disease develops.

Bile Acid Synthesis And Metabolism

In order to understand the ensuing discussion of gallstone pathogenesis, a preliminary discussion of bile acid chemistry is necessary. Bile acid synthesis is the primary method by which cholesterol is catabolized, or broken down. However, bile acids are not simply metabolites of an organic substance. They have their own unique roles—numerous functions which scientists are continuously clarifying and expanding. The fascinating phenomenon of bile acid synthesis converts a completely hydrophobic molecule into an amphiphile which can solubilize and transport nutrients, fats, and vitamins while also functioning as a powerful receptor ligand in various signaling pathways. The process of synthesizing bile acids from cholesterol is a multistep sequence catalyzed by a host of different enzymes. The liver is the only organ in the body that boasts all fourteen enzymes required for this de novo synthesis of bile acids (Chiang, 2009).

The first step in the classic pathway of bile acid synthesis is to add a hydroxyl group to position 7 of the cholesterol steroid nucleus. This reaction is catalyzed by the rate limiting enzyme, cholesterol 7α-hydroxylase, abbreviated CYP7A1. At this point, the pathway diverges to form the two most common human bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA). To create the former, sterol 12α-hydroxylase (CYP8B1) is employed to add a hydroxyl group to position 8 of the steroid nucleus. The precursors of CDCA do not need an additional hydroxylation and proceed to the next step in the sequence. Once the cholesterol double bond is reduced by enzymes in the cytosol of the hepatocyte, mitochondrial sterol 27-hydroxylase (CYP27A1) can add a hydroxyl group to position 27 of the cholesterol side chain. This alcohol is then further oxidized to an aldehyde, and finally to a carboxylic acid, whereupon it is transported to the peroxisomes of the hepatocytes for

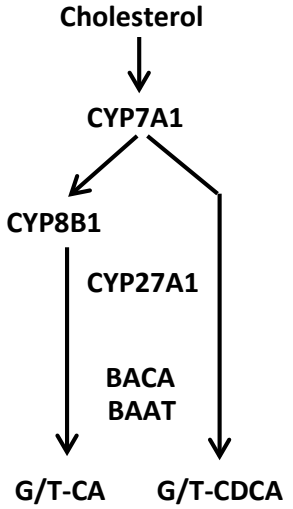

Figure 2. Bile acid synthesis (see text for explanation). (Adapted from Chiang, 2009)

side chain shortening. In the peroxisomes, a three carbon unit is cleaved from the original side chain to form the C-24 bile acid from the C-27 cholesterol molecule. The basic backbone of the bile acids has thus been created, but most are conjugated to hydrophilic side chains derived from amino acids to increase their solubility in bile. Glycine and taurine (derived from the amino acid cysteine) are conjugated to the carboxylic acid side chain of the bile acids by means of their amino groups. The enzymes BACA (bile acid-CoA synthase) and BAAT (bile acid-amino acid transferase) facilitate this process and create the glyco- or tauro-conjugated CA or CDCA (Monte et al, 2009).

An interesting point is that this developed synthesis pathway is only employed for 5% of the bile acids that are secreted into bile (Portincasa et al, 2009). This is due to the recycling effect known as enterohepatic circulation. The bile acids synthesized by the liver are termed primary bile acids and are further modified by intestinal bacteria to their secondary bile acid counterparts. Both primary and secondary bile acids are then actively absorbed in the distal ileum, the last part of the small intestine. A transport protein coupled to sodium reabsorption transports the bile acids across the epithelial cells lining the intestine to the hepatic portal vein (Chiang, 2009). Upon reaching the liver, bile acids are taken up by hepatocytes where they prevent further bile acid synthesis by inhibiting the rate limiting enzyme CYP7A1 (Monte et al, 2009). These recycled bile acids are then used again in the secretion of bile. While some bile acids are inevitably lost in the feces, over 95% are recovered via enterohepatic circulation, accounting for the minimal de novo synthesis of bile acids in the liver.

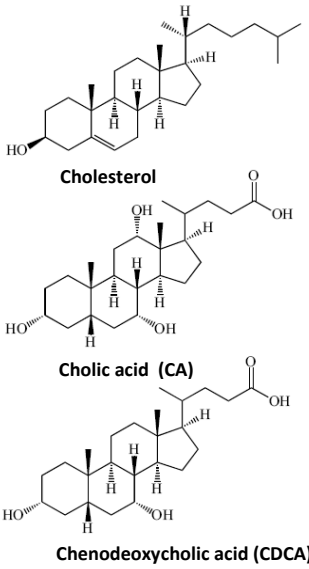

Figure 3. Chemical structures of cholesterol and the primary bile acids

Gallstone Pathogenesis

Gallstone disease is one of the most common and most costly digestive diseases affecting Western industrialized countries. In a large epidemiologic study, the third National Health and Nutritional Examination Survey (NHANES III) found that the overall prevalence of gallstones was 7.9% in men and 16.6% in women in the United States. The disease was more dominant among Native Americans than other ethnic groups, indicating that genetics may play a role. Incidence of gallstones was comparable in European countries but much lower among Asians and Africans (Paumgartner and Greenberger, 2009). With over one million new cases of gallstones being discovered every year, scientists continue to research the contributing factors of gallstone disease in the hopes of preventing future diagnoses (Portincasa et al, 2009).

All arrows seem to point to cholesterol as the primary culprit of gallstone disease, or cholelithiasis. Radiographic scans have enabled doctors and scientists alike to characterize 80% of diagnosed gallstones as cholesterol stones. The pathogenic mechanisms that contribute to the formation of gallstones, then, must be connected to cholesterol metabolism. Indeed, the hydrophobic cholesterol molecule is not soluble in bile and must be maintained in proper proportion with the other components of bile (namely, bile acids and phospholipids) in order to remain in solution (Portincasa et al, 2009).

Bile acids have been shown to be amphiphiles, that is, they are detergent-like molecules with both lipophilic and hydrophilic properties. As mentioned, the ringed steroid nucleus forms

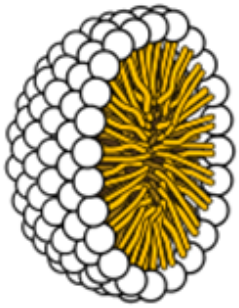

Figure 4. Micelle

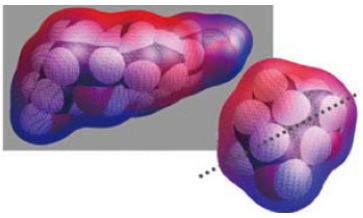

Figure 5. Space filling model of cholic acid showing hydrophobic face(red) and hydrophilic face(blue). (Portincasa et al, 2009)

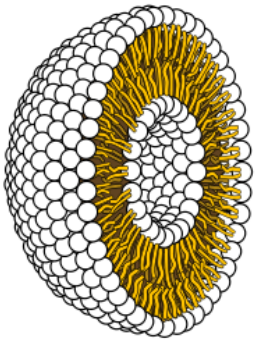

Figure 6. Vesicle

the lipophilic face of the molecule while the hydroxyl groups and conjugated side chain create a hydrophilic face. If the levels of bile acids exceed a certain value, termed the critical micelle concentration (CMC), they will self-assemble into simple micelles. These structures are sphere-like shapes consisting of a monolayer of bile acids surrounding cholesterol molecules sequestered within. The hydrophobic face of the bile acids faces inwards towards the lipophilic cholesterol, while the hydrophilic face is oriented outwards facing the aqueous medium of bile (Portincasa et al, 2009).

Phospholipids are another kind of amphiphile composed of long chain fatty acids bound to glycerol and polar phosphate groups on one end. They form a phospholipid bilayer that surrounds cholesterol molecules in unilamellar vesicles. These vesicles are unstable, but with the addition of bile acids, will form water soluble "mixed micelles" that can solubilize triple the amount of cholesterol that simple micelles solubilize (Portincasa et al, 2009). Thus, if all the biliary components are in proper proportion, cholesterol will remain in solution and no disease will occur.

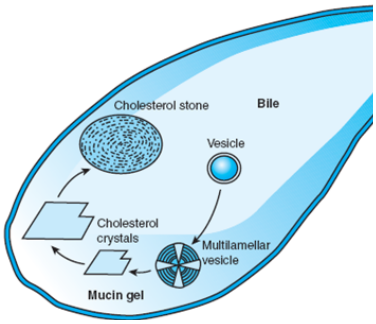

Figure 7. Gallstone pathogenesis (Paumgartner and Greenberger, 2009)

The problem arises when bile is supersaturated with cholesterol so that the cholesterol exceeds the solubilizing capacity of bile acids and phospholipids. This can occur due to excess cholesterol or insufficient amounts of bile acids or phospholipids. Whatever the reason, if all the unilamellar vesicles cannot be converted into the more stable mixed micelles, unstable cholesterol-rich vesicles remain in the bile. These vesicles are in a state of delicate equilibrium and can easily aggregate to form large multilamellar vesicles. The multilamellar vesicles are even less stable than their counterparts and cannot prevent the cholesterol molecules they hold from precipitating and forming cholesterol crystals (Paumgartner and Greenberger, 2009). Cholesterol crystals have traditionally been described as rhomboid monohydrate crystals with a notch missing from one corner. Recent studies have shown that in the early stages of crystal formation, cholesterol may form anhydrous crystals shaped like needles, spirals, or tubules (Dowling, 2000). Cholesterol crystals that get stuck in a mucin gel lining the gallbladder will aggregate, giving rise to mature gallstones (Paumgartner and Greenberger, 2009).

Different proteins have been identified as being responsible for transporting each specific element of bile from the interior of the hepatocyte to the bile canaliculus. These proteins are part

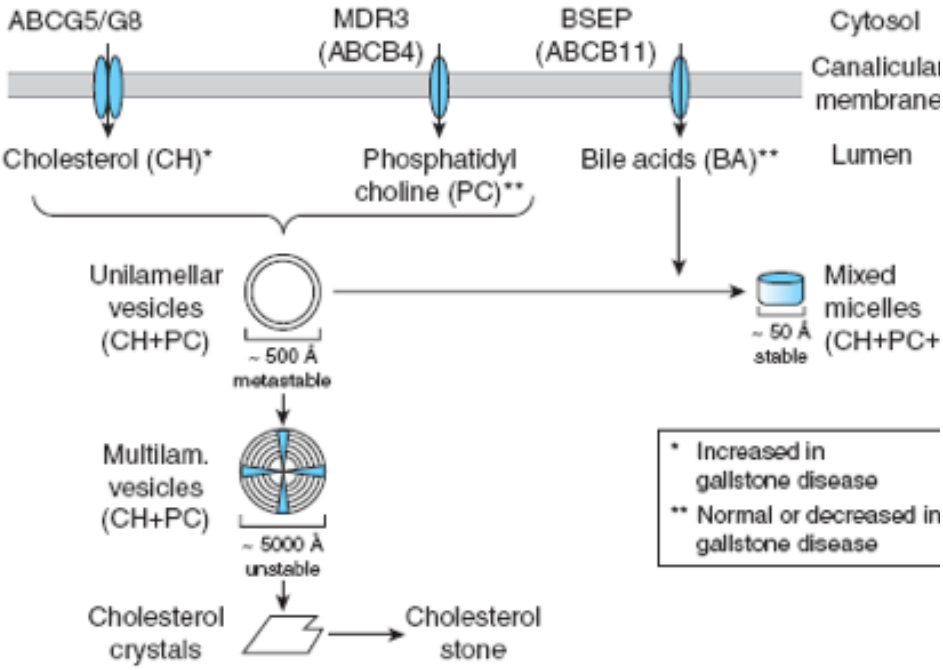

Figure 8. Biliary transport proteins and gallstone pathogenesis.
(Paumgartner and Greenberger, 2009)

of a superfamily of transport proteins called ATP-binding cassette transporters (ABC transporters) that use the energy from ATP hydrolysis for translocation of various substances across a biological membrane. Cholesterol secretion into bile is controlled by ABCG5/G8, while the bile salt export pump (BSEP; ABCB11) regulates the secretion of bile acids. Multidrug resistant p-glycoprotein 3 (MDR3;ABCB4) transports phosphatidylcholine, the primary phospholipid in bile, from the inner leaflet to the outer leaflet of the canalicular membrane. Emerging studies reveal that a genetic defect in the cholesterol transporter, ABCG5/G8, predisposes its carrier to gallstones (Paumgartner and Greenberger, 2009). Additionally, one team identified a mutation in the gene encoding the ABCB4 transporter protein (Wang et al, 2009). This mutation results in decreased phospholipid levels in bile and has been named "gallbladder disease 1" (GBD1). Other risk factors of gallstone disease include obesity, where excessive amounts of cholesterol supersaturate the bile, and pregnancy, where increased estrogen levels also result in elevated levels of cholesterol in bile (Paumgartner and Greenberger, 2009).

One final factor implicated in the pathogenesis of gallstones is impaired gallbladder emptying. The sequence outlined above that accounts for the formation of gallstones from cholesterol-rich unilamellar vesicles cannot occur if these vesicles and the cholesterol crystals they produce are expelled from the gallbladder in a timely fashion. Normally, the time required for gallstones to develop from supersaturated bile is longer than the amount of time the bile sits in the gallbladder! However, if the contractility of the gallbladder is diminished in a condition known as gallbladder stasis, then prolonged retention of supersaturated bile allows gallstones to grow. This condition is associated with fasting and parenteral nutrition, where there are no nutrients in the intestine to stimulate the release of CCK, which will, in turn, stimulate the gallbladder to contract. Additionally, increased levels of progesterone during pregnancy inhibit smooth muscle contraction, as do certain drugs, thereby impairing gallbladder emptying (Paumgartner and Greenberger, 2009). Later sections will discuss options that have been proposed to increase gallbladder contractility, and thus prevent the formation of gallstones.

To Treat Or Not To Treat?

Most patients that are diagnosed with gallstones do not require any immediate treatment (Lanzini et al, 1994). Since many gallstones are found during imaging studies done for other gastrointestinal conditions, and the patients are otherwise unaware of their gallstones, these gallstones are considered asymptomatic (Paumgartner and Greenberger, 2009). Patients with asymptomatic gallstones have a very low likelihood of developing any pain or complications from their condition. Only a small subgroup of asymptomatic patients are at increased risk of developing complications due to individual characteristics. This group of patients will not be discussed in this paper. In general, then, treatment of asymptomatic gallstones is ill-advised, as the risks outweigh any potential benefits. The only recommendations offered are that patients be monitored by observation alone to ensure that they remain asymptomatic (Lanzini et al, 1994).

Symptomatic gallstone disease, on the other hand, is a definite indication for treatment. Patients with symptomatic gallstones typically present with intense pain in the upper right quadrant of the abdomen that often radiates to the angle of the right scapula and/or shoulder (Portincasa et al, 2009). The pain is usually persistent despite its common classification as biliary colic, a misnomer suggesting intermittent pain. Rapid onset of pain is common and duration of symptoms ranges from fifteen minutes to a few hours after which the pain subsides gradually or rapidly. Patients may complain of associated nausea and other nonspecific symptoms of indigestion and may feel the urge to walk. Many patients are awakened by nocturnal symptoms, often after eating fatty foods. The pain of symptomatic gallstone disease is a visceral pain triggered by gallstones lodged in the cystic duct, common bile duct, or ampulla of Vater (the enlargement of the common bile duct and pancreatic duct as they enter the duodenum at the sphincter of Oddi). As the gallbladder contracts and releases bile, gallstones may migrate along with the bile into the aforementioned passageways. When they get stuck in the ducts, they block the normal flow of bile, thus causing distention of the biliary tract and gallbladder. Such distention activates visceral sensory neurons that communicate a feeling of pain to the patient (Paumgartner and Greenberger, 2009). Once pain sets in, patients should commence fasting to prevent release of CCK and further contraction of the gallbladder causing more pain. Narcotic analgesics, such as meperidine, or non-steroidal anti-inflammatory drugs (NSAIDs), such as ketorolac or ibuprofen, are indicated to relieve biliary pain (Portincasa et al, 2009). Although direct relief of biliary pain is a vital component of managing symptomatic gallstone disease, this paper will focus on methods of eliminating or reducing the gallstones themselves in an effort to relieve their associated pain.

Laparoscopic Cholecystectomy

As the opening paragraph suggests, surgical removal of the gallbladder is considered first line treatment for symptomatic gallstone disease. In the days of open cholecystectomy, many patients were understandably concerned about the mortality risks, prolonged hospital stay, extended recovery time, and unattractive scars of the operation. The tendency to seek alternative, medical options to treatment was therefore high. However, with the advent of laparoscopic cholecystectomies in the late 1980s, attitudes towards surgical treatment have dramatically improved (Lanzini et al, 1994).

As opposed to the large incision required for open cholecystectomy, laparoscopic technique only necessitates four small incisions (5-10 mm) in the abdomen. The surgeon then inserts a thin, lighted tube, called a laparoscope, connected to a video monitor, and the tools that he will use during the operation (Brandon et al, 1991). This technique requires considerable surgical skill,

but has not been shown to increase the inherent risks of mortality and complications involved in surgically resecting the gallbladder. One landmark study even noted decreased risks of some complications after laparoscopic cholecystectomy, as compared with its traditional counterpart. "Lap chole," as it is known, effectively reduces the hospital stay of open cholecystectomy patients by up to three days and recovery time by up to three weeks. By returning to work early, laparoscopic patients have reduced the overall costs of the operation by 18% (Paumgartner and Greenberger, 2009). This minimally invasive procedure has demonstrated cosmetic and economic appeal over the conventional open cholecystectomy but an obvious question remains. To what degree does the surgery guarantee relief of biliary pain, its primary indication?

One study evaluated 118 patients who had undergone cholecystectomies, whether laparoscopic or traditional, and found that 93% of participants were satisfied with the results of the procedure. Although a significant number of patients reported persistent abdominal pain even after the operation, Vander Velpen et al classified these symptoms as unrelated to gallstone disease. By comparing the number of patients with preoperative symptoms to the number complaining of postoperative symptoms, the researchers determined that fat intolerance, flatulent dyspepsia, and nagging abdominal pain were unrelated to gallstone disease. They claimed, in keeping with earlier studies, that these symptoms were caused by unrelated gastrointestinal disturbances prevalent in gallstone patients and were therefore unaffected by removing the diseased gallbladder. In contrast, nausea, vomiting, colicky abdominal pain, back pain, and heartburn appear to be secondary to gallstone disease and were more reliably relieved by cholecystectomy (Vander Velpen et al, 1993).

Postcholecystectomy diarrhea may be the most distressing symptom that develops as a result of cholecystectomy. Vander Velpen et al found that 18% of their patients complained of PCD and traced it to malabsorption of fats due to changes in release of bile (1993). In a recent published work, Fisher et al offered their own pathogenic factors for PCD, but admitted that they could not be substantiated (2008). They found a similar incidence of PCD and correlated this increased incidence with male patients, younger than 50 years of age, with elevated BMIs. Despite the disruptive nature of PCD, Vander Velpen et al have shown that most patients are nevertheless pleased with the outcomes of their cholecystectomies (1993).

Besides the specific nature of PCD, a persistence of nonspecific abdominal symptoms after surgical resection of the gallbladder is known as postcholecystectomy syndrome, or PCES. PCES is not well defined but may be associated with sphincter of Oddi dysfunction resulting from scarring during cholecystectomy. Patients who complain of continued abdominal pain also do not realize that gallstones may still be forming in the common bile duct, even though their gallbladder has been removed. Endoscopic tests must be performed to diagnose and treat PCES properly (Comstock, 2008).

In one study, a group of 100 patients awaiting laparoscopic cholecystectomy was followed over the course of their operation and beyond. After evaluating individual characteristics in patients that remained symptomatic after surgery, Luman et al were able to identify specific predictors of PCES (1996). They urged surgeons to reconsider accepting patients with preoperative constipation, bloating, or psychotropic medication histories. These patients seemed more likely to develop PCES, and for good reason. Histological studies of the resected gallbladders revealed that many of them were not inflamed. Therefore, the abdominal pain that was diagnosed as biliary pain may have been due to underlying gastrointestinal disorders, such as irritable bowel disease. This finding matches the opinion of Vander Velpen et al cited above, and is confirmed by a history of anxiety and depression among many IBD patients (Luman et al, 1996).

Just over a year ago, another analysis confirmed these results and advocated more individual patient assessment before lap chole is categorically advised. Mertens et al proposed the novel notion that expectant management of gallstone disease may be preferred to surgical treatment in a group of high risk patients (2010). As found previously, individuals with dyspeptic symptoms (bad taste, heartburn, under abdominal pain, diarrhoea and flatulence) and those using psychotropic medications are at increased risk of unsuccessful cholecystectomies and should therefore be evaluated differently (Mertens et al, 2010).

In general, then, lap chole is considered the safe and effective gold standard for treating symptomatic gallstone disease. However, the decision to undergo surgery has to be made on an individual basis. Health care practitioners must educate patients on the potential risks of unsuccessful relief of symptoms or the onset of new symptoms, such as PCD. This is especially the case in patient populations with characteristics known to predict PCES and the like. Patients can then weigh the pros and cons of laparoscopic cholecystectomy and decide whether or not to go ahead with the procedure. Since gallstone disease is relatively benign, and watchful waiting has no increased risk of complications, patients may prefer such an option (Konikoff, 2003). The rest of this paper will discuss medical alternatives that patients and providers may wish to pursue as alternative to surgery in the treatment of symptomatic gallstone disease.

Ursodeoxycholic Acid (UDCA)

In 1936, a Japanese team identified the structure of ursodeoxycholic acid, the 7β-hydroxy epimer of chenodeoxycholic acid (Hofman and Roda, 1984). Where the hydroxyl group on C7 of CDCA has an alpha configuration (dashed wedge) and faces down and out of the plane of the steroid nucleus, the C7 hydroxyl group in UDCA acid has a beta stereochemistry (solid wedge) in line with the steroid nucleus. This minor change in stereochemistry has led to major differences in clinical findings, which will be discussed shortly. Ursodeoxycholic acid is found to only a limited degree in the bile of healthy individuals (Portincasa et al, 2009). However, it is the major bile acid of the black bear, explaining the origin of its unusual name. The curative nature of bear bile has been known for centuries, thus the Japanese who first established UDCA sought to investigate its medicinal effects (Hofman and Roda, 1984). As UDCA became more popular, other scientists joined in this effort, in the hopes of finding a new oral therapy to treat symptomatic gallstone disease. While CDCA had been the oral bile acid in use since the 1970s, it was later proven to be associated with increased liver enzymes, elevated LDL, and the development of bile acid-induced diarrhea (Portincasa et al, 2009). The manufacturer's information for chenodiol, the generic name for CDCA, now contains a black box warning with this evidence ("Chenodiol"). Scientists hoped that UDCA would preserve the efficacy of CDCA in dissolving cholesterol gallstone, while minimizing its adverse effects.

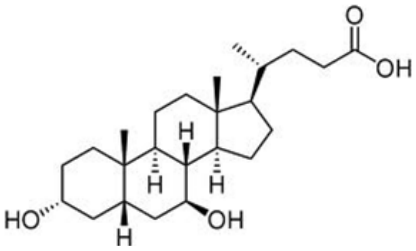

Figure 9. Chemical structure of UDCA

Indeed, if patients are carefully selected for treatment with UDCA, complete dissolution of gallstones can be accomplished in about 90% of cases. Proper inclusion criteria for UDCA treatment include the presence of radiolucent cholesterol stones, preferably less than 5 mm, and a functioning gallbladder (Portincasa et al, 2009). Cholesterol stones are generally radiolucent, thus they allow the passage of x-rays and cannot be seen on regular x-ray films. If the gallstones are com-

posed mostly of pigments or calcium, such radiopaque stones will show up on x-rays and are not indicated for oral dissolution with UDCA (Bouchier, 1983). Patients with smaller gallstones have a greater chance of complete dissolution in a reasonable time frame (around six months), because of the greater surface area and greater exposure to dissolving effects of the drug (Lanzini et al, 1994). Although patients with stones up to 10 mm are indicated for UDCA treatment, their length of therapy extends to one year and likelihood of complete dissolution drops to about 50% (Portincasa et al, 2009). Finally, a functioning gallbladder is necessary for UDCA treatment, to ensure that the drug will work properly and that its effects can be monitored radiologically (Bouchier, 1983). Thus, if patients meet the inclusion criteria for UDCA therapy, they can hope to benefit from the newer bile acid on the market. To confirm this hypothesis, Meredith et al established that UDCA is at least as effective as CDCA in treating gallstones, but that it does so at lower doses and with fewer side effects (1982). All that remains to be seen is the mechanism by which ursodeoxycholic acid succeeds in dissolving cholesterol gallstones.

If supersaturated bile is a prerequisite for gallstone formation, as discussed above, then unsaturated bile is a prerequisite for dissolution of preformed gallstones. Indeed, UDCA has been found to unsaturate gallbladder bile, although the exact mechanism is still unclear. It seems that by inhibiting cholesterol reabsorption from the intestines, cholesterol secretion into bile is decreased, and an unsaturated bile results (Portincasa et al, 2009). One team suggested that UDCA simply improves the incorporation of cholesterol into mixed micelles, thereby reducing biliary cholesterol saturation (Guarino et al, 2007). When cholesterol crystals are exposed to unsaturated bile in the gallbladder, they slowly dissolve over time. Fig. 9 compares typical cholesterol crystals in patients with gallstones(A,B) to the atypical crystals observed during UDCA therapy(C,D). The atypical nature of the bottom crystals indicates that they have been chipped away at by the unsaturated bile created by UDCA treatment (Portincasa et al, 2009).

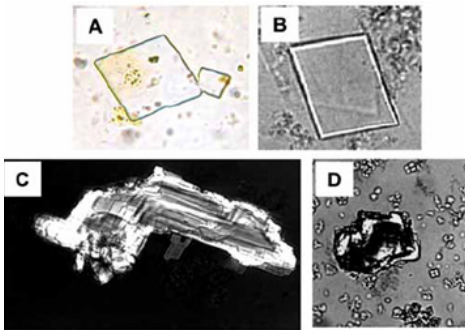

Figure 10. Cholesterol crystals (see text for explanation). (Portincasa et al, 2009)

One interesting theory proposed to account for UDCA-dependent unsaturation of bile involves the ABC transporters mentioned earlier. The genes that regulate the expression of BSEP and MDR3 are in turn regulated by transcription factors. Transcription factors are receptors that bind to specific ligands, undergo a conformational change, and migrate to the nucleus where they regulate expression of specific genes. The ligand-activated transcription factor that is responsible for the expression of BSEP and MDR3 is known as farnesoid X receptor, FXR. FXR has been shown to upregulate BSEP and MDR3, thus creating increased levels of bile acids and phospholipids in gallbladder bile (Portincasa et al, 2009). Since UDCA is a ligand, albeit weak, of FXR, its binding to FXR may explain its unsaturation of gallbladder bile (Sauter et al, 2004). If the ratio of bile acids and phospholipids to

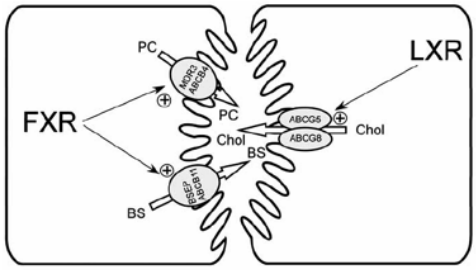

Figure 11. Transcription factors regulating biliary secretion. BS=Bile salts, PC=Phosphatidylcholine, Chol=Cholesterol. (Portincasa et al, 2009)

cholesterol is increased, then more cholesterol can be dissolved in mixed micelles and the bile is considered unsaturated.

While supersaturation of bile certainly accounts for the pathogenesis of gallstones, gallbladder stasis is also implicated in this phenomenon. Therefore, improving the contractility of gallbladder can prevent gallstones from forming. In terms of UDCA therapy, its effects on the saturation of bile and gallbladder motility are related. By reducing cholesterol saturation in bile, UDCA prevents cholesterol from impairing gallbladder motility. Whatever the reason for UDCA-dependent unsaturation of bile, the end result is diminished levels of cholesterol in bile. Guarino et al proved that excess cholesterol in patients with gallstones tends to accumulate in the plasma membrane of muscle cells. The cholesterol molecules aggregate in caveolae, "high-cholesterol domains containing caveolin proteins and a variety of molecules involved in signal transduction pathways," and sequester receptors located in these regions (2007). When ligands such as CCK attempt to stimulate receptors in the caveolae to cause gallbladder contraction, they are blocked by the excessive of amounts of cholesterol surrounding the receptors. Reducing levels of cholesterol will therefore prevent the accumulation of cholesterol in the plasma membrane of muscle cells. This will allow CCK to bind to its receptors and restore normal gallbladder contractility (Guarino et al, 2007). In vitro studies have substantiated these theories, showing increased contraction with UDCA treatment, especially during long term therapy (Mas et al, 2007). Thus, by reducing levels of cholesterol, UDCA can effectively improve gallbladder motility.

Other authors have suggested a different mechanism by which UDCA improves gallbladder contractility. In this case, it not linked to a reduction of cholesterol saturation, but to a reduction of deoxycholic acid. DCA is secondary bile acid and is formed by dehydroxylation of the primary cholic acid. A hydrophobic bile acid, DCA is associated with impaired gallbladder emptying and cholesterol hypersecretion into bile (Dowling, 2000). Recent findings suggest that UDCA may prevent these detrimental effects of DCA (Portincasa et al, 2009). Specifically, UDCA has been shown to induce expression of CYP3A4, an enzyme responsible for bile acid metabolism. By increasing levels of CYP3A4, UDCA may increase breakdown of toxic, hydrophobic bile acids into less toxic, hydrophilic bile acids. UDCA can thus decrease levels of DCA and prevent gallbladder stasis and the attendant formation of gallstones (Paumgartner and Beuers, 2004).

The optimal dosage for UDCA therapy is 10-15 mg/kg/day. This dose is best administered at bedtime to maximize the effect of the drug (Portincasa et al, 2009). Normal physiology dictates that enterohepatic circulation is interrupted at night. This is will decrease the secretion of bile acids into the bile and increase the proportion of biliary cholesterol. However, UDCA maintains the secretion of bile acids overnight, thereby preventing secretion of supersaturated bile. It is therefore recommended to take a bedtime dose of UDCA in order to potentiate its effects (Lanzini et al, 1988).

As opposed to CDCA, treatment with UDCA has little if no side effects. In a comparative study of chenodiol and ursodiol, the incidence of hepatotoxicity with UDCA was low and none of the patients developed diarrhea (Meredith et al, 1982). However, other studies indicate that a small percentage of patients may develop calcified rims surrounding their cholesterol gallstones. This would render the stones radiopaque and incompatible with further UDCA therapy. The biggest disadvantage of oral dissolution of gallstones with UDCA is the high risk of recurrence. Up to 50% of patients treated with UDCA may find recurring gallstones as soon as 3-5 years after treatment. Therefore, UDCA therapy is only advised for patients who refuse surgery, are not candidates for surgery, or have transient risk factors such as pregnancy or rapid weight loss (Portincasa et al,

2009).

Extracorporeal Shock Wave Lithotripsy (Eswl)

Lithotripsy is the practice of using soundwaves to break stones into smaller fragments. This technique was originally developed for use in treating kidney stones but was pioneered for use in gallstone treatment by Paumgartner and colleagues in the 1980s (Paumgartner, 2010). The theory behind this treatment was to reduce larger gallstones to a diameter of less than 3 mm that would then be indicated for treatment with UDCA. Lithotripsy is therefore an adjuvant, or supplementary, procedure meant to improve the efficacy of UDCA therapy (Lanzini et al, 1994). Performing ESWL before UDCA also increases the amount of patients who eventually qualify for UDCA therapy. Indeed, the selection criteria for ESWL with subsequent UDCA therapy are broader than those for UDCA therapy alone. In order to qualify for ESWL + UDCA, patients must have one radiolucent gallstone less than 20 mm and a functioning gallbladder (Paumgartner, 2010). If these criteria are met and a high energy lithotripter is used, the treatment is effective in two-thirds of the cases (Lanzini et al, 1994).

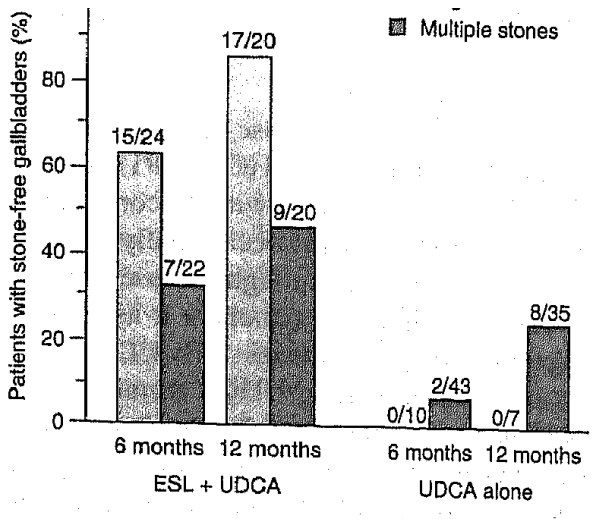

Figure 12. Percentage of patients with complete gallstone dissolution at 6 and 12 months after extracorporeal shockwave lithotripsy (ESL) plus UDCA therapy (left), and UDCA alone (right) for single and multiple stones. (Lanzini et al, 1994)

Before the advent of laparoscopic cholecystectomy, ESWL was preferred over traditional cholecystectomy because of its enhanced safety profile. However, like oral bile acid therapy, the recurrence rate was high. The incidence of recurrence of gallstones at two years and ten years after ESWL treatment was 11-29% and 60–80% respectively (Paumgartner and Greenberger, 2009). Darzi et al concluded that "while ESWL has minimal side-effects it is associated with more limited selection criteria, a more prolonged treatment time, a higher failure rate, and a higher recurrence rate when compared with laparoscopic cholecystectomy" (1994). Therefore, when lap chole became the gold standard for treating symptomatic gallstones and greatly reduced hospital stays and recovery time after surgery, ESWL became obsolete. Currently, ESWL is reserved for treating bile duct stones that have not responded to endoscopic treatment (Paumgartner, 2010).

Ezetimibe (Ezt)

Ezetimibe is a potent member of a new class of drugs that inhibit intestinal absorption of cholesterol. Although it is currently only indicated for reducing LDL levels in hyperlipidemia, research is underway to determine its potential for preventing and dissolving gallstones. Cholesterol absorption from the intestine is a key step in the process of secreting cholesterol into bile. Although the liver can synthesize cholesterol from scratch, this de novo synthesis only contributes 15% to the overall biliary cholesterol (Porincasa et al, 2009). The majority of biliary cholesterol is derived from dietary and recycled biliary cholesterol that are absorbed in the small intestine. After absorption, cholesterol is packaged with triglycerides into lipoprotein particles called chylomicrons. These particles lose their triglycerides to tissues as they migrate through the bloodstream, and are finally absorbed by hepatocytes as cholesterol-rich particles (Malloy and Kane, 2009). By reducing intestinal absorption of cholesterol, ezetimibe can effectively reduce the amounts of cholesterol that reach the liver for secretion into bile.

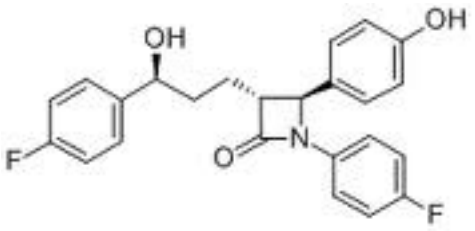

Figure 13. Chemical structure of ezetimibe

The mechanism of action of ezetimibe centers around a transport protein located in the brush border lining the small intestine. This protein has been recently characterized as NPC1L1 (Niemann-Pick C1-like 1) and facilitates intestinal absorption of cholesterol through a multistep process (Fig. 14) (Portincasa et al, 2009). Ezetimibe binds to and inhibits NPC1L1, thereby effectively inhibiting absorption of dietary and biliary cholesterol. This, in turn, results in decreased

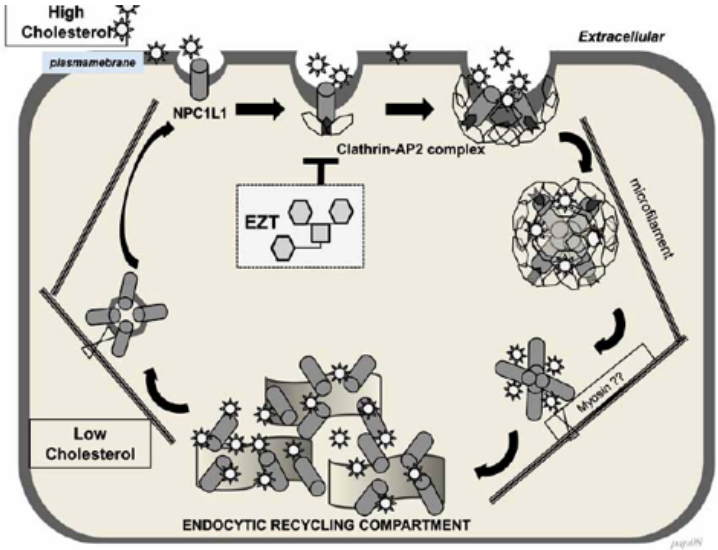

Figure 14. The NPC1L1 protein recycles between the plasma membrane facing the extracellular space and the endocytic recycling compartment. In the presence of high extracellular cholesterol, cholesterol is incorporated into the plasma membrane and is sensed by cell surface-localized NPC1L1. Both NPC1L1 and cholesterol are then internalized together through clathrin/AP2-mediated endocytosis and stored within the endocytic recycling compartment. If the intracellular cholesterol level is low, endocytic recycling compartment-localized NPC1L1 moves back to the plasma membrane along microfilaments and new cholesterol is absorbed. The key role of the inhibitor ezetimibe (EZT) is shown at the center of the cell. EZT prevents NPC1L1 from entering the AP2-mediated clathrin-coated vesicles. At this stage, the endocytosis of NPC1L1 is inhibited and cholesterol absorption is decreased (Portincasa et al, 2009).

secretion of biliary cholesterol and an unsaturated bile. Studies in mice proved that ezetimibe accomplishes dissolution of gallstones by forming unsaturated micelles. As cholesterol present in the preformed gallstones is transferred to the unsaturated micelles, the stones become smaller and eventually dissolve. Additionally, reduced levels of biliary cholesterol can improve gallbladder motility in the same way that UDCA achieves this goal (Wang et al, 2008). Although the effects of ezetimibe on cholesterol saturation of bile have yet to be corroborated by studies in humans, its effects in mice have shown potential for a novel therapy in the treatment of gallstone disease.

Dietary Phospholipids

Based on an understanding of gallbladder physiology and biliary secretion, studies have been conducted to evaluate the effects of phospholipids on formation of cholesterol gallstones. One experiment found that in vitro supplementation of gallbladder biles with disaturated phospholipids prolonged cholesterol nucleation time (Jungst et al, 1993). Nucleation time is understood as the number of days required for cholesterol monohydrate crystals to first appear in bile (Abeysuriya et al, 2010). Jungst et al proposed that this prolonged nucleation time was due to two factors (1993). Firstly, increasing levels of phospholipids serves to decrease the proportion of cholesterol in bile. This lowers the cholesterol saturation of bile and slows formation of cholesterol gallstones. The second interesting phenomenon Jungst and his colleagues discovered was that phospholipids stabilized cholesterol vesicles by shifting the phase equilibrium towards mixed micelles (1993). As mentioned earlier, cholesterol-phospholipid vesicles are considered unstable while mixed micelles consisting of cholesterol, phospholipids, and bile acids are more stable and therefore less likely to allow the cholesterol to precipitate into crystals. Thus, in vitro supplementation with phospholipids has been shown to create a biliary environment better able to solubilize cholesterol and prolong formation of gallstones. While other studies confirm these beneficial results, clinical practitioners cannot yet advise their patients to supplement their diets with phospholipids (Moschetta et al, 2001). Before such a recommendation can be made, further studies are needed in order to investigate whether the published results can be reproduced in vivo.

Summary Algorithm

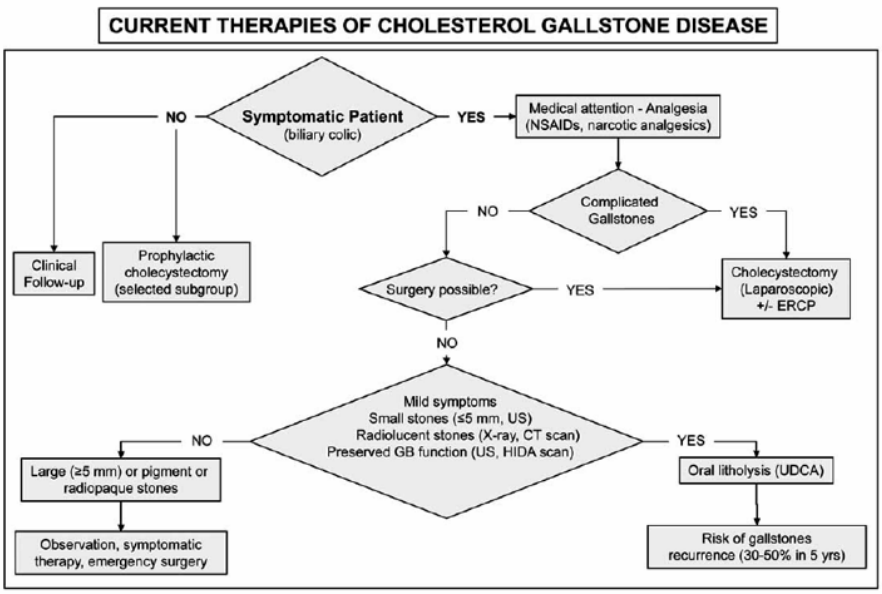

Figure 15. Algorithm for the management of cholesterol gallstone disease. (Portincasa et al, 2009)

CONCLUSION

The high prevalence of gallstone disease in Western countries renders it an important topic of discussion. Unfortunately, many people can relate to the opening question of whether or not to consent to surgical removal of the gallbladder. In light of this paper's analysis of gallstone treatments, this decision is no doubt easier. Those with asymptomatic gallstones need no active treatment as long as they are routinely monitored and remain asymptomatic. It is clear that laparoscopic cholecystectomy is considered first line treatment for symptomatic gallstone disease. Although it poses a slight risk of mortality and complications, it is the most efficacious procedure for preventing recurrence of biliary pain and gallstones. Even the complaints of persistent abdominal pain following surgery have been shown to be associated with underlying conditions not related to gallstone disease. While UDCA and ESWL used to be standard alternatives to surgery, their prolonged length of therapy and high recurrence rate have rendered them virtually obsolete. They are reserved for patients who refuse surgery or for whom surgery poses increase risks. Novel therapies such as ezetimibe and dietary phospholipids are still being investigated and may prove to be mainstay treatments in the future.

The bottom line emerging from the clinical studies canvassed in this paper is the need to evaluate each individual case before recommending an option for treatment. Personal characteristics may indicate whether surgery is advisable or whether a medical option might be more successful. Effective communication between the informed patient and the well-versed physician is crucial for selecting the most beneficial treatment option. If the suggestions presented here are employed, we may yet see a decline in the incidence of gallstone disease.

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The Role of Ghrelin and Leptin in Obesity: Is Exogenous Administration of these Hormones a Possible Drug Therapy?

PERI ECKSTEIN

ABSTRACT

Ghrelin and leptin are two hormones that have been recognized to have a major influence on energy balance. Leptin is a mediator of long term regulation of energy balance, suppressing food intake and thereby inducing weight loss. Ghrelin, on the other hand, is a fast acting hormone, playing a role in meal initiation. As a growing number of people suffer from obesity, understanding the mechanisms by which various hormones and neurotransmitters influence energy balance has been a subject of intense research. This paper provides background on leptin and ghrelin hormones, their role in food intake and body weight in humans, and their mechanism of action. Possible abnormalities in the leptin and ghrelin systems that may contribute to the development of obesity will be mentioned. The role of gut hormones on hunger and satiety as well as the effect of sleep deprivation on these hormones will be briefly described. Finally, the potentials of leptin and ghrelin as drug targets will be described (Klok, et al., 2006).

INTRODUCTION

The incidence of obesity has increased dramatically worldwide. The prevalence of obesity is a major health problem because excessive body weight is a risk factor for development of chronic diseases such as cardiovascular disease and type II diabetes mellitus (Park, 2010). Overweight is a result of a deregulation of calorie intake and energy expenditure. An individual's genetics and the environment create a system that controls appetite and energy expenditure. Throughout the past few decades the type and cost of food has changed. The food industry encourages people to eat fast foods which are relatively inexpensive but high in calories. Snacks and beverages high in sugar have also been added to the daily diet. Physiologically, the body secretes hormones which are responsible for controlling appetite and satiety. Genetic defects in this control system manifest itself in obesity (Skelton & Rudolph, 2007). The overwhelming percentage of overweight people created the need to develop new treatments. In pursuit of this goal, researchers have dissected the mechanism through which satiety and hunger manifests (Jayasena & Bloom, 2008). Further research has led to the discovery of gut hormones and the role of adipose tissue as participants in controlling the body's physiologic and pathologic processes (De Luis, et al., 2008).

Body weight is regulated by a complex system. Two hormones that play an important role in the regulation of food intake and body weight are leptin and ghrelin. Both hormones are part of the peripheral nervous system and signal the brain, specifically the arcuate nucleus (ARC) of the hypothalamus, through different pathways. In the hypothalamus, activation of leptin and ghrelin

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receptors set off different signals leading to changes in food intake (Klok, et al., 2006). One of the pathways consists of neurons that express neuropeptide Y (NPY) and agouti gene related protein (AgRP), stimulators of food intake. Other arcuate neurons express proopiomelanocortin (POMC), cocaine and amphetamine- regulated transcript (CART), which suppress food intake (Nogueiras, et al., 2004).

DISCUSSION

Leptin and its Functions

The human obese (OB) gene and its product leptin were discovered in 1994. The OB gene is on chromosome 7 (Klok, et al., 2006). It encodes leptin, a protein consisting of 167 amino acids which is secreted primarily from adipocytes. Leptin is also secreted by skeletal muscles, the placenta, and the stomach (Feldman,Freidman, & Brandt, 2010). Leptin acts through the leptin receptor (OBR). The OBR gene is located on the first chromosome and encodes a protein consisting of 1162 amino acids. OB-Rb, a part of the OBR gene, is expressed in the hypothalamus and the cerebellum. Leptin functions through a feedback mechanism that signals regulatory centers in the brain to inhibit food intake and to regulate body weight and energy homeostasis (Klok, et al., 2006). Studies on rodents have shown that the hypothalamus is the primary center for body weight and food intake regulation. After leptin is released by adipose tissue, it crosses the blood brain barrier and binds to the hypothalamic leptin receptors, conveying information regarding the status of body energy stores. Leptin inhibits the expression of orexigenic, or appetite stimulating neuropeptides, and stimulates anorexigenic, or appetite inhibiting neuropeptides in the arcuate nucleus of the hypothalamus (Neary, et al., 2003).

Anorexigenic	Appetite inhibiting
Orexigenic	Appetite stimulating

Figure 1.

When leptin is secreted, it activates anorexigenic peptides proopiomelanocortin and cocaine and amphetamine-regulated transcript neurons and inhibits orexigenic peptides neuropeptide Y and agouti-related protein neurons which results in inhibition of eating and an increase in energy expenditure (Nogueiras, et al., 2004).

Leptin is involved in different signaling pathways in the hypothalamus. Leptin is a member of the cytokine family of signaling molecules. There are different forms of leptin receptors throughout the body. The “long form” leptin receptor is a type of cytokine-receptor, which is located in the hypothalamic nuclei. Leptin binds to and activates Janus Kinase (JAK-2), tyrosine kinases involved in intracellular cytokine signaling, beginning a positive feedback mechanism. Activation of JAK-2 leads to phosphorylation of members of the signal transduction and transcription family of proteins (STAT). As a result, STAT proteins activate transcription of leptin target genes (Figure 2). The “long form” of leptin receptor is required for normal energy homeostasis. Studies have shown that mutations of this gene result in obesity in rodents (Kronenberg, et al., 2008)

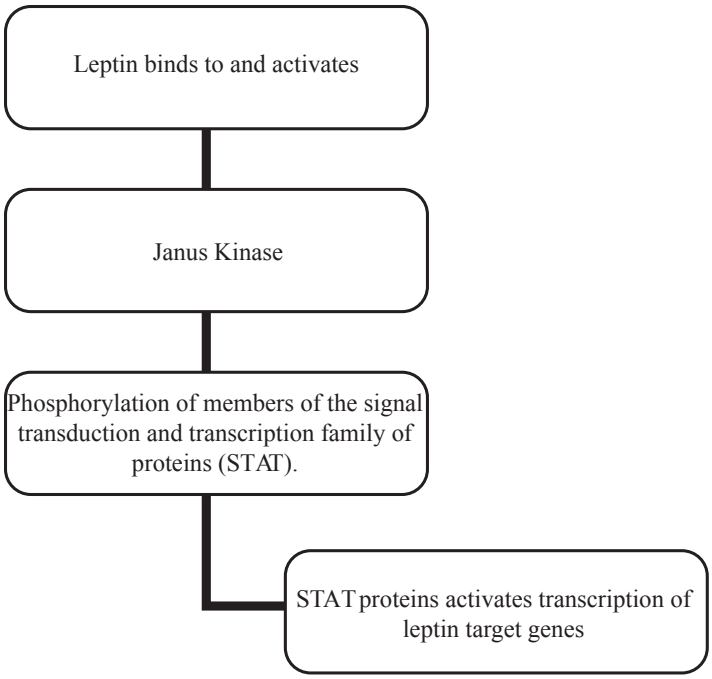

Figure 2. The Pathway of leptin through a positive feedback mechanism

Leptin also activates other pathways in the hypothalamus. Leptin activates the insulin receptor substrate phosphatidylinositol-3 OH kinase (IRS PI 3-Kinase) pathway in the hypothalamus which is also required for leptin regulation of food intake. Leptin also increases hypothalamic mammalian target of rapamycin (mTOR) activity, an enzyme which regulates growth. This pathway is required for leptin-induced anorexia, or lack of appetite. Signaling through these pathways in the hypothalamus is necessary for leptin to function to reduce food intake and body weight.

In addition, the "long form" of leptin receptor is expressed in the hippocampus, hindbrain, and mesolimbic areas. The mesolimbic areas are involved in reward and include the ventral tegmental area (VTA) and the substantia nigra. This leads to the possibility that leptin may reduce food intake through direct action on brain motivation/reward circuitry, in addition to the action of leptin on areas of the brain involved with energy homeostasis (Morton, et al., 2009).

A study was performed to examine the mechanism by which leptin signaling in the VTA reduces food intake (Morton, et al., 2009). Two questions arose: Firstly, does leptin activate the Janus Kinase - signal transduction and transcription family of proteins, insulin receptor substrate phosphatidylinositol-3 OH kinase, and hypothalamic mammalian target of rapamycin signal transduction pathways in the ventral tegmental area and substantia nigra as it does in the hypothalamus? Secondly, is activation of these pathways required for VTA leptin-induced anorexia? The data collected suggested that JAK-2 signaling in the ventral tegmental area is activated by leptin. JAK-2 is required for anorexia induced by VTA leptin action, but leptin signaling of IRS-PI 3-kinase and mTOR do not participate in ventral tegmental area leptin induced anorexia.

The experiment was performed on adult male Wistar rats held in a temperature controlled room and provided with ad libitum access to water and food. A stainless steel cannula was placed in the third cerebral ventricle in the animals to examine the signal transduction pathways activated by intracerebroventricular administration of leptin. In experiments with intra ventral tegmental area injections, a bilateral stainless steel cannula was implanted to the ventral tegmental area. The rats had 7 days to recover after the surgery, and daily intake of food and body weight was recorded. An hour before the injection of recombinant mouse leptin into the VTA, the food was removed. A dosage of 0.05, 0.25, or 0.50 micrograms was administered. To determine the role of JAK-STAT signaling in VTA leptin action, the leptin injection was counterbalanced with either a pretreatment of JAK-2 inhibitor or, as a control, its vehicle (a substance of no therapeutic value used to convey an active medicine for administration).

It had been demonstrated previously that this inhibitor blocks the central effect of leptin to reduce food intake and body weight. The food was then replaced. Similar injections were administered to determine the role of insulin receptor substrate phosphatidylinositol-3 OH kinase and hypothalamic mammalian target of rapamycin on leptin action in the ventral tegmental area.

The results showed that bilateral intra VTA leptin administration in rats reduced food intake by 25%. The reduction of food intake was accompanied with a decrease in body weight in the animals that received leptin as opposed to the control group. In the case of the JAK-STAT pathway, it was found that central administration of leptin activated the JAK-STAT pathway in the ventral tegmental area and the substantia nigra. To determine if leptin acts directly in the VTA to activate the pathway, leptin was delivered directly into the VTA. It was noted that intra-VTA administration of leptin induced tyrosine phosphorylation of STAT3, a mediator of leptin receptor signaling in this part of the brain. However, intra-VTA pretreatment of the JAK-2 inhibitor, when administered alone blocked leptin in the VTA from reducing food intake and decreasing body weight.

The experiment continued to determine whether leptin in the VTA signals through the IRS-PI 3-Kinase pathway. The question to address was, does intracerebroventricular leptin or direct administration of leptin in the ventral tegmental area increase phospho protein kinase B, a marker of PI 3-Kinase activation? The result was that leptin administration failed to induce phospho protein kinase B in the VTA. An additional group of rats was used to determine if the mTOR pathway is affected by leptin action in the VTA. Neither intracerebroventricular nor direct intra-ventral tegmental area administration of leptin activated the mTOR signaling pathway in the VTA. Although the hypothalamus plays a major role in leptin signaling and activation, this experiment supports the growing body of evidence that leptin receptors are expressed in extrahypothalamic sites as well, particularly the VTA (Morton, et al., 2009).

Leptin and Obesity

Researchers suggest that obesity in humans is due to leptin resistance. Leptin resistance is thought to involve a period of overeating. Overeating results in an increase in leptin levels, which may damage the hypothalamus. As a result, the hypothalamus becomes less sensitive to leptin, leading to a sustained increase in leptin levels (Klok, et al., 2006).

The Role of Ghrelin

Ghrelin plays a central role in weight regulation. In 1999, attempts to identify the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) led to the discovery of ghrelin (Higgins, et al., 2007). The gene that codes for human prepro-ghrelin is located on chromosome 3.

Prepro-ghrelin consists of 117 amino acids, and the mature ghrelin constitutes 28 amino acids, with a fatty acid chain on the third amino acid. Ghrelin peptide was first isolated in the stomach and is most abundant in the gastric fundus, but is also found in the pancreas and adrenal cortex. Ghrelin is produced by endocrine cells known as P/D1 cells that are categorized as opened or closed. The open cells are exposed to the lumen of the stomach and are in contact with gastric contents. The closed type is in close proximity to the capillary network of the lamina propria. Both types of cells secrete the hormone into the bloodstream (Feldman, et al., 2010). In the brain, ghrelin producing neurons are found in the pituitary, hypothalamic arcuate nucleus, and between the dorsal, ventral, paraventricular, and arcuate hypothalamic nucleus (Klok, et al., 2006).

Ghrelin stimulates growth hormone secretion, increases food intake, and produces weight gain. Ghrelin is a member of the motilin family of peptides, stimulates gastric contraction and enhances stomach emptying (Feldman, et al., 2010). Ghrelin levels show preprandial (prior to meals) increase and postprandial (after meals) decreases. Ghrelin levels are also low in obesity, while high during fasting and in anorexia nervosa. The postprandial reduction of ghrelin levels is smaller in obese subjects than in subjects of normal weight. This explains how ghrelin leads to excessive eating. First, the diminished reduction of postprandial ghrelin levels increases the length of time that subjects feel hungry. Second, because of relatively higher ghrelin levels, the speed of gastric emptying may not be reduced, which causes a lack of feeling satisfied. Without feelings of satiety, obese individuals eat more than they need and gain weight. Furthermore, ghrelin levels are influenced by age, gender, BMI, growth hormone, glucose, and insulin. Leptin also affects ghrelin levels. Ghrelin plays a central role in neurohormonal regulation of food intake and energy homeostasis.

The expression of orexigenic peptides in the hypothalamus regulates eating behavior. Obesity may develop from deregulation of these pathways. Intravenous and central administration of ghrelin stimulates hunger and food intake. Ghrelin levels are decreased in obese subjects, which are thought to be the body's response to reduce hunger and food intake in those with excessive energy balance.

In the following study, the diets of healthy lean men were supplemented with a moderate amount of fat-rich food, to establish the effect of overfeeding on plasma ghrelin levels. Six men between the ages 21-34 with a BMI of 21.4-24.3 were included in the study. Over a period of three weeks, the participating students visited the lab for 8 experimental visits, 4 visits for postprandial fat loading tests and 4 visits for the measurement of gastric emptying. The day before postprandial testing, subjects were given a low fat dinner, and refrained from exercise. The day of the testing, an oral fat tolerance test consisting of 125 ml of dairy cream was administered and blood samples were taken at 30 minute intervals for 2 hours and then at 4 and 6 hours postprandially. The same preparation was done the day before gastric emptying measurement: a low fat dinner, and no exercise the night before. In the morning of the testing, subjects consumed 500 ml of water. A nasogastric tube was positioned. A 300 ml intralipid was injected into the stomach over a period of 2 minutes. The contents of the stomach were mixed by aspiration and 20-30 ml was reinjected 10 times using a catheter tip syringe. This cycle continued for 60 minutes. Following the initial tests, subjects were provided with a high fat dietary supplement to be eaten daily for 3 weeks. Subjects were tested on days 7, 14, and 21 for postprandial testing, and on days 8, 15, and 22 for gastric emptying measurements. Blood samples were collected to analyze plasma fatty acids, leptin, ghrelin, and pancreatic polypeptide. During the study, the mean weight gain was 2.1 kg. Results showed that the inhibitory response of ghrelin to oral fat was enhanced after overfeeding. Before

the study, maximal suppression of ghrelin occurred 90 minutes postprandially. After only one week into the study, the maximal suppression occurred at 120 minutes. Leptin concentration was increased after the 3 week dietary supplementation. Triglyceride concentration was also changed by dietary supplementation. It has been suggested that ghrelin levels respond to changes in body weight and not to changes in energy supplied to the GI tract. However, these results imply that changes in ghrelin concentration begin before significant increases in body weight, since a weight gain of 3% was associated with an 18% decrease in ghrelin concentration in the study.

The Mechanism of Action of Ghrehlin

Ghrelin is considered a natural growth hormone secretagogue (GHS), or a substance that stimulates the secretion of growth hormone. Generally, the administration of growth hormone secretagogues, which are synthetic peptide and nonpeptide compounds, stimulates the release of growth hormone. Administration of growth hormone secretagogues synthetic peptide also stimulates feeding behavior. Growth hormone secretagogues receptor (GHS-R) is expressed in the pituitary gland, hypothalamus, and several other areas. In the hypothalamus, GHS-R mRNA is expressed in neuropeptide Y, agouti-related protein, proopiomelanocortin, and growth hormone releasing hormone (GHRH) containing neurons.

Similarly, research shows that ghrelin administration increases food intake in rodents. An experiment was done on male Sprague –Dawley rats, weighing 250-280 grams. They were given food and water ad libitum. A stainless steel cannula was implanted into the right lateral ventricle. Only the rats whose cerebrospinal fluid flowed into the cannula were used. The rats were injected

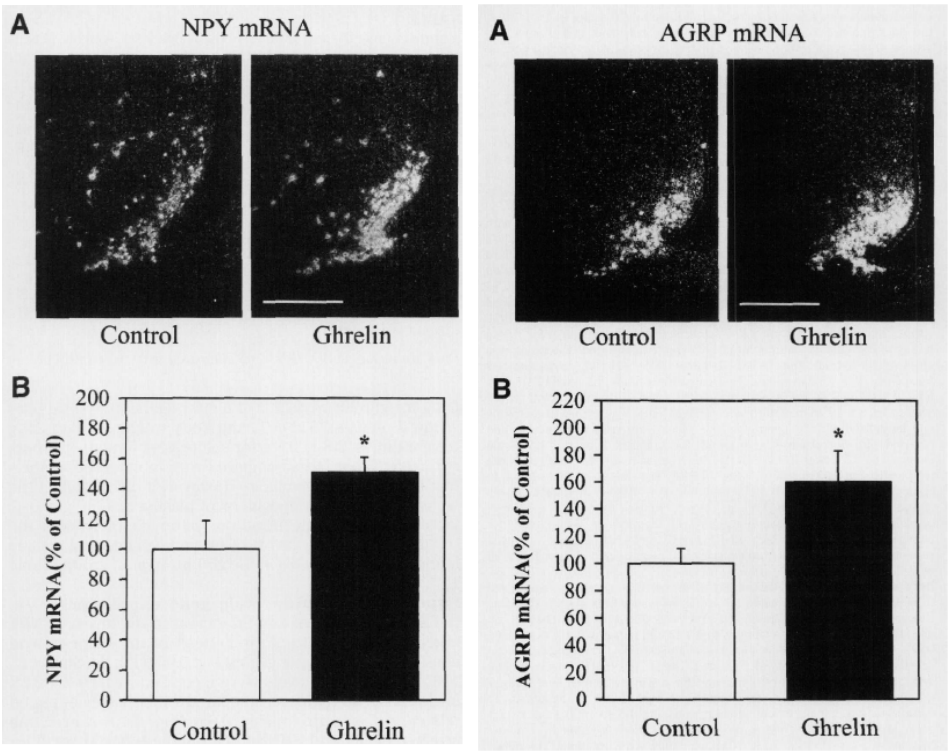

Figure 3. These two diagrams show the result of the above experiment, indicating that the primary hypothalamic target of ghrelin are NPY and AGRP, proving that ghrelinis an orexigenic peptide (Kamegai, et al., 2001).

with the peptide 14 days after the cannula was placed. Rat ghrelin or saline was injected every 12 hours over a 72 hour period. Food intake and body weights were measured daily. Around 4 hours after the last ghrelin injection, the rats were killed. The blood was taken to test glucose, insulin, leptin, and growth hormone levels. The brains were preserved, and then coronal sections were cut and mounted onto slides. The results revealed an increase in food intake; however, they did not affect plasma insulin, glucose, leptin, and GH concentrations. Figure 3 shows that neuropeptide Y mRNA levels were increased, as well as arcuate nucleus agouti-related protein mRNA levels in ghrelin treated rats. However the levels of proopiomelanocortin and growth hormone releasing hormone mRNA in the arcuate nucleus did not differ between the ghrelin treated and saline treated rats. These results show that the primary hypothalamic targets of ghrelin are neuropeptide Y and agouti-related protein neurons and that ghrelin is an orexigenic peptide in the brain and stomach. The fact that ghrelin administration did not alter GHRH levels or the release of growth hormone indicates that the orexigenic action of ghrelin is not likely to be due to GH secretion (Kamegai, et al., 2001).

In contradiction to this experiment, experiments on Zucker rats led to the conclusion that ghrelin may be GH dependent (Nogueiras, et al., 2004). The purpose of this study was to investigate a number of properties affecting ghrelin.

1. Leptin may alter the expression of Growth hormone secretagogues receptor, thereby inhibiting the effect of ghrelin.
2. The effect of leptin administration on the expression of Growth hormone secretagogues receptor in the arcuate nucleus and ventromedial nuclei.
3. The influence of ghrelin administration on Growth hormone secretagogues receptor expression.
4. The influence of ghrelin on the expression of leptin receptors Ob-Rb.
5. The role of growth hormone in the effect of ghrelin.

The rats used were male between the ages of 8 and 12 weeks. For short term ghrelin and leptin effects, intracerebroventricular cannulas were implanted in the lateral ventricle of the rats. One group of rats was fed ad libitum, and the other group was deprived of food for 48 hours. The rats were then injected with a single injection of ghrelin or its vehicle. In a second experiment, rats were injected with 0.5, 2.0, or 5.0 microgram of rat ghrelin or its vehicle. In both cases, the rats were killed 2 hours after the injection. In another experiment, rats were fed ad libitum or fasted for 48 hours. A single injection of recombinant human leptin or its vehicle was given, and the animals were killed 2 hours after. For long term ghrelin effects, a cannula was placed in the lateral ventricle. Rats received ghrelin or its vehicle for 24 hours, 48, hours, or 7 days. For leptin testing, either human recombinant leptin or its vehicle was infused for a period of 7 days into the lateral ventricle. The last 48 hours of the infusion, the rats were either fed ad libitum or fasted.

The results showed the following. The GHS-R expression was higher in the arcuate nucleus of the obese Zucker rats than the lean rats, but the expression in the ventromedial nuclei did not differ significantly. Plasma ghrelin levels were unchanged in the fatty rats in comparison to the lean ones. The GHS-R mRNA of the arcuate nucleus in fasted rats was higher than in ad libitum fed rats. No change was seen in its expression in the ventromedial nuclei.

The effect of leptin on GHS-R differed for short and long term infusions. A single injection of leptin did not affect the level of GHS-R expression in the arcuate nucleus or ventromedial nuclei in the fed animals. However, the long term infusion of leptin decreased food intake and body weight. Additionally, long term infusion of leptin caused a decrease in GHS-R levels in the ARC

but not in the ventromedial nuclei in both ad libitum and fasted animals. The effect of leptin on GHS-R was dose dependent. No change was seen at 1 microgram/ day, but at 5 and 15 microgram/ day, GHS-R expression was lower.

Ghrelin also had a significant effect on GHS-R expression in the ARC. After short-term treatment with ghrelin, GHS-R mRNA levels increased in the arcuate nucleus. The administration of ghrelin caused an increase in food intake. The increase in GHS-R gene expression was also seen in the fasting animals. No change was seen in the ventromedial nuclei in any of the treatments. The effect of ghrelin seemed to be dose-dependent: gene expression of GHS-R was higher after 5 micrograms compared with 2 micrograms. In long term infusion of ghrelin, GHS-R mRNA was only significantly increased after 1 week and not after 24 hours or 48 hours.

Interestingly, short term treatment of ghrelin did not increase GHS-R mRNA levels in the arcuate nucleus of growth hormone deficient rats. It was concluded that ghrelin and leptin are both involved in the regulation of GHS-R in the arcuate nucleus, but not in the ventromedial nuclei. Intracerebroventricular ghrelin increases GHS-R expression while intracerebroventricular leptin decreases GHS-R mRNA. During fasting (high levels of ghrelin and low levels of leptin) and in obese rats insensitive to leptin, GHS-R mRNA expression is increased in the arcuate nucleus. GHS-R mRNA seems to be regulated by ghrelin through a GH dependent mechanism since ghrelin fails to stimulate GHS-R expression in the absence of GH (Nogueiras, et al., 2004).

Ghrelin is released from the GI tract and provides input to the brain through three mechanisms.

1. Directly, via the blood stream, by entering the anterior pituitary gland and other areas of the brain not protected by the blood brain barrier.
2. Directly, by crossing the blood brain barrier, via a saturated transport system.
3. Indirectly, via the vagus nerve. Ghrelin levels rise before a meal due to the reduced inhibitory controls of the vagus nerve on ghrelin.

Ghrelin acts in the arcuate nucleus, a part of the brain important in the regulation of feeding and appetite. Growth hormone secretagogues receptors are found in the arcuate nucleus on neurons that release neuropeptide Y and agouti-related protein, stimulators of weight gain, where ghrelin acts to increase the output from these neurons. GHS can be found on presynaptic nerve endings, influencing the release of neurotransmitters.

Ghrelin promotes appetite in two ways. It can depolarize the orexigenic neuropeptide Y and agouti-related protein neurons, or it can increase the inhibition exerted by the NPY and AgRP neurons over the anorexigenic proopiomelanocortin and cocaine and amphetamine-regulated transcript neurons. Both of these enhance appetite (Higgins, et al., 2007). Ghrelin stimulates the activity of neurons expressing neuropeptide Y, agouti related protein, and orexin. On the other hand, ghrelin inhibits proopiomelanocortin neurons and corticotrophin releasing hormone producing neurons (Figure 4) (Klok, et al., 2006).

Gherelin and Obesity

Ghrelin functions as a meal-initiation signal in the system for short term energy balance regulation. This is demonstrated by two things. Firstly, the preprandial increase in ghrelin levels initiates meals voluntarily even in the absence of food. Secondly, an intravenous injection of ghrelin induces hunger and food intake in healthy and obese humans (Klok, et al. 2006). In addition to the involvement of ghrelin in short term regulation of energy balance, it also plays a role in long term energy balance regulation. Ghrelin concentration is negatively correlated with BMI. Ghrelin

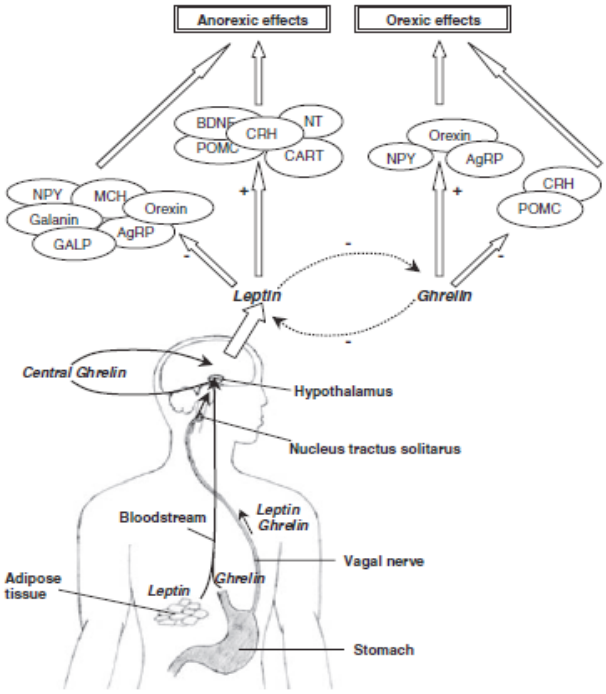

Figure 4. This drawing shows the pathways by which ghrelin and leptin may reach the hypothalamus in order to have an effect on food intake and body weight. Leptin is secreted by adipose tissue and ghrelin is secreted by the stomach. Both hormones may enter the brain through the bloodstream. In addition, ghrelin and leptin may reach the hypothalamus through the vagal nerve and nucleus tractus solitarus. Leptin and ghrelin both stimulate (+) and suppress (-) hypothalamic neurons resulting in anorexic or orexic effects on energy balance (Klok, et al. 2006).

levels change in response to dieting to maintain body weight. This is seen in obese and anorexic subjects. When obese people lose weight, these levels increase, and they decrease when anorexic patients gain weight (Klok, et al. 2006).

Gut Hormones and their Effect on appetite

Although ghrelin is the only gut hormone that stimulates food intake, there are other hormones in the GI tract that have an effect on hunger and satiety (Jayasena & Bloom, 2008). In 1982, Peptide YY (PYY) was isolated from colonic extracts. Peptide YY is a 36 amino acid peptide with tyrosine residue at both the C and the N terminals. PYY is secreted from the endocrine L cells of the small and large bowel. Two forms of peptide YY exist, PYY 1-36 and PYY 3-36. The maximum concentration is found in the rectum, while lower concentrations are found in the small intestine, terminal ileum, and colon. In contrast to ghrelin, PYY levels are suppressed in a fasting state, but increase after a meal. PYY levels in the body are lowest in the morning, and steadily increase throughout the day, reaching a peak after the evening meal (Neary, et al., 2003). Peptide YY is released into the blood following a meal, in proportion to the calorie intake. PYY acts on the hypothalamus as a satiety signal. It has multiple effects that slow the passage of nutrients through the gut. PYY decreases food intake by delaying gastric emptying, and inhibiting gallbladder contraction, pancreatic exocrine secretions, and gastric acid secretion (Jayasena & Bloom, 2008.)

Peptide YY reduces the appetite stimulating hormone ghrelin, and diminishes the preprandial rise in ghrelin. Low levels of peptide YY in obese subjects point to PYY deficiency contributing to obesity (Popovic & Duntas, 2005)

Another gut hormone involved in the increase or decrease of food intake is cholecystokinin (CCK). CCK is a peptide hormone expressed in the small intestine and is released after a meal. Cholecystokinin stimulates pancreatic and gallbladder exocrine secretions, inhibits gastric emptying, and increases intestinal mobility. Cholecystokinin also acts as a neurotransmitter. Injections of cholecystokinin decrease the amount eaten but increase meal frequency without any change in body weight. However, because the ability of cholecystokinin to reduce food intake does not last long, it is not the best target as a drug to treat obesity (Jayasena & Bloom, 2008).

Ghrelin and Leptin as Drug Therapy

Because of the increasing cases of obesity throughout the world, scientists found a need to find an effective drug therapy to aid in weight loss. This has led to extensive research on recombinant hormones, as well as lipase inhibitors as possible paths to increase or decrease ghrelin and leptin as needed.

Current Drug Therapy For Obesity

Pharmacotherapy options to treat obesity are very limited. There are two weight loss medications that are approved in the United States and Europe, Orlistat® and Sibutramine®. Very recently, Rimonabant® was approved in some European counties. The FDA recommends pharmacotherapy only after dieting and exercise have failed and the BMI is greater than 30kg/m2. Orlistat® (Figure 5) was approved by the FDA in 1999.

Orlistat® is a gastrointestinal lipase inhibitor. It decreases fat absorption by binding to pancreatic lipase, which blocks hydrolyses of triglycerides into fatty acids and monoglycerides, and increases fat excretion in fecal material by 30%. In clinical trials, weight loss was 3% greater in subjects taking Orlistat® compared to those taking the placebo. Orlistat® has gastrointestinal side effects such as oily fecal splotting, fecal urgency, abdominal pain, and fecal incontinence. In addition, Orlistat causes a loss of fat soluble vitamins.

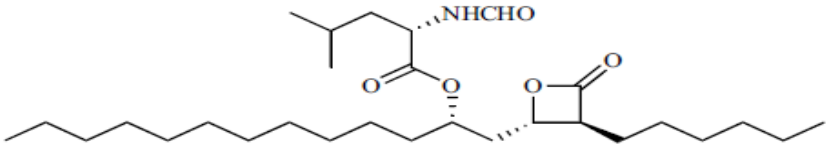

Figure 5. Chemical Structure of Orlistat® (Isidro & Cordido, 2009).

Sibutramine® (Figure 6) was approved by the FDA in 1997. It inhibits serotonin and norepinephrine reuptake. Sibutramine® was originally created as an antidepressant and reduces food intake by reducing appetite. It has been demonstrated, that long term Sibutramine® along with a reduced calorie diet resulted in weight loss over a period of 1-2 years. In clinical trials, weight loss was 5% greater in subjects taking Sibutramine® than those taking the placebo. Maximum weight loss was achieved after six months, and was dose related. 10 mg/day resulted with an average of 7.4 kg compared

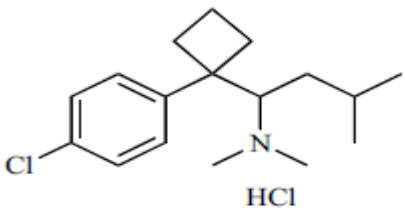

Figure 6. Chemical structure of sibutramine (Isidro & Cordido, 2009).

with 3.6 kg for the placebo, and 15 mg/day resulted in 10.3 kg weight loss compared with 1.3 kg in the placebo group. Because Sibutramine® can increase blood pressure and heart rate, patients taking it are monitored carefully.

Although these anti obesity drugs do promote weight loss of 3-5% of initial body weight, most patients are still overweight or obese after treatment. In addition, they regain the weight lost after drug withdrawal. Therefore different strategies are being researched to achieve weight loss, such as stimulating anorexigenic signals and blocking orexigenic signals. Possible antiobesity therapy currently being investigated includes leptin receptor superagonists, neuropeptide Y receptor antagonists, peptide YY analogues, ghrelin receptor antagonists, and growth hormone receptor agonists (Isidro & Cordido, 2009).

A study was done comparing leptin and ghrelin levels in obese subjects who took Orlistat® and those only on a dietary regimen of 55% carbohydrates, 25% each of fat and protein over a span of 12 weeks. Twenty-one obese patients, 6 males and 15 females, and ten control subjects were involved in the experiment. The obese patients were divided into two groups: one group consisting of 11 people who took Orlistat® 3 times daily, while the second group of 10 received dietary treatment only. At the end of the 12 week period, there were decreases in BMI, weight, waist perimeter, glucose, insulin, and cholesterol in the Orlistat® group, while there was only a decrease in BMI, weight, and insulin levels in the dietary treatment group. Ghrelin levels were increased in both groups. Leptin levels were reduced in the Orlistat® experimental group, but did not decrease significantly in the dietary treatment group. The results of this study have shown that patients who change their lifestyle and eating habits have similar success rates as those who are treated with Orlistat®. The elevated leptin levels and reduced ghrelin in obesity are reversed by weight loss regardless of the type of treatment, Orlistat® vs. dietary treatment (Ozkan, et al., 2009). However, the above experiment does not have a proper control and experimental group. The group that was given Orlistat® was also on dietary treatment, making it difficult to discern whether the weight loss and change in BMI, glucose and insulin levels were due to changes in food habits or to the treatment of Orlistat®.

Leptin As Pharmacotherapy

Leptin directs the onset and termination of appetite and restraint between meals at two sites. At the first site, leptin opposes the orexigenic action of ghrelin directly and indirectly by restraining the output of excitatory neuropeptide Y signaling in the arcuate nucleus- paraventricular nucleus axis. Secondly, leptin suppresses ghrelin output from gastric glands to restrain appetite between meals. Deficiency in this complex system of leptin restraint leads to weight gain in humans and rodents. Several experiments were performed to try to prevent an insufficiency of leptin with the goal that it will assist those with obesity.

One experiment was done with gene transfer technology with viral vectors. The non-replicative, non-immunogenic, and non-pathogenic vector, recombinant adeno-associated viral vector encoding the leptin gene was engineered for testing in rodents. It was found that a single intravenous injection increased circulating leptin and normalized body weight in obese leptin mutant mice. Similar effects were produced when it was injected centrally into either intracerebroventricularly or into hypothalamic sites on leptin mutant rats and mice (Kalra, 2007).

Another experiment was done to determine whether leptin administration affects body weight. As mentioned earlier, leptin signals regulatory centers in the brain to inhibit food intake and regulate body weight. Overeating can cause a lack of sensitivity to leptin receptors and signal-

ing resulting in obesity. This insensitivity is thought to be overcome by administration of exogenous recombinant methionyl human leptin in high doses.

A previous study had shown that a minimum of 3 injections daily of recombinant leptin was required to have an effect; however, this dose was intolerable. Therefore, this study (Zelissen, et al., 2005) was done to determine whether a lower dosage of leptin would be tolerated better. The recombinant leptin would be injected at night to mimic the normal diurnal rhythms. Diurnal variation in endogenous leptin peaks between 12:00 and 2:00 AM, and is less pronounced in obese subjects. It was hypothesized that diurnal variation could be improved by recombinant leptin administered once daily in the evening, which might result in greater weight loss. For 3 weeks, patients with a BMI between 27.5 and <37.5 kg/m2 were given a 2100 calorie diet. All participants were given 2-ml injections of a placebo. Those that lost more than 5 kg over this period were excluded from the rest of the experiment. Patients were divided into three groups of 12 week treatment regimens.

- Group 1: administered once daily in the morning; 70 patients with recombinant leptin and 25 with placebo.
- Group 2: administered once daily at night; 70 patients with recombinant leptin and 24 with placebo.
- Group 3: administered twice daily; 71 patient with recombinant leptin and 24 with placebo.

The results did not show overall significant changes between the placebo groups and the leptin groups. Although only slight, the difference in weight change was greater in the twice daily recombinant leptin and placebo than in the once daily doses. The weight loss of subjects with low serum leptin levels was not much different from those with high serum leptin levels. Similarly, feelings of hunger were compared between the groups, and no change was found. The same was true for calorie intake from before the study and the end of the study. Other studies have shown that when pegylated leptin was administered in addition to a restricted diet, greater weight loss was observed (Zelissen, et al., 2005). As in Ozkan, et al.'s study (2009), more research has to be done to conclude whether physical activity and diet restriction combined with leptin administration is a valuable treatment for obesity.

Just as in the previous study done by Zelissen, et al., (2005) in which the use of recombinant leptin was used to determine its affect on weight loss, the following study (Hukshorn, et al., 2002) was done using pegylated recombinant leptin (PEG-OB) to examine if its administration will decrease food intake and promote weight loss. Twenty-eight obese subjects, 16 women and 12 men, partook in the study. Subjects were between the ages of 18-65 years and women had to be either sterile or postmenopausal. Anyone with a medical condition requiring pharmaceutical treatment was excluded. After a 4 week lead in diet, only those who lost 1.75 kg or more were allowed to continue treatment. Treatment consisted of 60 mg pegylated recombinant leptin or a matching placebo, which was administered once a week over 8 weeks. In addition to the injections, subjects were on a hypoenergetic diet designed to reduce energy intake by 3200kJ/day. Body weight and height were measured weekly, as well as the BMI. Blood and urine were collected throughout the study. The results after 8 weeks were similar in the pegylated recombinant leptin group and the placebo. Both groups lost about the same amount of weight, and no real difference in change of BMI between the two groups was recorded. There was also no significant difference in the decrease of cholesterol, triglycerides, glucose and insulin levels between the treatment groups. However, pegylated recombinant leptin levels and total leptin levels showed an increase a week after the last dose. These results show that exposure to 60 mg of PEG-OB weekly for 8 weeks did not influence

weight loss in obese subjects, even though pegylated recombinant leptin levels were elevated after the study. Although this dosage of PEG-OB was ineffective, it may be possible that when PEG-OB is administered during a severe energy restriction or total leptin deficiency, it will cause weight loss. In addition, the small number of subjects studied, as well as the short duration of the study, may also explain the lack of effectiveness of pegylated recombinant leptin administration (Hukshorn, et al., 2002). It is interesting that the pegylated recombinant leptin did not produce a weight loss, while gene transfer in a viral vector did contribute to an increase in leptin levels and weight loss.

The Effect of Sleep on Ghrelin and Leptin

Average sleep duration in the United States has declined over the past several decades. At the same time, obesity rates have increased. Studies indicate that sleep may regulate hunger and satiety through hormones including ghrelin and leptin. Previous studies have indicated that sleep deprivation leads to an increase in ghrelin and a decrease in leptin, which affects appetite and hunger. It is hypothesized that sleep brings about these changes by changes in the hypothalamic control of autonomic nervous system activity which influence ghrelin and leptin secretion.

A study was done (Littman, et al., 2007) on moderate to intense physically active post menopausal women to establish the relationship between exercise, sleep, BMI, ghrelin, leptin, and weight change over a 12 month period. Before the experiment, the scientists hypothesized what the outcome would be.

1. Improvement in sleep might lead to greater weight loss by influencing satiety and hunger hormones.
2. Exercise induced decreases in leptin and increases in ghrelin would be greater in those whose sleep worsened as compared to those whose sleep remained the same or improved.

Participants in the study were aged 50-75 years who exercised less than 60min/week of moderate or vigorous activity. Participant's BMI had to be greater than 25.0 kg/m2. The exercise group had 87 subjects, while the control group contained 86. Blood was taken after a 12 hour overnight fast at the baseline, 3 months, and 12 months. Participants in the experimental group performed a minimum of 45 minutes of moderate to intense aerobic exercise 5 days a week for 12 months. Those in the control group attended a 45 minute session of stretching once a week for a year. Subjects in both groups maintained their usual diet. The results revealed that the average BMI in women who slept more than 9 hours per night was higher than that of women who slept 8 or fewer hours per night. Ghrelin levels increased during the 12 month trial among both groups. This increase was mainly due to weight loss. The difference in ghrelin levels was greater in those who experienced improved sleep as opposed to those whose sleep remained the same. Leptin decreased more among the exercise group than the control group. Leptin increased with worsening of sleep quality in the experimental group. The difference in leptin levels in both groups was less among those with improved sleep quality. The observed trends were in the opposite direction of the proposed hypothesis. The weight loss difference between the groups was greater for those who slept less. Ghrelin levels increased and leptin levels decreased among those with improved sleep compared with those with decreased sleep quality. In addition, exercise induced weight loss was greater in those who slept < 6 hours per night in direct opposition to the hypothesis that improved sleep would increase weight loss (Littman, et al., 2007).

On the other hand, recent studies have shown that short sleep duration not only increases BMI, but also reduces circulating leptin levels which suppress appetite and increase ghrelin levels

that promote hunger. Experiments on subjects who slept 4 hours in comparison to 10 hours for two consecutive nights revealed that they experienced decreased leptin levels and increased ghrelin levels, overall increasing feelings of hunger and appetite. To further determine the influence of short term sleep curtailment on leptin and ghrelin secretion, fasting morning levels of these hormones were taken from healthy men after nights of different amounts of sleep.

The study was done (Schmid, et al., 2008) on nine men whose ages ranged from 20- 40 years. The BMI of these subjects fell between 20.7 and 25 kg/m2. Subjects had normal sleep cycles and were not following any particular diet. Average sleep was 7-8 hours with bedtime between 10:00pm-1:00am and waking time 6:00-8:00am. Subjects were examined after three conditions, spaced 2 weeks apart. 1) a night of total sleep deprivation. 2) a sleep restriction to 4.5 hours during the first part of the night. 3) a night with 7 hours of sleep. Participants in the study did not drink caffeine for 10 hours before and alcohol for 24 hours before the experiment. In addition, subjects were told to avoid intense physical activity. Blood samples were taken, and then subjects rated their feelings of hunger on a scale from 0 (not at all) to 9 (severely).

Results showed (Figure 7) that subjects had stronger feelings of hunger after total sleep deprivation than after 7 hours or 4.5 hours of sleep. The difference between 7 and 4.5 hours in hunger ratings was not significant. Ghrelin levels were 22 + 10% higher after total sleep deprivation than after 7 hours of sleep. After 4.5 hours of sleep, ghrelin levels were increased 11 + 5%. Interestingly, leptin levels were completely unaffected by sleep restriction or deprivation. A single night of sleep deprivation increased feelings of hunger and plasma levels of the hunger-promoting hormone ghrelin (Schmid, et al., 2008). There are still factors that have to be taken into account and further researched. What would be the effect of sleep deprivation and restriction on women? In addition, this study was done only over a short period of time and after one night of each category of sleep restriction. The question still remains, what effect will long term sleep deprivation have on ghrelin levels as well as leptin levels?

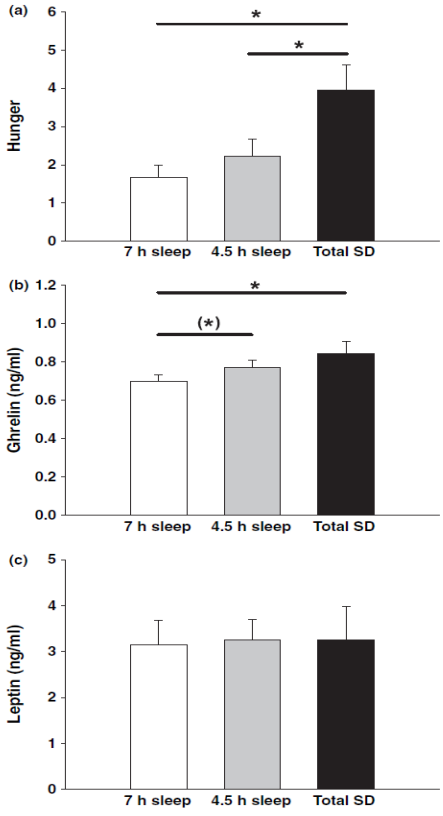

Figure 7. (Schmid, et al., 2008).

In the study done by Littman, et al. (2007), leptin levels did decrease in those that experienced less sleep, while in the study done by Schmid, et al., (2008), leptin levels were unaffected by sleep change. It is possible that the physical activity done by subjects in the former experiment was the driving factor behind the change in leptin levels, and not actual change in sleep. It is unclear, since sleep and exercise were combined into one experiment. Furthermore, the changes in ghrelin levels show an opposite trend in the two studies. In Littman, et al., (2007), ghrelin levels increased with improved sleep, while in Schmid, et al., (2008), ghrelin levels increased when subjects experienced less sleep.

Obstructive sleep apnea syndrome, (OSAS) is a common disease associated with obese subjects. The actual effect of obstructive sleep on ghrelin and leptin levels is further complicated

by the following study whose results seem to differ from the research done by Littman, et al. (2007) and Schmid, et al. (2008). The subjects of the study were 65 obese men with obstructive sleep apnea syndrome. Subjects underwent polysomnography overnight to measure the degree of obstructive sleep. All subjects had fasting blood samples taken between 7:00-8:00 AM. Cholesterol, triglyceride, leptin, and ghrelin levels were tested. Thirty of the 65 obese subjects had moderate to severe obstructive sleep apnea syndrome. Twenty two subjects did not have OSAS and became the control group. The results showed that leptin levels were higher in patients with moderate-severe obstructive sleep apnea syndrome compared to the control group. There was no difference in ghrelin levels between the two groups; however, ghrelin levels were correlated with BMI, while leptin levels were not (Ciftci, et al., 2005). This study is another factor pointing to the relationship of sleep, obesity, and corresponding ghrelin and leptin levels.

CONCLUSION

Leptin and ghrelin both play major roles in energy balance in humans. It is unclear whether abnormalities in the leptin or ghrelin systems contribute to the development of obesity. However, disturbances in these systems do maintain obesity. Obese patients are leptin resistant, and it is therefore necessary to develop a treatment that overcomes it or bypasses normal leptin functioning. Ghrelin also is seen as a potential drug target for weight regulation, as obese patients are ghrelin-sensitive. Diet and exercise have significant effects on energy homeostasis. The use of therapeutic drugs alone is not sufficient to treat obesity. As seen from a number of experiments, the most effective treatment is provided by a combination of diet and exercise. The best strategy to accomplish long term changes in body weight is the use of potential anti-obesity agents in combination with a low fat diet and sufficient exercise.

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A Proposed Mechanism for Drug-induced Nightmares

ISAAC BREZNER

INTRODUCTION

The fields of neuropharmacology and psychopharmacology are known to be highly connected, despite our severe lack of knowledge in these fields. One of the many overlaps between the two fields is sleep, which itself entails many mechanisms and events which are, as of yet, unexplainable. From the perspective of either field, one of the most mysterious events occurring during sleep is dreaming. From the cause of dreams to their content, little is known about them or their more sinister subclassification: nightmares. In this field of disturbed dreaming, neuropharmacology makes a large intrusion into the organized theory of psychology. It has been shown via drug studies that some drugs can cause disturbed dreaming as a side effect of their neuropharmaceutical action or as a backlash of withdrawal (Pagel, 2010a, 2010b). Additionally, it has been shown that REM sleep is linked to dream production (Nofzinger, 1997). These windows into the dream world leads to the suggestion that part of the mechanism for causing dreams is disturbed by certain drugs resulting in hyper-bizarre psychotic dreaming. Perhaps, the specific mechanism that tries to fit the nonsensical stream of neurological data to with the brain's compilation of expected physical action and reaction has been faulted by the drug, thereby producing such psychotic dreaming known as nightmares.

Link between Drugs and Dreaming

Pharmacological disturbances of dreaming are a rare but proven phenomenon. Disturbances have been reported by many psychiatric drugs, as well as by the antihistamine Chlorpheniramine, nicotine and others (Pagel, 2010a, 2010b). Barbiturates and alcohol, on the other hand, cause disturbed dreaming upon withdrawal of administration (Barrett and McNamara, 2007c). Drugs with neurological pharmacodynamics are most likely to cause disturbed dreaming (Pagel, 2010a, 2010b). In one extreme case, Chantix, a drug designed to help smokers quit, caused the death of one of its users (Westfall). Patients using Chantix have described dreams which are both vivid and hyper-bizarre. Clearly, there is some connection between sleep, dreaming and drugs.

Proposed Mechanism for Onset of sleep

Sleep begins with and is defined as the loss of consciousness. The brain gradually reduces its level of activity through sleep stages one through four, also known as NREM or non-REM sleep. Instead of remaining deactivated until awakening, the brain goes into the fifth stage of sleep known as REM sleep for its characteristic Rapid Eye Movement. This statement is a gross understatement of the complicated neurological and biochemical processes which occur during the transition into REM sleep.

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While awake and asleep, cells perform metabolism. They derive energy through the complex processes of glycolysis and the Kreb's cycle which convert one molecule of glucose to six of carbon dioxide and six of water (Merdian, 2009).

$$C6H1206 \rightarrow 6\ CO2 + 6\ H20 + 38\ ATP$$

The energy is transferred in the bond of a third phosphate group in the molecule adenosine triphosphate (ATP). The bond is broken to release energy which is used to drive many intracellular reactions via the following reaction (Merdian, 2009):

$$ATP \rightarrow ADP + Pi + Energy$$

Adenosine triphosphate is converted to adenosine diphosphate (ADP) and an inorganic phosphate group (Pi). The process can be repeated, converting the adenosine diphosphate to adenosine monophosphate (AMP) with an additional inorganic phosphate group.

$$ADP \rightarrow AMP + Pi + Energy$$

This second process is not naturally desired but can be forced by chemicals like caffeine (Zhang, 2009). Since while awake the body produces more metabolites than it can cart away, some of these metabolites build up. The increased concentrations of ADP and AMP cause the cell to slow its own metabolism to give itself time to lower the concentration of these metabolites and regenerate the ATP necessary to drive many cellular processes (Roth and Schwartz, 2008).

Another way the body causes the unconsciousness we call sleep is through the buildup of inhibitory neurotransmitters. γ-aminobutyric acid (GABA) and glycine (a derivative of α – ketoglutarate, an intermediate in the Kreb's cycle) are two inhibitory molecules whose effects can be felt throughout the brain. It has been shown that while awake, the concentrations of these chemicals rise, perhaps because they are metabolites of normal cellular processes. Since metabolism rates increase in times of intense use, the implication is that the rate of the production of metabolic byproducts increases. Therefore, in times of intense thought, GABA and glycine have an increased production. Eventually, when levels reach a specific threshold, the inhibitory effects become noticeable; slowing thought and, in high enough concentrations, induce unconsciousness (Watson et. al., 2010; Datta and McLean, 2007).

A similar sequence occurs with the prostaglandin (PG) known as PGD2. This particular chemical is produced via the cyclooxyenase (COX) pathway. For whatever reason this pathway is activated, whether because of an interaction of a glial cell with a neuron or because of another glial function, the levels of PGD2 peak before sleep. Interestingly enough, the same peak forms for two cytokines, Tumor Necrosis Factor A (TNFa) and Interleukin-1 beta (IL-1β). It is not surprising then that these chemicals have been said to be involved in the production of non-REM sleep.

Activation Synthesis Model

REM sleep, on the other hand, has a more subtle cause (Watson et. al., 2010; Datta and McLean, 2007). REM sleep is not triggered by the production of a specific chemical; rather, it is the absence of specific neurotransmitters that trigger the awakening of REM sleep (Pace-Schott et. al. 2003). The REM sleep trigger begins at the beginning of sleep, when the levels for certain neurotransmitters begin to fall from waking levels, causing a change in the chemical balance in the brain. The levels continue to fall throughout the brain's descent through the first four sleep stages. When the levels for serotonin and norepinephrine, neurotransmitters implicated in memory and understanding, fall past a specific inhibitory threshold, neurons in the pontine brainstem reticular formation begin to produce an excitatory neurotransmitter (Hobson and McCarley, 1977). These neurons produce acetylcholine which is involved in memory and attention. The acetylcholine be-

gins to wake up the brain, moving in a wave anteriorly and superiorly. The next brain section to wake up is the rest of the reticular formation, which responds by firing inhibitory signals to all sensory input. All motor neurons also receive hyper-inhibitory messages with the exclusion of those whose axons project into the intrinsic and extrinsic muscle of the eyes (Datta and McLean, 2007).

The process of keeping the brain locked within itself is an active suppression of the brain. The net effect keeps the mind resting and prevents it from becoming conscience while the brain itself becomes active to waking levels. This process moves in step with the wave of activation that sweeps up the brain. During and after the cholinergic self-activation of the brain, the activated neurons send out random signals which the now-activated brain treats as outside input, even though the sensory neurons are inhibited as previously stated. The mind attempts to fit this to a common storyline, but it lacks serotonin and norepinephrine, two chemicals which assist the brain in organizing data in a coherent manner. The resulting fusion with the brain's own signal results in the presentation of the bizarre data stream known as a dream (Hobson, 2010)

AIM Model

The REM sleep model just described is known as the Activation-Synthesis model (Hobson, 2010). It states in short that REM sleep is composed of the activation of the brain, followed by the synthesis of internally generated information. A newer model describes sleep in terms of a three-dimensional cube (Hobson, 2010). See figure 1, slightly modified from their source.

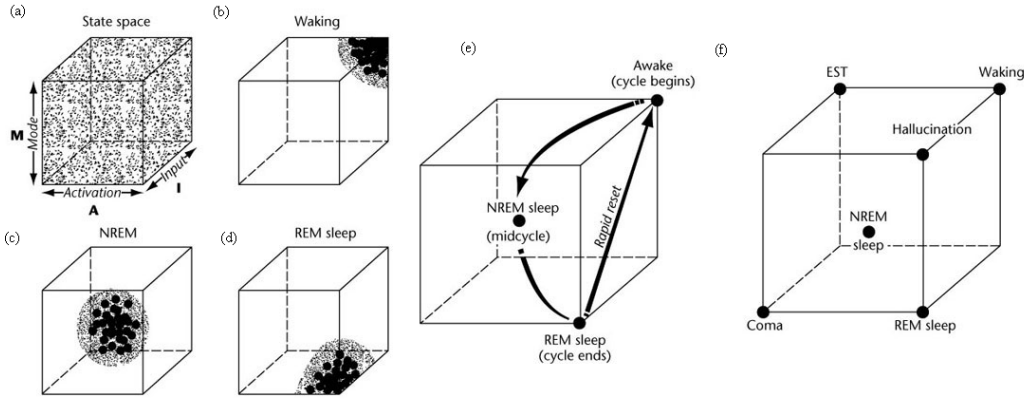

Figure 1: A visual representation of the three aspects of the AIM model when superimposed upon a cube (Hobson, 2010).

The model postulates that there are a theoretically infinite number of possible states for the brain to exist in. The space is a cube whose three dimensions are A (activation), I (input) and M (mode or modulation)(Figure 1a). Activation refers to the level of neuronal activity in comparison to waking levels. Input is indicative of the amount of outside input the brain receives. Modulation describes the likelihood of the events in the dream sequence being written to the person's memory. In theory, this could be determined by the ratio of serotonin and norepinephrine to acetylcholine in the brain, but a safe way to test for it has not been found for humans (Pagel, 2010a).

When all three dimensions are put together, it is found that a healthy waking brain would be found in the upper right corner, farthest away from the observer, with high levels of A,I and M (Figure 1b). A sleeping brain not in REM would be somewhere in middle, representing some A, I and M (Figure 1c). In REM sleep, the brain represents with high A but very low I and M (Figure 1d). A typical sleep cycle is also represented (Figure 1e). Hallucination, a period of high memory

and activation but low input (high A, M and low I), might be illustrated as being in the upper right corner closest to the observer (Figure 1f) (Pagel, 2010a).

Drugs or adverse conditions would force the brain to deviate from the path it normally travels. This could easily be demonstrated on this graph of sleep trajectory, showing how useful this model is (Pagel, 2010a). The one problem with using this model as a sole description of REM sleep is that it cannot describe the reason for the deviation or, for that matter, the trajectory in general. It tells about the state of the brain, not what the brain is doing. It can tell us what state is most likely to have dream occurrence, but it cannot tell us about the dream content. At the moment, it can show what state the brain is in, not why it is there. It is a factual model, not an explanatory model. To explain the effects of drugs on dreaming requires an explanatory model of the causes of dreaming.

RAT Theories

The set of theories that is most easily derived from the REM sleep mechanism explained above is known as the Random Activation Theories or RATs. These refer back the events following the activation of the reticular formation before REM sleep. It is simplest to say that random neuronal firing in the reticular formation activates the corresponding sectors in the brain, which in turn generate the random images that the brain then attempts to filter down and make sense of. Valli and Revonsuo (2009), in their study on sleep, ask two obvious questions. If the brain is randomly activated, they should be nowhere near as organized that they actually are? They should be highly chaotic and completely nonsensical. Secondly, why can sleep researchers point to specific events or characteristics that repeat in multiple people? If the brain really was entirely randomly generated, there should be no clearly identifiable sleep structures.

Attractor Model

A newer model, the Attractor model, posits the polar opposite of the random activation theories. It claims that not only is there a structure to dreaming, but that the structure itself is the hypothesized "fit mechanism" which fits a dream to conventional rules of observed reality. Memories from the waking hours may be delivered as input for the brain via the Continuity Hypothesis, which is a new hypothesis beginning to form around a strong correlation between waking events and their presence in dreams (Valli and Revonsuo, 2009). Input delivered in this manner or derived from some other source finds its way into the brain. The input is recognized by the brain which activates the input's own neuronal network. However, since recognition, even while awake, is based in some degree or another on the context of the recognition, it can require more input to activate a specific network over one very similar to itself. For example, little additional information is needed to recognize the difference between a hamster and a goldfish. Much more information, in other words much more activation is necessary to differentiate between a pair of identical twins. Enough input must be gathered to make the distinction. The recognition, depending on the power of activation, shows up in conscious level of the brain, feeding itself and its own context in the dream sequence. Items in the dream sequence receive activation signals in power levels proportional to the extent and power with which they show up in the dream sequence. Activated items, in turn, activate their contexts which results in the activation of a situation likely to follow the current context. These self-activated contexts show up in the sequence while simultaneously, the events which are unlikely to follow receive deactivation signals. If left to itself, this cycle would, in theory, generate an entire storyline which would make plenty of sense (Barrett and McNamara, 2007a).

Practically speaking though, there are streams of input coming to the brain from a variety of sources. Incoming items, therefore, in their respective contexts may have enough power to tip the sequence away from a previously activated network. Since this network is no longer all that likely because of the tip of balance, it begins receiving a few deactivation signals. The network becomes even less likely because it is being deactivated and in time, loses enough power so that it drops out of the sequence entirely. This answers the question of how to get a semi-sensical dream and defines a system which the brain uses while awake to utilize learned connections (Barrett and McNamara, 2007a).

This system, as any other neurological event, uses specific brain regions to carry out its task. However, it has been demonstrated that during sleep, loss of connection between these regions can occur resulting in the malfunction of the attractor give and take. The normal activations and inhibitions do not occur, resulting in illogical connections (Barrett and McNamara, 2007b). Logically, enough broken connection would result in a bizarre, jerky and radically changing dream sequence.

AND Model

The Attractor model, as just described, has been incorporated in a slightly modified form into a more general dream and nightmare model referred to as the AND (Affect Network Dysfunction) model. This model is unlike the models seen before in that it follows the path of a specific unit. The working unit of the AND model is known as the fear memory. It may be formed via classic Pavlovian conditioning (Knight et al., 2009). This type of conditioning entails the pairing of a stimulus to an otherwise abnormal response. The paradigm example of classical conditioning was performed on a dog. One normal reaction of a dog is to salivate with the presentation of food. The experiment was conducted by ringing a bell every time food was given. The dog salivates, as per its normal reaction. With the constant repetition of this food-presentation, bell-ringing pair, the animal begins to associate the ringing of the bell with the appearance of food. Eventually, when this coincidence has been repeated a sufficient number of times, at the mere ringing of a bell, the dog will begin to salivate because it now associates the bell with food presentation. This is known as classical Pavlovian conditioning (Cohen, 2010).

Fear memories, as stated above, may be formed by a normal conditioning pattern with the pairing of an event or memory with fear. Such memories can also be formed by one sudden traumatic event such as the case with Post Traumatic Stress Disease (PTSD) patients. Levin and Nielson (2007a, 2007b, 2009), in their explanation of the AND model, describe a three step process of events which occur in nightmare formation. The first step, Memory Element Activation, works in a manner very similar to the Attractor model as described above. The single difference between the two models is that, in contrast to the Attractor model, in the AND model, only elements of a scene are activated, but the entire scene will not be activated simultaneously. Typically though, as long as the memories activated are not fear-causing elements, the second phase is not required, and the process is likely to remain at the first phase until fear-causing elements come along (Levin and Nielson 2007a, 2007b, 2009).

When fear-causing elements are activated, the process continues to the next step. This second segment is called Memory Element Recombination. The mechanism by which it occurs is not well understood but functionally it combines the fear-causing elements with other unlikely elements. This recombination is then displayed in the dream sequence. The fear-causing elements still

cause some level of fear, but continued cycles of recombination and reality simulation somehow defuse the fear (Marin and Quirk, 2004).

It is thought that the reason for the presentation of the recombined memory in a simulation so real as to parallel reality is to induce the awake but contained brain to exhibit the same responses it would if it were fully awake. Additionally, it allows the brain to change the memory in the method it was formed, creating a more permanent fix. What Levin and Nielson cite as proof to the mechanism they propose, demonstrates the reality of this concept. A highly similar process is now being used in the treatment of PTSD. Exposure therapy - imagining the scene in which the trauma occurred - seems to works extraordinarily well in the reduction of nightmares in such patients (Levin and Nielson, 2009).

Table 1.

Neurotransmitters and their Effects on the Brain

Neurotransmitter	**Effects**
ACH (acetylcholine)	Injection in the pontine reticular formation increases REM sleep, while intravenous administration yields the prevention of REM sleep
Dopamine	Increases wakefulness while decreasing NREM sleep
GABA	Increases sleep, but injection into the pontine reticular formation results in overall sleep reduction
Glutamate	Injection into select areas (research does not detail specific areas) gives increased wakefulness and REM sleep while reducing NREM sleep
Norepinephrine	Generally increases wakefulness and inhibits overall sleep. However, bilateral injection yields an increase in REM sleep
Serotonin	Injection of chemicals that synergize with (works together with or increase concentration of) serotonin tend to increase wakefulness and decrease overall sleep.

(Levin and Nielson, 2007b)

The third step in the AND model, Emotional Expression, is not per se a next step, it is more like a parallel description of one byproduct of the model. The source of the emotion is simply the expression of the attempted fear extinction (Levin and Nielson 2007a, 2007b, 2009). As proof of the fact that the emotional display occurs, a nightmare is progressing. There are differences whether the nightmare is traumatic or not, but the actual differences are irrelevant to this discussion. In either case, fear memories, instead of being peacefully extinguished, can behave in one of a few ways. They can resist extinction, increase the amount of fear causing elements connected to itself or even cause the next nightmarish scene to arise in the dream sequence (Levin and Nielson, 2007b).

Resistance may be caused by a fault in one of a few places. One fault which may occur is that instead of following the normal path and activating scene elements, the brain (seemingly preferentially) activates the entire scene, causing the emotion content to be displayed and simultaneously preventing the extinguishing mechanism from acting. Alternatively, a dream may be activated correctly but during recombination, integrate more fear producing elements, causing a dream more fearful than its predecessor. The brain, effectively, enforces a dream scarier than the one it is trying to extinguish. A similar problem can occur with integrating distress-causing elements as well. When the dream is reactivated, instead of just the non-extinguished fear elements displaying themselves, the distress now inherent in the dream shows up as well. Since the distress may cause other dreams based around the same themes, this could be the beginning of a cyclic series of nightmares (Levin and Nielson, 2007b).

The AND model described above is one of two prerequisites to understand the hypothesis which this paper describes. Attention must now be turned from the overall theoretical understanding of the production of nightmares. The second prerequisite, knowledge of the neuropharmacol ogy of dreaming and nightmare production, is required. ACH (acetylcholine), dopamine, GABA (γ-aminobutyric acid), glutamate, norepinephrine and serotonin are all mimicked by drugs (Levin and Nielson, 2007b). It is for this reason that they will be examined in terms of their effects on the sleep and nightmare production models.

Table 2.

Brain Sectors Discussed in the AMPHAC model	
Brain Sector	**Effect or Purpose**
Amygdala	Required for the acknowledgement and response to fear stimuli.
MPFC (Medial PreFrontal Cortex)	Produces fear extinction, helps in reducing activity in the amygdale
Hippocampus	Controls amygdala and MPFC in the regulation of fear conditioning by regulating fear contexts
ACC (Anterior Cingulate Cortex)	Regulates the severity of distress produced by distress components during emotional expression

(Levin and Nielson, 2007b)

AMPHAC Model

Based on the REM sleep model described above, many necessary logical derivations can be made provided that the basic premise of the AMPHAC model is understood. The AMPHAC (Amygdala, Medial Prefrontal cortex, Hippocampus and Anterior Cingulate cortex) model is an attempted explanation of dreaming and nightmares not from the cognitive theoretical standpoint of the AND theory but from an examination of neuronal structures involved in the process. The exact details will not be discussed; however, a brief summary of the four brain sectors involved is provided in the chart above. (Levin and Nielson, 2007b).

Proposition of Mechanism

Combining both the AMPHAC and AND theories with logical deduction, one can easily understand how an imbalance in neurotransmitter levels may cause disturbed dreaming. It can be said that an excess of a neurotransmitter with an excitatory function may cause the hyperactivation of the amygdala, resulting in an above normal fear expression, while an excess of a neurotransmitter with an inhibitory function may cause disturbed dreaming by overly down-regulating any of the control or regulatory sectors, blocking the ample production of fear extinction. On the other hand, logically, a lack of neurotransmitter has the same effect as an excess of a neurotransmitter of the opposite effect. A classification of the neurotransmitters may now be made.

ACH promotes wakefulness and REM sleep. By promoting activity, ACH demonstrates an excitatory effect. In the pontine reticular formation, where sleep or inactivity is stimulated, an inhibitory role is played. The same logic prevails through the entire derivation. Dopamine increases waking and REM sleep. Since it serves the role of lengthening activation, it can be presumed to play an excitatory role. GABA, in contrast to ACH, generally plays an inhibitory role. In the pontine reticular formation however, it does exert an excitatory force. Glutamate increases wakefulness and REM sleep with the inhibition of NREM sleep demonstrating an excitatory role. Norepinephrine is excitatory, inhibiting sleep and increasing wakefulness. In the case of bilateral injection, norepinephrine increases REM sleep, fulfilling an exhibitory role. Serotonin is unusual as it increases wakefulness and decreases sleep. It could most likely be defined as excitatory.

Combining the nightmare model and the basic knowledge of neurotransmitters, a very logical conclusion arises. It is common knowledge that drugs affect neurotransmitter uptake, breakdown and concentration. For instance, L-dopa, a drug used in the treatment of symptoms for patients with Parkinson's disease is converted to dopamine (MedicineNet.com, 2004), Motrin prevents enzymes from working (Cerner Multin Inc, 2009b) and Klonopin increases the effects of GABA (Cerner Multin Inc, 2009a). It therefore stands to reason that drugs cause nightmares by effecting the neurotransmitter concentrations. The imbalance disturbs the nightmare model, inducing fear-filled dreams which we call nightmares.

This stands to explain why most drugs reported to cause nightmares are psychiatric drugs, for they affect the brain directly. Additionally, it is also understood why specific people report seeing nightmares. The normal pharmaceutically significant idiosyncratic effects of drugs (in other words the normal uncertainties of drug use) show their hand. Some people may not have such an interaction with the drug, while others may have too low of an M value (as per the AIM model as described above). There are many uncertainties with using drugs. Each one may contribute towards experiencing or not experiencing a drug-induced nightmare.

This hypothesis also serves to explain the post treatment nightmares. Drug tolerance, via the normal drug pharmacology, imitates the effects of neurotransmitters, producing the same re-

sults as an imbalance. The same idiosyncratic uncertainties which occur during drug treatment occur again. However, as the time since the last drug application increases, the neurons lose the tolerance, reducing the tolerance-caused imbalance. This would imply that as the time lengthens, the drug-induced nightmares would occur less frequently or with less fear experienced when a nightmare does come.

As implied in the paragraph above, this proposal should reveal itself to testing. Although dream and nightmare testing is fraught with faults as the patients themselves may color and disturb the actual events, the test would not involve specific details which could be susceptible to confusion. Such a test would involve asking volunteers to record nightmare occurrences following the termination of drug application. A high occurrence asymptotically approaching the normal nightmare levels would indicate that indeed, post treatment drug nightmares are being caused by the reducing tolerance. The implication of the statement would be that the same effect would be present during the actual drug application period.

CONCLUSION

The reported effects of Chantix and other drugs lay out rather bluntly that a drug interaction which causes nightmares and disturbed dreaming does exist. Dreaming, as explained above, is produced by the activation and attraction of similar scene contexts. Activated dream contexts rise into the dream sequence being experienced. If the activated scene contexts contain fear-causing elements, the brain subconsciously attempts to disarm these fear elements. At times, this mechanism can be faulty, resulting in a reinforcement of more fearful memory or a memory which incorporates distress elements as well. These distress elements, when remembered by day cause the distress that they activate possibly causing a nightmare cycle. The faulty mechanism could possibly be blamed on an imbalance of neurotransmitters. Such an imbalance could also be formed by the neuropharmaceutical effects of drugs, displaying itself as drug-induced nightmares. Naturally forming drug tolerances imitate the effects of a neurotransmitter imbalance as proposed above. This is the hypothesis presented in this paper and may be the mechanism for the production of drug-induced nightmares. Further testing would be necessary although empirically this hypothesis fits soundly. With this knowledge in hand, doctors and pharmacists can devise drug combinations which would reduce the causes of drug-induced nightmares thus making drug application all that much easier on the patient and hopefully lead to an easier and more complete recovery.

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The Effects Of Smoking On Pregnant Women

MEIR COHEN

ABSTRACT

Smoking during pregnancy is accepted as the most significant avoidable risk factor for an unsuccessful pregnancy result. Smoking is linked with fetal growth restriction, and increasing evidence also suggests that smoking can cause stillbirth, preterm birth, placental abruption, and possibly sudden infant death syndrome. Smoking during pregnancy is also associated with enhanced risks of spontaneous abortions, ectopic pregnancies, and placenta previa, and it might increase risks of behavioral disorders in childhood. Studies have shown with randomized controlled trials, that smoking intervention during pregnancy has had limited success. Smoking during pregnancy continues to be an important risk factor for maternal and fetal outcomes during pregnancy.

INTRODUCTION

The earliest cigarettes were mostly impossible to tell apart from their predecessor, the cigar. Cigarette smoking first began in Central America around the 9th century in the form of reeds and smoking tubes. The Aztec and the Maya tribes smoked tobacco and various drugs in religious rituals and regularly depicted priests and deities smoking on pottery and temple engravings. The cigarette and the cigar were the most common methods of smoking in the Caribbean, Mexico and Central and South America until recent times. In the South and Central Americas, plantation workers used various plant wrappers to wrap their tobacco in order to smoke it. In Spain, maize wrappers were introduced, which evolutionized into fine paper. The resulting product was called papelate. In France this product became known as the cigarette. British soldiers made smoking popular in the English-speaking world, after they saw Russian and Turkish soldiers use it. They brought this habit back home to Britain. It became a habit in Britain and later in the United States.

Cigarette smoking is the leading avoidable cause of death in many countries. Lung cancer is now a more frequent cause of death among U.S. women than breast cancer (U.S. Department of Health and Human Services [USDHHS], 2001b). The high occurrence of smoking among young women highlights not only smoking-related risks of adverse pregnancy outcomes, such as spontaneous abortions, stillbirth, preterm birth, and fetal growth restriction (USDHHS, 2001b), but also possible smoking-related long-term effects on infants, including neurodevelopmental disorders (Fergusson, et al, 1998) and cancers (Schwartzbaum, et al,1991). Usually, pregnant women are concerned about fetal well-being, so a good time to stop smoking would be before attempting pregnancy. Smoking termination should diminish the mothers' pregnancy risks (infertility, spontaneous abortion, ectopic pregnancy, and placental disorders) and also the long-term risks of developing lethal diseases such as cancers and cardiovascular diseases that are caused by smoking.

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Time trends in smoking among women

Starting in 1935, all of the yearly estimates of smoking popularity in the United States were only made for commercial purposes. Among females, daily smoking increased steadily, from 20% in 1935 to around 50% in 1965 (USDHHS, 2001b). The increase was especially prominent during and after World War II.

The increase in smoking popularity among women is most likely due to a couple of reasons. First, smoking popularity among women increased significantly in the younger birth cohorts. For example, in the birth cohort of white women born in 1900–1904, around 10% smoked at age 25, while among women born in 1920–1924, 40% smoked at age 25 years (USDHHS, 2001b). During WWII there were more women in the workplace than there ever was before. They were needed to help out due to all the men who were out at war. This definitely contributed the increase of women smoking. Second, it appears that the tobacco industry's successful promotion of smoking as a fashionable thing to do, during and after World War II, influenced all age groups (Brandt, 1996; Ernster, 1985). A good example of this would be the successful marketing of the Marlboro Man. Therefore, in the 1900–1904 birth cohort, the prevalence of smoking continued to increase until age 50 years, when more than 20% of white women smoked, while in the 1920–1924 birth cohort, smoking prevalence peaked at around age 35 years, when close to 50% of all white women smoked (USDHHS, 2001b). This increase occurred among African-American women as well but it was less prominent than among the white women. Among Hispanics, the smoking prevalence rates were not as high and there was no real difference between birth cohorts (USDHHS, 2001b). Another big factor of the female smoking increase came through the cinema. Many actors and actresses smoked in the movies that they acted in.

After 1965, the National Health Interview Survey has provided yearly estimates of smoking prevalence in the United States among Whites and Blacks. In 1979 they started to give smoking prevalence rates for Hispanics, Asians or Pacific Islanders, Native Americans, and Alaskan natives as well. Since the sixties, the prevalence of smokers among women aged 18 years or older has been fairly constant at around 40% (USDHHS, 2001b).

However, the rate of current smoking altogether has dropped from 34% in 1965 (USDHHS, 2001b) to 21% in 2000 (Trosclair, et al, 2002). This drop is observed in most ethnic groups, including non-Hispanic Whites, Hispanics, non- Hispanic Blacks, and Asians or Pacific Islanders. Meanwhile, smoking prevalence has not decreased among Native Americans and Alaskan natives.

The decrease in smoking prevalence during recent decades is especially noticeable among women in their reproductive years. In 1965, 38% of women aged 18–24 years smoked, as did 44% of women aged 25–44 years. In 2000, corresponding figures were 25% and 23%, respectively (USDHHS, 2002). However, during the past decade, the reduction in smoking prevalence among young women in the United States appears to have leveled off.

Since the mid-1960s, the popularity of smoking has dropped in the United States in all educational groups. The decline has been most distinct among highly educated women. Among women who held a bachelor's degree or higher, 26% smoked in 1974. In 2000 however, only 10% smoked (USDHHS, 2002). The smallest drop in smoking was among women with only a high school education. In 1974 32% smoked, while 27% smoked in 2000. Smoking is also popular among women below the poverty line, compared with those at or above it (USDHHS, 2002).

Most smokers now quit smoking. In the United States, the percentage of ex-smokers among adult females (18 years or older) doubled from 20% in 1965 to 40% in 1998 (USDHHS, 2001b). There are a few possible reasons for the decrease. Society has been educated in the health issues

that smoking causes. The many preventive measures against smoking seem to have worked. Some of these measures include prohibiting indoor tobacco use at work or in restaurants, advertisements, age limits for buying cigarettes, and higher tobacco taxes (Wakefield and Chaloupka, 2000).

More than 80% of current smokers started smoking before age 18 years (USDHHS, 2001b). Also, smokers who start to smoke early are less likely to quite later. In the United States, current smoking among high school seniors decreased from 39% in 1976 to around 33% in 1998 (USDHHS, 2001b).

Smoking behavior during pregnancy

Estimates of smoking during pregnancy are usually dependent on self-reported information. Substantiation of this information is done with biochemical markers such as cotinine. These biochemical markers have also shown that pregnant women may conceal their smoking (Ford, et al, 1997; Klebanoff, et al, 1998). Therefore, self-reported information of smoking during pregnancy is probably underestimated. Since disapproving attitudes toward smoking during pregnancy are greater , the legitimacy of self-reported smoking information may be a concern.

A Swedish study investigated the connection between self-reported smoking in early pregnancy and the risk of small-for-gestational-age (SGA) births in the 1980s and early 1990s (Cnattingius, 1997). The frequency of self-reported smoking in early pregnancy declined from 30% in 1983–1985 to 24% in 1990–1992. The risks of a SGA birth were almost identical for pregnancies in 1983–1985 compared with 1989–1992. There were also no differences found in risks connected to moderate (1–9 cigarettes per day) or heavy (10 or more cigarettes per day) smoking between these time periods.

In the United States, the National Natality Survey reported that 40% of white pregnant women smoked in 1967, compared with only 25% in 1980, while corresponding figures among black women were 33% and 23% (Kleinman and Kopstein, 1987). Since 1989, the information on the prevalence of smoking during pregnancy is accessible from the U.S. Standard Certificate of Live Birth. Birth certificate data of live births show that in 1989 close to 20% of American pregnant women smoked. Smoking declined since then, and in 2000 only 12% smoked (USDHHS, 2002).

The decline in smoking during pregnancy is probably mostly due to a decrease in women smoking in general as opposed to an improvement of mothers quitting before or during pregnancy (Cnattingius, 1997; USDHHS, 2001b, Wisborg, et al, 1996).

Smoking during pregnancy is highly affected by the education given to the mothers. For example, in the United States, only 2% of college-educated women reported smoking during pregnancy in 2000, while 25% of women who attended but did not complete college smoked (Martin, et al, 2002). Smoking during pregnancy is most common among Native Americans and Alaskan natives (20% in 2000); also, 16% of non- Hispanic Whites, 9% of non-Hispanic Blacks, and 4% of Hispanics smoke (Martin, 2002). Heavy smoking during pregnancy has become less common in the United States (Cnattingius, 1997; USDHHS, 2001b).

Smoking cessation among pregnant women

Almost all pregnant women who stop smoking do so because of concerns about fetal and infant health. Observation studies reported that 20%–40% of smokers quit during pregnancy (Cnattingius, et al, 1992; Fingerhut, et al, 1990; Wisborg et al. 1996). Of those who stop smoking, the majority do so in early pregnancy (Cnattingius, 1992; Fingerhut 1990; Wisborg et al., 1996).

The risk for continued smoking during pregnancy is greater in women who have had previous pregnancies than women who are in their first pregnancy. Women who started smoking at a young age, heavy smokers, and women exposed to second hand smoke are also more likely to continue to smoke during pregnancy (Cnattingius, USDHHS, 2001a, Wisborg et al., 1996). Although the connection between smoking and cancer and cardiovascular diseases is well known, most women who quit smoking during pregnancy usually resume smoking within 6 months after giving birth (USDHHS, 2001b).

It has been suggested that pregnancy may be an ideal time for smoking intervention. Pregnant women are concerned about fetal well-being, and pregnant women also repeatedly visit prenatal care clinics during pregnancy. This can help end the mothers' smoking addiction habits.

In an intervention trial done in Holland, pregnant women who smoked at least 10 cigarettes per day were randomized to receive a nicotine patch or a placebo patch. Patches were used for 11 weeks, and smoking habits were evaluated using salivary cotinine. Observance was low in both groups, and the authors found no differences in cotinine values between the groups (Wisborg, et al, 2000A). So far, smoking intervention strategies during pregnancy have had little success, and no effective methods exist for cheap intervention in regular prenatal care.

Reproductive outcomes

Smoking has been reported to affect the ability to conceive and increases the risk of infertility, which is normally defined as the inability to conceive after 12 consecutive months of unprotected intercourse. Other risks are pregnancy termination during the first 3 months, either as a miscarriage or an ectopic pregnancy; the risk of placental complications; the risks of other harmful pregnancy outcomes, including fetal growth restriction, preterm birth, fetal or infant death, and congenital malformations; and the associations between smoking during pregnancy and subsequent risks to the child, including risks of hospitalization, behavioral problems, psychiatric diseases, and childhood cancers.

Delayed conception and infertility

Pregnancy rates over defined periods of time are lower among smokers than nonsmokers (Florack, et al, 1994; Olsen, 1991). However, conception rates among nonsmokers and former smokers appear to be comparable (Curtis, et al, 1997). In studies of women who are not very fertile and are going through in vitro fertilization treatment, smoking seems to be connected with reduced fertility as well (Hughes and Brennan, 1996). Smoking seems to have anti- estrogenic effects (Baron, et al, 1990). Ovarian stimulation studies have shown that smokers exhibit a lower peak serum estradiol levels than nonsmokers (Gustafson, et al, 1996). Nicotine and the toxic products of cigarette combustion also can interfere with the structure of a corpus luteum, tubal transportation, or implantation (Gindoff and Tidey, 1989).

Ectopic pregnancy

Approximately 10% of maternal deaths in the United States are because of ectopic pregnancies (Atrash, et al, 1986). An ectopic pregnancy, is a complication in which the pregnancy implants outside the uterine cavity. Almost all ectopic pregnancies do not work. It can cause internal bleeding which is extremely dangerous for the mother. Most ectopic pregnancies occur in the Fallopian tubes, but the implantation can also occur in other areas such as the cervix, ovaries, and abdomen. Pelvic inflammatory disease (PID) is one of the most important risk factors for ectopic

pregnancy. Cigarette smoking is known to increase the risk of PID, providing a rationale for the association of smoking and ectopic pregnancies (USDHHS, 2001b). However, there may be a greater risk of ectopic pregnancy without a connection to PID. Smoking is thought to damage the tubal transport of the ovum. This increases the risk of ectopic pregnancies in women who smoke (Phipps 1987).

Spontaneous abortions

While many studies have found a modest association between a mother smoking and the risk of spontaneous abortion, there are studies that did not show any connection at all between cigarette smoking and the risk of a miscarriage (USDHHS, 2001b). Cotinine is a metabolite of nicotine that can be assessed and measured in many human tissues. It is considered to be a more correct determinant of tobacco exposure than self-reported tobacco intake. Ness (1999) discovered that the risk of miscarriage related to smoking is considerably underestimated when using self-reports as opposed to the use of cotinine in hair samples. Smoking is not associated with any fetal anomaly where the fetus will have the wrong amount of chromosomes. Rather, the smoking-related risk of spontaneous abortion may occur mostly among spontaneous abortions with the normal fetal karyotype. This is supported in part by the findings of Kline et al. (1995).

Placental complications

Placental abruption, which is the premature separation of the placenta from the uterine wall, occurs in less than 3% of all pregnancies and is one of the most common causes of maternal and perinatal death, the latter being defined as fetal death occurring at 28 weeks or later or death within the first week of life (Kyrklund-Blomberg, et al, 2001; USDHHS, 2001b). In pregnancies with placental abruption, perinatal mortality rates range from about 10% to 25% (Kyrklund- Blomberg 2001). Smoking during pregnancy is consistantly linked with placental abruption. Smokers face a twofold-increase in the risk of placental abruption, and the risk increases with amount smoked as opposed to nonsmokers (Ananth, et al, 1999; USDHHS, 2001b). The risks of placental abruption caused from smoking can apply to both single and multiple pregnancies (Ananth, et al, 2001). Women who stop smoking during pregnancy have a lower risk of placental abruption as opposed to women who continue to smoke (Naeye, 1980). In pregnancies complicated with placental abruption, there has been a connection to smoking increasing the risk of perinatal death (Kyrklund-Blomberg , 2001).

Smoking may raise the risk of placental abruption in numerous possibleways. There are degenerative and inflammatory alterations of the placenta that are found in maternal smokers (Rasmussen, et al, 1999). Women with placental abruption also have decreased levels of blood ascorbic acid, which is important in collagen synthesis. Plasma ascorbic acid levels are lower in smokers than in nonsmokers, which can affect the placenta to separate early in smokers (Faruque,et al, 1995). Smoking also is linked with the preterm rupture of the membranes, which can increase the risk of placental abruption (Kyrklund-Blomberg, 2001).

Placenta previa, which is defined as a placenta that covers the internal cervical os, occurs in around 5 per 1,000 deliveries (Iyasu et al., 1993). Women with pregnancies complicated by placenta previa are at increased risk for severe vaginal bleedings before or during delivery, preterm birth, and maternal death. The risk of perinatal death is increased as well. Cigarette smoking is connected with placenta previa. A smoker's level of carbon monoxide is increased at the expense of oxygen. Therefore, a smoker will have less oxygen attached to hemoglobin in the blood than nonsmokers.

Smoking-induced chronic hypoxemia has been suggested to result in placental enlargement, which increases the risk that the placenta reaches the cervical os.

Pregnancy-induced hypertensive diseases

Preeclampsia is defined as pregnancy-induced hypertension combined with proteinuria. If preeclampsia is complicated with seizures, the condition is called eclampsia. Preeclampsia is the leading cause of maternal mortality in the United States (Berg, et al, 1996) and can cause reduced fetal growth, placental abruption, and perinatal death (Cnattingius, 1997). One surprising and not very well understood findings is that smoking is inversely related to risk of preeclampsia, and most studies find no dose-response relationship between amount smoked and reduction in risk (USDHHS, 2001b). Compared with preeclampsia, gestational hypertension (pregnancy-induced nonproteinuric hypertension) is generally associated with lower maternal and fetal risks (Naeye, 1981; Seshadri and Venkataraman, 1997). It is not really known if smoking can also reduce the risk of gestational hypertension. Most, but not all, studies find a moderate reduction in the risk of gestational hypertension associated with cigarette smoking (Ros, et al, 1998).

McGillivray (1983) addresses the lowered risk of preeclampsia and/or gestational hypertension for smokers than for nonsmokers. He writes that there would be less extension of plasma volume in smokers than in nonsmokers. Cigarette smoke also includes thiocyanate, which has hypotensive effects (Klonoff, et al, 1993). Moreover, nicotine can restrain the production of thromboxane, which is a potent vasoconstrictor and a platelet aggregation stimulator (Ylikorkala, et al, 1985).

While smoking may reduce the risk of preeclampsia, once a smoker does develop preeclampsia, smoking may exacerbate the ill effects of the disease. Preeclampsia also may lead to a reduction in oxygen levels in placental tissue (placental hypoxia). In preeclamptic pregnancies, vasoconstriction leads to increased arterial blood pressure and uteroplacental resistance, which may cause placental hypoxia (Harris, 1988). If smokers develop preeclampsia, the placental hypoxia may be especially pronounced, leading to substantially increased risks of fetal hypoxia, placental abruption, and fetal death.

Fetal growth restriction

Simpson and Linda (1957) reported many years ago that infants born to mothers who smoked 10 cigarettes or more per day weighed 200 grams less than infants born to nonsmoking mothers. Recently, there have been studies that showed the smoking-related reduction in birth weight is caused primarily by fetal growth restriction (Kramer, 1987). We now know that there is a connection between smoking and fetal growth restriction. This connection between smoking and SGA births has been proven time and time again. The literature on this is solid. It is also proven that the risk of SGA births rises with the amount of cigarettes smoked. It has also been shown that when the mother stops smoking the fetal growth improves. Nordstrom and Cnattingius (1994) watched mothers' smoking habits in two births each. They noticed that for women who smoked during their first pregnancy and then stopped smoking during their second pregnancy, the second birth weight was similar to the women who never smoked. This was noticed as well in mothers who quit smoking early in the pregnancy. The birth weight of the baby in the mother who quit early was similar to that for the mothers who never smoked. (MacArthur and Knox, 1988). There are other factors that can combine with the effects of smoking to affect the fetal growth. For instance,

when a mother is older, smoking can affect the fetal growth and cause a SGA birth more than it would in a younger mother (Cnattingius, 1989; Fox, et al, 1994, Wen, 1990).

It is not known what causes the smaller birth weight. It is known that nicotine can cause contraction of the placental blood vessels (Suzuki et al., 1971). It is also known that carbon monoxide binds to fetal hemoglobin, which would result in fetal hypoxemia (Lambers and Clark, 1996). The mother's weight gain during pregnancy is also linked with fetal growth. Nonsmoking mothers gain more weight during pregnancy than mothers who do smoke. However, even though nonsmokers gain more weight than smoking mothers, smoking while pregnant will raise the risk of an SGA birth (Spinillo et al., 1994).

Preterm birth

Preterm birth is defined as a birth occurring at least 4 weeks before the estimated date of delivery. The major cause of neonatal mortality and morbidity is when the baby is born early. However, because of improvements in neonatal care, most deaths usually occur when the baby is born very early i.e. at 32 weeks (Berkowitz and Papiernik, 1993). There have been intervention programs, which try to prevent preterm births. These programs have not been successful (Goldenberg and Andrews, 1996). Smoking prevention might be a way to help lower preterm births.

Smoking has been linked to preterm birth and if there is less smoking it can help lower preterm birth (Chan, et al, 2001, Zeitlin, et al, 2001). Smoking is associated with the increased risk of intrauterine infection. It has also been shown that the mother's immunity is impaired when there is smoking (Naeye, 1978). Smoking can also increase the risk of preterm labor through greater production of prostaglandins in fetal membranes (Hoffman, et al, 1990).

Preterm premature rupture of membranes is when the amniotic fluid leaks before 37 weeks of gestation. This is the second most common cause of preterm delivery (Spinillo, Nicola, 1994). Smoking has consistently been associated with preterm premature rupture of membranes (Lee and Silver, 2001). Smoking reduces serum copper and ascorbic acid in blood plasma. These are important for collagen synthesis and maintenance (Hadley et al., 1990). A lack of serum copper and ascorbic acid might result in reduced elastic properties of the fetal membranes, but the smoking-related increased weakness to infections also may increase the risk of preterm premature rupture of membranes (Holt, 1987). The smoking-related risk of elective preterm delivery is mediated largely by other smoking- related risks. As stated previously, smoking increases the risk of placental abruption, placenta previa, and fetal growth disturbances, conditions that may require elective preterm delivery.

Although the smoking-related risk of preterm birth is relatively small, there are consistent findings that smoking might be associated with preterm birth. Also, women who stop smoking between pregnancies reduce the risk of preterm birth in the next pregnancy, to a point that it is comparable to that of nonsmokers in both pregnancies (Cnattingius, et al, 1999).

Perinatal mortality

Perinatal mortality is when there is a fetal death at 28 weeks or later in gestation. Smoking is consistently linked with an increased risk of perinatal mortality. There is an interesting twist where babies of smokers have a lower perinatal mortality rate than babies born to nonsmokers when the birthweight is less than 3 kilograms. However, the risk is switched in babies that are heavier than 3 kg. When looking at the overall picture, infants born to smokers have a greater risk of perinatal mortality in overall relative birth weights (Wilcox, 1993). There are two possible ways

that smoking affects perinatal mortality. The first is due to the fetal growth restriction and the other is that smokers have a greater risk of delivering very small preterm infants.

Stillbirth

Stillbirth is officially defined as fetal death at 20 gestational weeks or later. However, over 80% of stillbirths happen during the preterm period. This is between 20 and 36 weeks (Copper, et al, 1994). Even when stillbirth is considered as a fetal death at 28 weeks or later, the majority of stillbirths are preterm (Stephansson, et al, 2001). Stillbirth is relatively uncommon, and when defined as fetal death at 28 weeks or later, rates range from 3 to 8 per 1,000 in Europe and North America (Andersen, 2001).

Smoking has consistently been associated with the risk of stillbirth. A study found that smoking was foremost associated with risk of unexplained stillbirth (Froen et al., 2001). Although the smoking related risk of stillbirth is modest, most studies find that the risk increases with amount smoked (USDHHS, 2001b). Smoking appears to influence the risk of primarily preterm stillbirth (Stephansson, 2001), and the proportion of growth-retarded stillbirths also is larger among preterm compared with term stillbirths (Gardosi et al., 1998).

The smoking-related fetal hypoxemia and the increase in vascular resistance may explain the connection between smoking and reduced fetal growth and also may be part of the increased risk of placental abruption (Kramer, 1987; Kramer, et al, 1997). Another study also showed that the elevated risk of stillbirth in smokers was due mostly to high rates of placental abruption and placenta previa (Meyer and Tonascia, 1977). Likewise, in Sweden, a study showed that smoking was associated with a 40% increased risk of stillbirth, and this risk was shown due to the smoking-related risks of fetal growth restriction and placental complications only (Raymond, et al, 1994). Another study found that women who stopped smoking in early pregnancy reduced the risk of stillbirth to that of nonsmokers (Wisborg, et al, 2001).

Neonatal mortality

Smoking has been linked with the increased risk of early neonatal mortality. This is when a baby is born and then dies during the first four week of life. (USDHHS, 2001b). Meyer and Tonascia (1977) found that the increased risk of neonatal mortality coming from a mother smoking comes from the increased risk of an early delivery.

Congenital malformations

There is no conclusive evidence that smoking can cause congenital malformation. Most studies have not found a link between maternal smoking during pregnancy and the overall risk of congenital malformation (Malloy, et al, 1989; Shiono, et al, 1986, Van den Eeden, et al, 1990). There has been research in regard to maternal smoking associated with the risk of oral face clefts. The risk of oral face clefts in a baby is slightly higher when the mother smokes. However, the evidence is not strong enough. This was observed with both cleft lip with and without palate. However, the evidence that maternal smoking is linked with cleft lip or with any other congenital malformity such as missing limbs and cardiac defects are inconclusive (USDHHS, 2001b).

SIDS and childhood morbidity

Sudden Infant Death Syndrome or SIDS, is when the death of an infant is sudden and the cause of the death remains unknown after a postmortem examination. The diagnosis of SIDS

is usually restricted to unexpected deaths among infants aged 4 weeks to 1 year. Over the past ten years the rate of SIDS has declined in most modern countries. This decline has mostly been attributes to the change from prone to back sleeping position (Mitchell, et al, 1999, Willinger, et al, 1994). It appears that the prevalence of SIDS continues to decline. In the United States, SIDS prevalence rates fell by 7% from 1999 to 2000 (Martin, 2002; Mathews, et al, 2002).

Even though the rates of SIDS is declining, it remains the most common cause of post neonatal death in developed countries, and most SIDS deaths occur at 2–3 months of life (USDHHS, 2001b). In the United States, SIDS rates vary considerably by ethnicity. For example, in 2000, SIDS rates per 100,000 live births were 29.4 among Asians and Pacific Islanders, 51.8 among Whites, 120.0 among Native Americans, and 122.1 among Blacks (Mathews et al., 2002). When compared with U.S. Whites, U.S. Blacks and Native Americans face a twofold increased risk of SIDS, while the corresponding risk among Asians and Pacific Islanders is reduced substantially. The reasons for these differences are not fully understood, but lifestyle factors may play a role (MacDorman, et al, 1997).

Although the decline in SIDS rates during the 1990s has been attributed to change in infants' sleeping positions, the decline also coincided in many countries with declining rates of cigarette smoking during pregnancy. Smoking during pregnancy has been associated with SIDS, and dose-response relationships have been reported in studies from different populations (MacDorman, 1997; Murphy, et al, 1982). The risk of SIDS among infants of daily smokers is commonly doubled or tripled, when compared with non- smokers, and sometimes more than tripled risks have been reported (Malloy, et al, 1992).

The smoking related risk of SIDS might be explained by examining the other differences between smokers and nonsmokers. However, many studies have shown that the association between smoking and SIDS has remained essentially the same even when measures of socioeconomic status, education, or alcohol were included (Nordstro¨m, et al, 1993; Wisborg, Kesmodel, 2000b), However, Malloy, Kleinman, Land, and Schramm (1988) found that the smoking-related risk of SIDS was reduced after evaluating other lifestyle differences and factors.

A relationship between smoking and risk of SIDS is observed consistently across various study designs and populations. However, because most women who smoke during pregnancy continue to smoke after birth, it has been debated whether the smoking-related risk of SIDS is caused by either prenatal or postnatal smoking exposure or both. Anderson and Cook (1997) performed a systematic review of the connections between maternal prenatal and postnatal smoking and risk of SIDS. In this review there was no conclusive evidence shown. Sixteen studies were performed and four studies reported on postnatal smoking after controlling for prenatal smoking. There were three studies that reported that maternal postnatal smoking increased the risk of SIDS independent of prenatal smoking (Klonoff- Cohen, 1995; Mitchell et al., 1993; Ponsonby, et al, 1995; Schoendorf and Kiely, 1992), and one study found that the association between postnatal smoking and SIDS was not significant (Blair et al., 1996). Mitchell et al. (1997) reported that the risk of SIDS was increased among infants born to mothers who were smokers and also shared a bed with their infants, while bed sharing among non- smokers did not influence risk of SIDS. Similar results have been reported by Fleming et al., (1996), although a recently published U.S. study found no evidence of an interaction between smoking status and bed sharing with regard to risk of SIDS (Hauck et al., 2003).

Dwyer et al. (1999) performed a study of close to 10,000 infants in a high-risk population, of whom 53 infants died of SIDS. They found that the cotinine level in the infants' urine was re-

duced by half if smoking mothers did not smoke in the infants' room, which indicates that smoking in an infant's bedroom does influence the infant's exposure to passive smoke. However, this finding was not accompanied by a reduced risk of SIDS. In a study of findings at autopsy, cotinine concentrations in pericardial fluid were analyzed in 67 infants who died from SIDS. About 25% of the SIDS infants had cotinine concentrations exceeding 30 ng/ml indicating tobacco exposure prior to death (Rajs et al, 1997). Thus, the evidence to date is that both prenatal and postnatal smoking exposure influence risk of SIDS (Anderson and Cook, 1997; Fleming 1996).

Risks of hospitalization and respiratory disorders during infancy and childhood

During the first years of life, children whose mothers smoked during pregnancy have an increased risk of hospitalization (Harlap and Davies, 1974; Rantakallio, 1978; Taylor and Wadsworth, 1987; Weitzman, et al, 1990; Wisborg, et al, 1999). This is because the smoking is associated with a high risk of respiratory sicknesses (Harlap and Davies, 1974; Taylor and Wadsworth, 1987; Wisborg, 1999). A study has shown that the maternal smoking can increase the risk of childhood asthma. (Weitzman et al., 1990). It has also been shown that smoking can increase the risk of hospitalization from gastrointestinal or dermatologic symptoms. However, it is unknown if these symptoms are from smoking exposure before or after the birth of the child (Wisborg et al., 1999).

Behavioral diseases in childhood

Maternal smoking during pregnancy and fetal growth retardation have been associated with many childhood behavior disorders. For example, it was found that compared with infants born to non-smokers, infants born to smokers faced a much greater risk of attention-deficit/hyperactivity disorder (ADHD), and this positive association remained significant after adjusting for socioeconomic status, parental IQ, and parental ADHD status (Milberger, 1996). In another study, children exposed to maternal smoking in utero had higher psychiatric symptoms rates for conduct disorder, alcohol abuse, substance abuse, and depression (Fergusson et al., 1998). Although smoking during pregnancy was associated with other factors, including socioeconomic disadvantage, impaired childrearing behaviors, and family problems, the smoking-related risks of adverse outcomes is still statistically significant after adjustments for these factors. These effects of maternal smoking were more pronounced for male than for female adolescents (Fergusson et al., 1998).

Autistic disorders in children are manifested by impaired social interactions, communication deviance, and stereotypical behavioral patterns. The cause of these disorders is thought to be largely genetically determined (Bailey, 1995), but there have been studies that showed children with autism to have an increased frequency of pre- and perinatal complications (Burd, et al, 1999). In one study, it was found that SGA infants had an almost tripled risk of having autism (Hultman, et al, 2002). However, when smoking was included in multivariate analysis, SGA infants faced a doubled risk of developing infantile autism, and compared with infants born to nonsmokers, those born to women who were daily smokers during pregnancy faced a much higher risk of developing autism (Hultman, 2002). Although the results from this study suggest that smoking and fetal growth might influence the risk of autism, these findings require confirmation.

Childhood cancers

Tobacco-specific carcinogenics, such as benzene and nitrosamines, probably pass through the placental barrier to the fetus (Norman, et al, 1996). Animal experiments support the hypothesis that tobacco smoke exposure during pregnancy may increase an offspring's risk of developing

tumors and hyperplasias (Pershagen, 1989). Animal experiments have shown that nitrosamine exposure during pregnancy may cause cancer in the central nervous system among offspring (Rice and Ward, 1982).

In four studies on risk of childhood cancer, information about smoking during pregnancy was collected before onset of disease (Golding, et al, 1990; Klebanoff, et al, 1996; Neutel and Buck, 1971; Pershagen, et al, 1992). Only one of these studies, including 33 cases, found that maternal smoking was associated with an increased risk of childhood cancer (Golding et al., 1990).

Results from case-control studies of smoking during pregnancy and overall risk of childhood cancer contradict each other. In a case-control study, which had over 500 cases, McKinney and Stiller (1986) found no connection between maternal smoking and childhood cancer risk. John, Savitz, and Sandler (1991) examined over 300 cases and found that smoking during pregnancy was connected with a 30% increased risk of childhood cancer.

Schwartzbaum (1991) reported a positive association between smoking during pregnancy and the risk of childhood cancer in a study that had 1,270 cases.

Because tobacco smoke includes leukogenic substances, maternal smoking during pregnancy has been studied mostly with regard to risk of leukemia and other cancers in blood and lymphatic tissue. Most studies have not found that smoking is connected with increased risk of childhood lymphatic leukemia (Tredaniel, et al, 1994). Results from studies on maternal smoking and myeloid leukemia are inconclusive. However this is probably due to the low number of included cases (Cnattingius, 1995; Shu et al., 1988).

Similarly, the association between maternal smoking and risk of childhood non-Hodgkin's lymphoma needs to be addressed in larger studies (Adami, 1996).

Most studies have not found a connection between maternal smoking and risk of childhood brain cancer (Linet, 1996; Norman, 1996).

CONCLUSION

The most well known effect of smoking with pregnant women is fetal growth restriction. However, there has been a lot of literature showing that maternal smoking is related to the risks of preterm birth, stillbirth, and placental abruption. Increased risks have been found in most studies. It also has been shown that the risks increase the more one smokes. For example, if a pregnant woman smokes half a pack a day (approximately 10 cigarettes) the risks will not be as great as those of a woman who smokes two packs or more a day (twenty or more cigarettes). It has also been suggested that babies who have mothers who smoke have a greater chance of dying from SIDS. However it is unknown whether the increased risks are from smoking before or after the baby is born (Anderson and Cook, 1997; Dwyer et al., 1999). It is possible that both can attribute to the increased risks. Many studies have shown that there is a definite connection between smoking and fertility problems, ectopic pregnancies, and placenta previa. There are some suggestions that smoking during pregnancy can cause changes in the way the future child behaves and it has even been suggested that maternal smoking can cause an increase in ADHD and other behavior disorders. However, I have not found enough literature to definitely say that this is the case.

Smoking in the United States has been on the decline for a long time now. This is the case as well in other industrialized countries. However, in many other countries smoking is becoming more and more popular among young women. This is similar to what happened to the United States in the fifties. In these countries, sixty years ago it was rare to see women in the work force.

Now it is a common sight to see young women in the work force. It is important that they receive proper education about the risks of smoking. Smoking is still one of the most important preventable risk factors for unsuccessful pregnancy outcomes.

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