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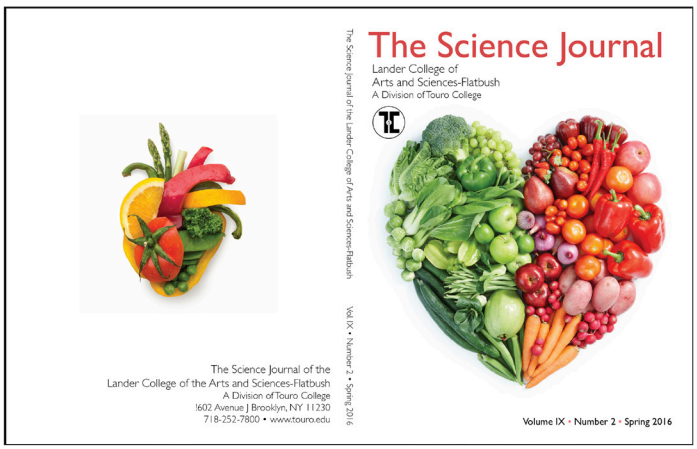

Cover pictures: These relate to the article entitled, "A Dietary Approach to Cardiovascular Disease and Cancer: Does a Plant Based Diet Help Prevent and Reverse Cardiovascular Disease and Cancer?" pages 132-139.

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Itch Mediation and How It Differs from Pain

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Abstract
Itch, to most, is a common nuisance, although when chronic it can negatively affect quality of life. It is obvious that itch is processed differently than pain, but how it differs is not clear. Researchers have been trying to find a path that specifically mediates itch. They have found that itch is mediated through at least two different pathways: histamine dependent and histamine independent. However, many of the mediators involved in the transduction of itch also mediate pain. Although some itch-specific neurons have been found, the majority of the pruritogenic neurons are also responsive to pain stimuli. Two theories that can explain how the brain processes itch and pain as different sensations are the specificity theory and the selectivity theory.

Introduction

Itch is defined as "a stimulation of free nerve endings, usually at the junction of the dermis and epidermis of the skin, that evokes a desire to scratch." (Encyclopedia Britannica) Although most assume itch to be harmless, chronic itch can cause much discomfort and affect quality of life. Pain and itch are obviously different to the one experiencing either feeling, but whether these sensations are processed differently is not so obvious. Since the late nineteenth century when pain was discovered to be a real sensation, itch was thought of as just a weaker form of pain (Von Frey cited in Handwereker, 2014). Later, scientists found certain unmyelinated C-fibres of the dorsal root ganglion that were sensitive specifically to histamine (Schmelz, et. al. 1997). Similar histamine sensitive neurons were found in lamina I of the spinal cord (Andrew, Craig, 2001). This led to the specificity theory for itch, also called the labelled line theory, which proposes that different sets of neurons exclusively mediate itch or pain. However, many of the receptors that respond to itchy stimuli are also involved in mediating pain and later studies show that most of the neurons that respond to itch also react to pain producing substances (Shmelz, et.al.2003, Simons, 2004, Davidson, et. al., 2007, Akiyama, et. al.2009, Akiyama, et.al. 2010). So although itch and pain have separate pathways, a lot of overlap seems to exist between them.

Methods

This review was written through the critical analysis of clinical research papers and peer reviewed journal articles. The papers were found in searches on Google Scholar and access was obtained through the Touro College Library.

Discussion

Researchers first thought of itch as a milder form of pain, a theory known as the intensity theory. According to this theory, pain and itch sensations are mediated by the same neurons; if the stimulus is strong, there is a pain sensation, and if the stimulus is weak, an itch sensation (quoted in Patel, Dong, 2010). The basis for this theory was that cordotomy, surgical disconnection of the spinothalamic tract, leads to loss of both itch and pain on the contralateral side of the cut, whereas the perception of touch is impaired on the ipsilateral side (Nathan, 1990). This led to the theory that itch and pain are conducted along the same ascending pathway whereas mechanosensation travels along a separate path (quoted in Handwerker, 2014). However, many well-known characteristics of itch and pain don't seem to fit with this theory. For one, itch can be quite intense and never turn into pain, (Tuckett, 1982) and similarly, a painful stimulus does not feel like itch when administered at a lower intensity (Ochoa, Torebjork, 1989). More, the reflexes in response to pain and itch are quite different. The pain reflex is withdrawal, whereas the itch reflex is scratching. These observations seem to suggest that pain and itch have separate neural pathways.

One of the first steps in the advancement of itch research was the discovery of histamine. Histamine is released from mast cells and white blood cells, as a response to allergens or inflammatory mediators. A "triple response" to histamine was found. There is a local reaction, swelling, and also erythema, a flare, skin reddening around the affected skin (quoted in Handwerker, 2014). Histamine could be applied to specific regions and therefore allowed for the identification of the neurons which responded to it.

The breakthrough came in 1997, when using the new computer-assisted marking technique in microneurography, one group was able to identify histamine-sensitive C-fibers. Previous research had shown that itch had sometimes been induced during microstimulation in skin nerves where C-fibers were recorded. But most C-fibers identified were insensitive or only weakly responsive to histamine. The new technique was able to identify even small units that could not be identified with the old methods. Researchers identified 56 fibers in the superficial peroneal nerve of 52 healthy patients. Units were classified as mechanically and heat-responsive (CMH), heat-responsive (CH), or unresponsive to mechanical and heat stimulation (CMiHi). All of the fibers were tested by iontophoresis of histamine and the subjects rated the itch feeling. After the application of histamine, itch was usually felt 30-60 seconds after, reached a maximum after 2-3 minutes, and lasted for about 10 minutes. Twenty-three of the fifty-six units studied were not at all excited by the histamine. Twenty were weakly activated but their discharges did not match up with the time response of the subjects. Eight of the units (5 CH and 3 CMiHi) showed lasting responses to the histamine that coordinated with the time response of the

subjects. These neurons were found to have a much slower conduction velocity than the non- histamine responsive neurons, suggesting that these neurons are a class onto themselves. The units with histamine responses had a mean velocity of .52m/sec., compared to the .9m/sec. of the other units. The slow velocity reflects small axon diameters. Additionally, the units were found to have unusually large innervation territories. This finding explains the flare that is found after application of histamine (Schmelz, et. al. 1997).

The next study that supported the idea of an itch-specific pathway was done on cats and found a population of histamine-selective lamina I spinothalamic tract (STT) neurons. The researchers obtained recordings from 190 lamina I STT neurons that had distal hind limb fields in the lumbosacral spinal cords of 33 cats. After categorizing these neurons into three main classes- nociceptive-specific, thermoreceptive- specific, and polymodal nociceptive- based on response to natural mechanical and thermal stimuli, there were 17 neurons left that were mechanically and thermally insensitive. Unlike the mechanically and thermally sensitive neurons, these neurons did not show spontaneous activity. These neurons were found to have a similar response time after histamine application to their innervation area of skin to the histamine selective neurons found in the previous study. The conduction velocities of these neurons were slower than the rest of the lamina I STT neurons, which is also consistent with the findings of the previously mentioned study. In addition, they all had large receptive fields, similar to the histamine selective fibers in the previous study. These characteristics that differed from the rest of the neurons seem to put these neurons into a class of their own. Some of the histamine-responsive neurons were tested with mustard oil, which causes a burning sensation, and some responded to it. Of those that responded to the mustard oil, most had responses that were much weaker than the response to histamine. The neuron that had a strong response to the mustard oil had been found to have a weaker response to histamine than the rest of the histamine-responsive neurons. Some of the histamine- responsive neurons did not respond at all to mustard oil, indicating that they are histamine-selective. (One should note, however, that the neurons were never tested with capsaicin, an algogen) (Andrew, Craig, 2001). This study and the one mentioned above both support the "specificity theory," as it seems like units were found that responded specifically to histamine.

Knowing that histamine is not the only pruritogen, a later study tested different pruritogens to see if the histamine-selective units would respond to them in accordance with pruritic potency of the pruritogen. They found that many histamine-selective units responded to the other pruritogens more than the non-histamine-selective units. However, they also found that the mechano- insensitive, histamine responsive units could also be excited by capsaicin and bradykinin, both algogens, albeit with a different response pattern than the CMH units. The mechano-responsive units had intense, short-lasting responses to capsaicin, whereas the mechano-insensitive units had longer lasting responses. This challenges the specificity theory as the itch units were activated by algogens (Shmelz,et.al.2003). Another study tested the responsiveness of STT neurons from the ventral posterior lateral nucleus of the thalamus in monkeys to different algogens and pruritogens. Neurons were found that responded to both histamine and capsaicin, another challenge to specificity, this time in the STT. (Simone, 2003)

As the pathway for itch becomes clearer and more defined, the question of how the brain processes the signals from the pathway as itch rather than pain remains uncertain.

The Histamine Dependent Pathway

Histamine binds with histamine receptors on free nerve endings. These receptors are classified into four subtypes, 1-4. In order to determine which of the four histamine receptors are responsible for the Ca2+ influx into the cell after the application of histamine, agonists of the different subtypes were applied with the histamine. The only agonist that stopped the calcium response was the histamine type-1 receptor (H1R) agonist, mepyramine (Nicolson, et.al. 2002). In pharmaceutical attempts to alleviate itch the H1R became the major target (Simons, 2004).

In the mediation of itch, H1R was found to activate phospholipase Cβ 3 (PLCβ3). PLCβ3 is expressed in neurons with histological markers for unmyelinated, C-fiber nociceptors. PLCβ3 is coexpressed with H1R in 90% of H1R neurons, suggesting that PLCβ3 may also be involved in the transduction of itch. Indeed, PLCβ3-deficient mice showed low Ca2+ release in response to histamine application, compared to the regular Ca2+ response to application of ATP, capsaicin, UTP, and bradykinin. In vivo testing showed that PLCβ3 deficient mice started scratching later and scratched less in response to histamine injection than wild-type mice. The PLCβ3 deficiency was most noticed when the H1R agonist, HTMT, was applied to the PLCβ3-deficient mice, and was compared to application of agonists of the other histamine receptors. The scratching response to HTMT was almost as low as the response to the control, saline injection. This indicates that PLCβ3 responds mainly to activity in the H1R receptor. Reduced scratching was also found in the response to

48/80, a substance that causes degranulation of mast cells and therefore the release of histamine (Han, et.al. 2006).

Activation of PLCβ3 causes increase in intracellular Ca2+ in the DRG neurons through the ion channel TRPV1. TRPV1, transient receptor potential vanilloid receptor-1, an ion channel, is a receptor that is found on free nerve endings and allows the action potential to occur by allowing Ca2+ into the neuron. Although TRPV1 was previously only known for its role in mediating pain, there were clues that it may also be involved in processing itch. For one, capsaicin, a major algogen that activates TRPV1, can cause itch when applied to the skin surface (Green, Shaffer, 1993). In addition, TRPV1 and histamine receptors are found on the same subset of neurons)Nicolson, et. al. 2002). Lastly, when high levels of capsaicin are applied to the skin, TPRV1 is desensitized and, interestingly, pruritus is also stopped (quoted in Shim, et. al. 2007). Indeed, studies show that TRPV1 has a role in mediating itch. When H1R or TRPV1 alone was transfected to HEK 293T cells, these cells did not respond to histamine. However, H1R and TRPV1 transfected together caused a large current in response to histamine application. Further, when capsazapine, a TRPV1 antagonist, was applied to the skin before histamine application, it caused smaller Ca2+ responses to histamine. In addition, TRPV1-deficient mice did not have a large increase in intracellular Ca2+ when histamine was applied, in contrast to wild-type mice who did. In vivo studies also proved TPRV1's involvement in the mediation of itch. Capsazapine given before histamine injection in mice resulted in reduced scratching to the histamine. Compared to wild-type mice, TRPV1-deficient mice also showed reduced scratching in response to histamine injection (Shim, et.al. 2007). These experiments demonstrate that TRPV1 plays a role in the transduction of itch.

The Histamine Independent Pathway

Although histamine plays a role in allergic itch, histamine is not the main pruritic mediator in most diseases of chronic itch (Klein, Clark, 1999). The use of H1R antagonists, antihistamines, are ineffective in stopping chronic itch (Twycross, et.al. 2003).

Chronic itch can be caused by the release of pruritogens from lymphocytes, mast cells, and eosinophils (Ikoma, et,al, 2006). In patients with atopic dermatitis, antihistamines did not suppress itch upon degranulation of mast cells. This suggests that there are mast cell itch mediators other than histamine (Rukweid, et.al. 2000). In addition, one of the regular characteristics of histamine induced itch, the flare, is missing in many types of itch (Ikoma, et. al. 2005). These findings point to another pathway for itch other than the mechano-insensitive C-fibres that mediate histamine induced itch.

Cowhage, the common name for the spicules that cover the tropical plant Mucuna pruriens, causes severe itch without a flare response (Johanek, et. al. 2007). It has been found in biochemical studies that mucunain, a proteinase in the cowhage, is what makes cowhage itchy (Reddy, et.al. 2008) In a study designed to test if histamine and cowhage activate the same C-fibres, all CMH units responded to cowhage, and none of the CMi, histamine responsive, units did, proving that cowhage and histamine do not activate the same fibres (Namer, et.al. 2008). Thus, cowhage has been used in many studies to understand histamine- independent itch.

Proteinases, such as mucunain, activate proteinase-activated receptors (PARs). A specific proteinase-activated receptor, PAR-2, has been identified on afferent nerve fibers (Steinhoff, et. al. 2000). PAR-2 is also activated by trypsin and tryptase. Tryptase is released by mast calls and trypsin is expressed in the skin (quoted in Shimada, et. al. 2006). The first study that found a connection between PAR-2 and itch was done on mice and found that PAR-2 played a major role in allergic dermatitis. PAR-2 deficiency led to reduced ear swelling. (Kawagoe, et.al. 2002) Patients with atopic dermatitis (AD) were found to have an increase in PAR-2 signaling, which was seen through higher levels of codeine-induced tryptase, PAR-2 expression on keratinocytes, and a greater response to PAR-2 agonist (Steinhoff, et.al. 2003). Another study found that itch was induced by PAR-2 agonist, a peptide, SLIGRL-NH2. When mice were injected with SLIGRL-NH2 they started vigorously scratching in levels much higher than when injected with saline vehicle. The application of an antihistamine, pyrilamine, did not reduce the scratching, confirming that PAR-2 is a receptor in non-histaminergic itch (Shimada, et.al. 2006).

One of the drugs used to treat malaria is chloroquine. Many patients complain about intense itch while taking chloroquine. The itch can be so intense that they stop taking the medicine. Antihistamines were ineffective in relieving the itch (quoted in Liu, et.al. 2009). Parts of the Mrgpr family (Mas related G-protein coupled receptors), a family of G protein-coupled receptors (GPCR), have been detected only on small-diameter sensory neurons in the DRG and TG. This would make them likely to be involved in the sensation of pain or itch. Itch induced by chloroquine was reduced in Mrpgr deficient mice, although the response to histamine induced itch was not significantly reduced in these mice. Further study found that the specific Mrgpr receptor for chloroquine was MrgprA3. BAM-822 was

found to excite MrgprC11 (Liu, et.al. 2009).

TRPA1, an ion channel, plays the same role in histamine- independent and chronic itch as TRPV1 plays in histamine dependent itch. That is, it allows the action potential to occur by allowing Ca2+ into the neurons. Researchers found that TRPA1 is greatly expressed in subset of TRPV1 positive neurons. Chloroquine and BAM-822 were found to activate the subset of TRPV1 neurons that also expressed TRPA1. Yet, neurons from TRPV1-deficient mice and neurons treated with capsazapine, a TRPV1 antagonist, both showed regular Ca2+ signaling in response to BAM and chloroquine. There was also no difference in action potential firing in response to chloroquine and BAM between TRPV1-deficient neurons and wild-type neurons. Thus, it is shown that the TRPV1 ion channel is not required for the mediation of BAM and chloroquine itch. However, TRPA1-deficient neurons had significantly decreased responses to both BAM and chloroquine. (As would be expected, they did have normal responses to histamine.) Further testing showed that the primary target for the MrgprA3 and MrgprC11 receptors was TRPA1. Interestingly, BAM and chloroquine have different mechanisms leading to activation of TRPA1. Thus, TRPA1 is downstream of different histamine- independent itch pathways (Wilson, et.al. 2011). Another study found TRPA1to be a vital mediator in chronic itch. Application of AEW (acetone/ether mixture with water) to the mouse cheek causes chronic itch-like symptoms in mice. It causes dry skin and increases scratching. It also causes epidermal thickening, a major symptom of chronic itch due to psoriasis in humans. Compared to wild-type mice, TRPA1-deficient mice showed significant reduction in scratching induced by AEW treatment. This is in contrast to TRPV1-deficient mice who after AEW treatment showed no decrease in scratching. Further, injection of TRPA1 inhibitor, HC-030031, into cheeck also caused reduction in scratching in AEW treated mice. These results show that TRPA1 is required in chronic itch. (Wilson, et.al. 2013).

One study used a novel approach to test if there are distinct histaminergic and non-histaminergic pathways. Instead of deleting certain receptors or neurons, this group used QX-314, a sodium channel blocker, to selectively "silence" specific receptors. When QX-314 was inserted into the mouse along with histamine, sodium currents in TRPV1 alone were blocked, thus temporarily "silencing" TRPV1. QX-314 inserted along with chloroquine had the same effect on TRPA1, silencing it. When TRPV1 was silenced, histamine induced scratching was stopped, but chloroquine induced scratching was regular, and when TRPA1 was silenced, chloroquine induced scratching was stopped. This confirmed the separate pathways for itch. (Roberson, et.al. 2013)

The above information has shown at least two DRG pathways for itch- histaminergic and non-histaminergic. A few other itch pathways were found, such as the pathway for β-alanine which is unique (Liu, et.al. 2012 b). Some other receptors have been found as well, such as toll-like receptor 7 (TLR7) and toll-like receptor 3 (TLR3). Although TLR7 knockout mice showed normal sensitivity to thermal and mechanical pain and showed normal scratching response to histamine-dependent pruritogens, scratching response to non-histaminergic pruritogens was significantly reduced (Liu, et.al. 2010). TLR3 knockout mice showed reduced scratching response to both histaminergic and non-histaminergic stimuli and TLR3 was found to be necessary in the development of chronic itch (Liu, et.al. 2012a). Another itch mediator found was the cytokine, interleukin-31 (IL-31). IL-31 is produced by T-cells and mice generated to overexpress it developed intense pruritus. IL-31 protein was fed to adult mice and within 3-4 days the mice developed severe pruritus (Dillon, et.al. 2004).

The separate histaminergic and non-histaminergic pathways continue into the spinal cord. In a study done on monkeys, 57 dorsal horn, STT neurons were tested for responsiveness to histamine and cowhage. Nineteen responded either to histamine or cowhage; none responded to both. This shows a continuation of the separate pathways. Interestingly, all pruritogen-responsive neurons also responded to capsaicin, further evidence against the specificity theory. As pointed out above, the study done on STT neurons that suggested the existence of histamine-specific neurons did not test for response to capsaicin, thus lacking evidence that those neurons did not respond to pain stimuli (Davidson, et. al., 2007).

Just like overlap was found in the neurons mediating pain and itch in the dorsal root ganglia, overlap was also found in the trigeminal ganglia (TG) and the trigeminal subnucleus caudalis of mice. Mice TG neurons were tested with different pruritogens, i.e. histamine, PAR-2 agonist, and 5-HT, and algogens, i.e. capsaicin and AITC. Using calcium imaging it was found that of 856 TG neurons, 15.4% responded to histamine and 5.8% to PAR-2 agonist. Although a small percentage of the pruritogenic neurons only responded to one pruritogen, the majority of those pruritogenic neurons responded to AITC or capsaicin as well, another finding that is inconsistent with the specificity theory. Consistent with the findings of different histaminergic and non-histaminergic pathways, most pruritogenic neurons were responsive to only one of the pruritogens. The majority of TG neurons that responded to pruritic stimuli were also responsive to algogens (Akiyama, et. al. 2010). Another study found neurons in the superficial dorsal horn that were responsive to PAR-2 agonist and 5-HT, yet most were also responsive to algogens (Akiyama, et.al. 2009).

As can be seen from the information presented above, many of the receptors in the itch pathways are also involved in the transduction of pain. TRPV1, PAR-2, TRPA1, and the TLRs all play roles in mediating pain. It has also been shown that algogens can activate pruritogenic neurons. It seems that there is no itch-specificity. However, two neurons have been found that seem to be itch-specific GRPR+ neurons and, more recently, MrgprA3+ neurons.

At the spinal level, gastrin-releasing peptide receptor, GRPR, has been found to be essential for mediating itch exclusively and not pain. GRP is expressed in a subgroup of dorsal root ganglion neurons. It is also colocalized with markers for unmyelinated fibers. In addition, approximately 80% of GRP+ neurons express TRPV1. GRP+ neurons are found only in the lamina I and II outer layer in the spinal cord. This data points to a possible involvement of the GRP signaling pathway in itch and pain. GRPR mutant mice were tested along with wild-type mice. GRPR mutant mice did not show a significantly different response from the wild-type mice to heat, pain, or mechanical stimuli. The mice were then tested with three different pruritogenic agents: 48/80, a PAR-2 agonist (SLIGRL-NH2), and chloroquine. After the separate injection of each pruritogen, a scratch count was taken from the mice. Although the GRPR mutant mice did scratch as a response to the injection, the number of scratches was significantly reduced, showing that GRPR mediates itch. To test whether activating GRPR at the spinal level would induce itch, GRPR agonist, GRP18-27, was intrathecally administered to the wild-type mice. It induced scratching behavior. As expected, the scratching response to GRP18-27 was significantly lower in GRPR mutant mice. In addition, when GRPR antagonist was inserted 10 minutes before the pruritogen in wild-type mice, scratching behavior was significantly reduced. Interestingly, the antagonist caused a lesser reduction in the effect of compound 48/80 than it had on PAR-2 agonist and chloroquine. This may be because PAR-2 and chloroquine act through a histamine-independent pathway, unlike compound 48/80. Since GRPR mutant mice showed regular pain responses to mechanical, thermal, and pain stimuli, but significantly reduced itch responses to pruritogens, GRPR+ neurons seem to be itch-specific (Sun, Chen, 2007). However, this does not rule out the possibility that GRPR neurons can also respond to pain and in GRPR mutant mice the loss of these neurons is compensated for by the other nociceptive neurons and therefore pain is still felt.

In order for something to be considered completely itch specific it needs to fulfill three criteria. First and most obvious, the neurons must respond to pruritic stimuli. Second, the loss of these neurons should only cause a loss of itch, not pain. Last, and most vital, only an itch response should be elicited when these neurons are specifically activated, not pain. The study done on the GRPR mutant mice fulfilled the first two criteria, but did not test for the third.

One group of neurons that was found to fit all the criteria is the MrgprA3+ neurons. These neurons only innervate the skin and were found to be absent from all other tissues, one clue that these neurons may be specific to itch, which is only felt on the epidermis. These neurons were coexpressed with TRPV1. They were also found to synapse with GRPR neurons in the spinal cord. These neurons responded to all types of itch other than β-alanine. When these MrgprA3+ neurons where ablated from a mouse line, the mice still showed regular responses to pain stimuli, though response to itch stimuli was greatly reduced. Mice have clearly distinct responses to itch and pain and therefore it is easy to know what sensation is being felt. Facial wiping with the forelimb is the response to pain, and scratching with the hindpaw is the response to itch (Shimada, LaMotte, 2008). (One interesting find was that the itch response to β-alanine was regular in MrgprA3+ -ablated mice, confirming that the neural pathway for β-alanine is unique.) In order to rule out the possibility that MrgprA3+ neurons are not necessary in pain response but can still be involved, MrgprA3+ neurons were specifically activated to see what response the neuron would elicit- pain or itch. The researchers used TRPV1-deficient mice and transfected TRPV1 only on the MrgprA3+ neurons. When capsaicin, an algogen, was injected into the cheek of these mice, the mice responded with scratching. This is in contrast to both wild-type mice that responded with wiping and TRPV1-deficient mice that did not respond at all. Thus it was shown that MrgprA3+ neurons coexpressed with TRPV1 elicit an itch response, regardless of the stimuli (Han, et. al. 2013). This is the first study to prove the existence of itch-specific neurons. No matter what the stimulus was, the sensation transmitted by those neurons was itch.

Conclusion

After the two initial studies that seemed to find itch-specific neurons, the support for the labelled line theory weakened. As seen above, many of the receptors for itch are also involved in the transduction of pain. Even more, itch responsive neurons in the DRG, TG, and the spinal cord also respond to algogens, such as capsaicin. However, with the finding of the MrgprA3+ itch-specific neurons, it is possible that the labelled line theory is correct for at least some of itch transduction. GRPR+ may also be part of this itch-specific group that mediates itch although further research would be necessary to confirm that. The study done on TG neurons also found a small population of pruriceptive neurons that only responded to pruritogens. Perhaps these pruriceptive-specific neurons are enough to mediate itch.

Even if itch is mediated through the few pruriceptive-specific neurons, it is still possible that the units that respond to both pruritogens and algogens also signal itch. Pruriceptive neurons are a subset of nociceptive neurons. Some hypotheses are that if the brain only receives input from the pruriceptive neurons, it processes the sensation as itch. However, if the brain is also receiving input from the nociceptive neurons, the brain only processes the pain and not the itch. So although those pruriceptive neurons are responsive to both pruritogens and algogens if there is no activity in the nociceptive-specific neurons when an itch stimulus is activating the pruriceptive neurons, itch will be felt (quoted in Patel, Dong, 2010). Along the same lines, another possibility is that the pain pathway activates mechanisms that inhibit itch transduction. Pain inhibiting itch is a familiar phenomenon, as it is well known that scratching relieves itch (Shmelz, et.al. 2003). A similar inhibition mechanism was suggested in the findings of a recent study. TRPV1 and TRPA1 both respond to algogenic stimuli. However, the study found that silencing one of them can cause algogens to elicit an itch response, i.e. scratching. This suggests that there can be inhibitors from the other ion channel that do not allow the brain to process the feeling as an itch (Roberson, 2013).

A final point for consideration is that most of these studies were done on animal models. But pain and itch may be mediated differently in different animals. Therefore, although research done on mice and other animals can give us an idea about the pathways in humans, further studies must be done to see how these mechanisms work in humans.

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A Dietary Approach to Cardiovascular Disease and Cancer: Does a Plant Based Diet Help Prevent and Reverse Cardiovascular Disease and Cancer?

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Abstract

Modern Western societies seem to suffer from a veritable epidemic of serious diseases, two of the most serious of which are cardiovascular disease and cancer. In contrast, hunter and gatherer groups have a very low incidence of such diseases. Despite the diversity of hunter and gather diets, they all share the same characteristic: the absence of a Western diet. This suggests that there is something uniquely inflammatory about a Western diet, which is high in both fats and carbohydrates. Departures from a Western diet appear to result in better health. Experimental studies have shown that heart disease can be reversed by adopting a very low-fat, high-carbohydrate, plant-based diet. The results for cancer are less clear. A low-fat, plant-based diet seems to offer promising results, but so does a low-carb, high-fat ketogenic diet. Perhaps forcing the metabolism into burning either fatty acids alone or glucose alone is beneficial, so long as one does not mix fat and carbohydrates.

Introduction

In Western societies, heart disease is the leading cause of death followed by cancer. In 2010, cardiovascular disease (CVD) caused a total of 788,000 deaths, which makes up nearly 32 percent of all deaths. Heart disease is the leading cause of death in blacks, but second to cancer in Hispanics, Asians, and American Indians. In the United States alone, a national survey from 2007 to 2010 showed that an estimated 83.6 million people either suffered from cardiovascular disease or were at risk for it. Of this number, 77.9 million people had hypertension and 15.4 million people had coronary heart disease (NHLBI, 2012). As for cancer, more than thirteen million people in the US had cancer in a survey taken in 2012 (Seer, 2012).

Although important advances have been made in the treatment and control of cardiovascular, lung, and blood diseases, these diseases continue to be a major burden on the nation. It has been estimated that in 2009, for example, we spent $313 billion on treating CVD--$192 billion of which took the form of direct health expenditures and the other $121 billion was in the form of the indirect costs of mortality (NHLBI, 2012).

Might there be a way to allay these financial and human costs? By way of an affirmative, this paper will explore the hypothesis that dietary measures can prevent and/or ameliorate cardiovascular disease and cancer.

Methods

Articles were located on the Touro library, University of Austin webpage, google scholar, and some journals found in the Touro library. The articles were critically read, reviewed, compared, and contrasted.

Discussion: Genes or Diet?

Genes, surely, have role to play in explaining susceptibility to various diseases. For example, one of the risk factors for heart disease, elevated cholesterol, sometimes has a genetic cause. And with the rise of medical inventions, such as penicillin, people who might once have died young from infectious diseases now live long enough to develop other diseases, such as cardiovascular ones and cancers. So it is possible that we are now seeing genetic dispositions come into play.

However, it is unlikely that genes are the whole story. When people of the same genetic stock change their diet, their risk of getting serious diseases changes. For example, when Asian men move to the United States, they develop prostate cancer at a higher rate than their peers at home. (Cook, et. al. 1999). Since the men who moved to the United States did not alter their genes, the increased incidence of prostate cancer is likely a product of a change of lifestyle, of diet, or of both. Further, in the United States the annual number of deaths from cardiovascular disease increased substantially from 1900 to 1970 and remains high(NHLBI, 2012) (Figure 2). While during this period, antibiotic use was on the rise, other changes were afoot. In particular, the way food was produced and consumed underwent enormous transformations. At the turn of the twentieth century, refrigerators designed for home use had not been invented and meals had to be made from scratch. By 1970, housewives were able to buy packaged foods and fast-food restaurants were becoming more and more popular.

It is plausible, then, that dietary and lifestyle factors might have greatly contributed to the development of serious diseases. Not only that, diet and lifestyle may also contribute to the disease's amelioration. If so, it is only logical that cardiovascular (and cancer) therapy should involve changes in diet and lifestyle.

The question then becomes: what type of diet and lifestyle is most protective against, and most able to ameliorate, cardiovascular disease and cancer?

Non-Western Groups, Western Diseases

One place to look for answers is among groups of people who have low rates of serious disease. One such place is among hunters and gatherers, who have astonishingly low incidences

of both cardiovascular disease and cancer. Weston A. Price's Nutrition and Physical Degeneration documents how agricultural and hunter-gather groups eating their traditional diets tend to have very low rates of degenerative diseases. This hypothesis has been supported by studies of particular groups. Only a few of them are listed here to give an idea of their lowered risk of disease. One study that surveyed blood pressure among Tsimani forager-farmers found that they had low blood pressure, and it remained low even as they aged—a finding that opposes the "normal" trend whereby blood pressure tends to increase with age.(Gurven, et al., 2012) Similarly, a study of traditional hunter-gatherer Cameroon Pygmies found that they had less aortic stiffness, lower LDL, and higher HDL than did semi-urbanized Pygmies and African Bantous.(Lemogoum, et al., 2012). The South Pacific commission of 1985 reported that the rate of cancer incidence among Fijiian Melanesians is quite low (Robertson, 1991). This finding is particularly striking since even the rates of lung cancer is one-fourth less than that of Polynesians and Micronesians, even though all groups smoke regularly. It is possible that the Melanesians' consumption of leafy green vegetables, such as bele and taro leaves, is protective (Robertson, 1991). And in the first half of the twentieth century, Canadian and Alaskan Inuits eating their traditional diet had very low cancer rates compared to Westerners or to Inuits eating a non-traditional diet (Steffanson, 1960). However, in the second half of the twentieth century, "Inuit have undergone noticeable dietary changes from a diet mainly based on fish and sea mammals towards a diet more dependent on imported food" and adapted, as well, to a Western way of life, at the same time, the incidence of cancer of the lung, breast, and colon, has increased (Friborg; Melbye, 2008).

What characteristics do groups such as these share that might be protective against heart disease and cancer? The answer is not a particular diet per se since diets among different non-Western groups tend to vary quite radically. A traditional Melanesian diet, for example, was mostly plant-based, which included both leafy greens and starchy foods such as taro, but also included some seafood and coconut. At the other end of the spectrum, the Inuits traditionally subsisted on a high fat, high-meat diet that included very little plants and other carbohydrates. Nevertheless, what they have in common is avoidance of a Western diet and lifestyle. According to this view, a Western diet and/or lifestyle is uniquely inflammatory and likely to increase risk factors for heart disease and cancer.

Untangling the Lifestyle and Diet Hypotheses

What might account for the tendency of Western diets and lifestyle to increase the incidence of cancer? There are many hypotheses ranging from an intake of excess calories to micronutrient deficiencies produced by processed foods One hypothesis, even, is that it is not a Western diet per se that increases cancer rates, but a Western lifestyle. In contrast to the high-stress Western lifestyle, non-Western groups tended to provide people with a cohesive community, a clear sense of purpose and/or identity, trust in a spiritual power, a more laid back life that is closer to the rhythm of the changing seasons and days. In support of this hypothesis, is the following evidence: Amish adults in Holmes County, Ohio had a cancer rate that was only 60 percent of the rate of cancer among non-Amish white adults in Ohio. (Westman, et al., 2010). However, since the Amish tend to prepare their own food, it is hard to know whether their lowered cancer rate was due to their lifestyle or to the benefits of a particular type of diet. Further complicating this scenario is a study of the Old Order Amish in Pennsylvania, whose mean total serum cholesterol was 212 (and a standard deviation of 45.2), a little above the recommended cholesterol number (200). (Pollin, et al. 1991, Table 1). However, the Old Order Amish are known for being exceptionally long-lived. (Sorkin, et al., 2005). This may be due to genetic mutation that protects them from the negative effects of high cholesterol. (Pollin, et al., 1991).

Given these complicating factors, one way to test the hypothesis that lifestyle alone and not diet is what determines cancer risk would be to study groups of people that have a cohesive community, sense of purpose, and belief in God but eat a typical Western diet. If we were to find out that people on such a diet experienced rates of cancer similar to the general population, then this would contradict the lifestyle hypothesis. But such communities are very difficult to find, especially since people who adopt a Western lifestyle—even religious groups of people—adopts, as well, the non-dietary accouterments of such a lifestyle, such as 9 to 5 jobs, smoking, and sedentary habits. For example, Seventh Day Adventists—who tend to be vegetarians—tend to have lower mortality rates from serious diseases compared to the rest of the population. (Heuch, et al., 2005). But it is hard to untangle the factors that might explain this, since in the Seventh Day Adventist, this may be due to non-dietary factors, e.g. they are discouraged from smoking.

In the absence of such a stringent test, the next-best test would be to compare differing religious groups that each have a strong sense of community, purpose, and belief in God but that have different diets. Such a study has, in fact, been attempted in Denmark. A study of 11,580 Baptists, who have no dietary restrictions, and Danish Adventists, who are lacto-ovo vegetarians, found, first, that both groups, especially, the Seventh Day Adventists had cancer rates that were lower compared to the general Danish population (66.95 percent for men and 85 percent for women), and that, second, overall "Adventists had lower hazard rates than Baptists" (Thygesen, et al., 2012). These

results suggest that both lifestyle and diet are protective or carcinogenic, since it turns out, first, that being part of a religious group might, in fact, be somewhat preventive against cancer and that, second, nonetheless certain kinds of diets may be more protective than other kinds of diet (and perhaps even more protective than the type of lifestyle one adopts).

Seventh Day Adventists generally tend to consume a mostly vegetarian diet, suggesting that it might be a beneficial diet even among people who follow a Western lifestyle.

Heart Disease: Causes and Characteristics

While some heart attacks are due to arrhythmias or faulty valves, endothelial injury, inflammatory oxidative stress, foam cell formation, and development of plaque can all be an introduction for cardiovascular disease. In obstructive heart disease, high cholesterol sometimes oxidizes, attaches to fatty acids, and begins to clog the arteries (atherosclerosis). These plaques sometimes calcify in the body's attempt to stabilize them, but this has the effect of further occluding the arteries. Occluded arteries make it harder to pump blood through them, and so blood pressure increases, in an attempt to ensure that adequate blood reaches vital organs. When the arteries are obstructed, or when the plaque is too unstable such that a clot forms and breaks off, then that blocks the flow of blood. This prevents enough oxygen from reaching the heart (as in ischemic heart disease), the brain (as in strokes), the lungs (pulmonary heart disease), or other vital organs.

In this scenario, high cholesterol starts an inflammatory cascade. Dietary cholesterol is often the source of high blood cholesterol, as well as of the fatty acids that the cholesterol transports, but the liver, too, can produce cholesterol and fatty acids. Further, when we take in too much sugar and starch than we need or than can be delivered to our cells, the liver converts them to fat. And insulin resistance, which prevents adequate disposal of glucose, is a predictor of heart disease (Yip, et al., 1998). Insulin resistance shifts our metabolism from burning glucose to burning fat, which means that the blood will have elevated levels of circulating free fatty acids, which have also been implicated in heart disease.

This picture is perhaps an overly simplified one. While it begins with the premise that the problem is elevated cholesterol, in a healthy body, ingested cholesterol generally should be converted to pregnenolone, estrogen, testosterone, progesterone, and cortisol. This raises the question: what is stopping or slowing down such a conversion? We may not be able to fully understand the genesis of heart disease without answering this question, but perhaps the answer is simply that we were not designed to ingest as much fat, sugar, and cholesterol that we currently do. So our bodies cannot convert all of it to various hormones.

Given these dynamics, we would expect that diets that have an abundance—not only of fat and cholesterol—but of sugar, fat, and cholesterol, to be ones that are effective at increasing the incidence of heart disease—which is the case in Western countries. Conversely, we would expect that diets that reduce our intake of fat and sugar would be beneficial at preventing and reversing heart disease.

Heart Disease and a Mediterranean Diet

There have been many attempts to find therapeutic diets that might prevent and reverse heart disease. One commonly studied diet is the Mediterranean diet, which comprises of mostly grains, fruits, vegetables, olive oil, nuts, some fish, dairy, and some occasional red meat. Fat makes up roughly 30 to 40 percent of the caloric intake and the fat itself was mostly monounsaturated (McKeown, et al., 2010). The Lyon Diet Heart Study was the first attempt to use this diet on 275 patients who had survived a first myocardial infarction. A test group was to follow the Mediterranean diet, while a control group was to follow the diet prescribed by their doctor. In patients who followed the test diet, the rate of cardiac death was 1.32/100 patients after 27 months and 1.24 after 46 months. This was an impressive reduction in mortality compared to the control group, which had a mortality rate of 5.55 and 4.07 after 27 and 46 months, respectively (de Lorgeril, et al., 1999). Many further studies have attempted to replicate the success of the Lyon Diet Heart Study, with varying success. One review of observational studies estimated that a Mediterranean diet reduced the risk of heart disease by 8 to 45 percent (Panagiotakos, et al., 2004).

It might seem that favoring the intake of monounsaturated to saturated fats might account for these somewhat favorable results, but regardless of whether patients ate either type of fat, the disease spread just at the same rate (Castelli, 1996). Further, while compared to a control group, the results of adhering to a Mediterranean diet seem impressive, we must keep in mind that while it reduced the rate at which people died from coronary heart disease, it did not actually stop or reverse the progression of the disease. The disease continued to progress, albeit at a slower rate compared to the control group (de Lorgeril, et al, 1999).

Heart Disease and a Plant-Based Diet

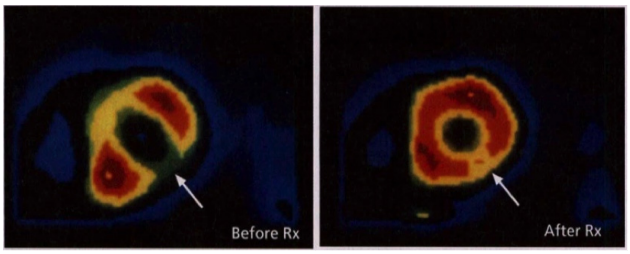
It is possible that the Mediterranean diet is not restrictive enough or permits too much animal-based foods. In support of this hypothesis, we can point to how during World War II, when the Germans confiscated Norwegians' livestock, the Norwegian diet was effectively limited to plants. During this time, the death

rates from strokes and heart attacks dropped considerably and then returned to pre-war levels when livestock was abundant again. This is an epidemiological study, but there is other evidence that supports the use of a plant-based diet to control heart disease. First, on a jocular note, African green monkeys apparently find a plant-based diet to be heart protective, especially when used in conjunction with cholesterol-reducing medication. Over a five-year period, these monkeys saw a halt to the progression of coronary artery disease (Rudel LL et al. 1995).

But clinical studies have yielded positive results for human beings, too, who adopt a plant-based diet. In one study, patients put on a plant-based diet that had less than 10 percent fat saw a drop in their cholesterol: an average of fifty points, from 227 mg/dL to 172 mg/dL. And there was a regression of heart disease in 82 percent of the 28 patients (Ornish et al., 1990). In this study, in addition to making dietary changes, the test patients had to exercise for three hours each week, as well as spend an hour a day on some type of stress-reducing exercise, such as meditation.

It would have been useful to compare the effects of a regimen of stress-reduction alone to a dietary regimen only; this would allow us to better tease out the degree to which diet accounts for the results. But we have a semi-answer in the form of clinical studies undertaken by Caldwell Esselstyn at the Cleveland Clinic. Twenty two patients with cardiovascular disease were put on a plant-based diet consisting of legumes, whole grains, fruits, and vegetables; the total fat intake was around 8 percent of total caloric intake. The 17 patients who adhered to this diet all witnessed a complete halt to the disease, and in 4 of them there were signs that the disease was reversed. One patient, who was limping from obstruction of the arteries in the leg, known as claudication, and who also had a low pulse volume experienced total pain relief after ten months of being on a plant based diet. Figure 3, below, presents the PET results of the myocardium of another patient before the diet and three weeks after: the restricted myocardial blood flow showed signs of restored blood flow (Esselstyn, 2014).

Figure 3



Restoration of blood flow to myocardium after three weeks on plant-based diet

While this study was a small one, a study consisting of a larger sample size, a cohort of 198 patients was performed. The patients had medical issues ranging from hyperlipidemia, hypertension, and diabetes. These patients were then counseled on the guidelines of the core of a plant-based diet. They were instructed to eat only whole grains, legumes, and fruits and vegetables. They were encouraged to take a multivitamin and a B12 supplement and then assured that these food and vitamins would provide all of their amino acid needs. Omega-6 and omega-3 essential fatty acids were provided in the form of flax seeds so were therefore also recommended as part of the diet. Foods that contained oil, fish, meat, fowl, dairy, avocado, nuts, caffeine and sugar were foods that were prohibited. Out of these patients, twenty one patients were not adherent to the diet. Out of these twenty one patients, 13 (62 percent) experienced at least one cardiovascular disease event including sudden cardiac death, heart transplants, and ischemic stroke. This is in glaring contrast to the patients who did follow the diet. Out of the 198 patients, 177 (89 percent) followed these guidelines and ninety-three percent of these adherent patients experienced improvement or even resolution of symptoms. There was only one major cardiovascular event that was related to disease progressions within the patients who adhered to the diet (Esselstyn, 2007). This is a 0.6 percent recurrent event rate. This contrasts sharply to the 62 percent recurrence rate recorded from the nonadherent patients.

Because adherence is generally the hardest factor to maintain in a diet, this study provided the patients with a five hour counseling seminar before the start of the diet to inform the patients of the risks involved when ingesting animal products. The patients were then called and given psychological support during a 3.7 year follow up to ensure they were adhering to the diet. This may have contributed to the success of the study. But there are some limitations to this study. First, it did not include a control group. Second, the patients who made up the cohort were very specific. They fell into a certain age range and all had history of cardiovascular disease. Further study would be needed to determine if complete prevention of cardiovascular disease can be accomplished in patients who do not have a history of cardiovascular disease. Third, the sample size, even though larger than the sample included in the Cleveland Clinic study, is still rather small.

Despite these limitations, in both of Esselstyn's studies, the percentages of patients in which cardiovascular disease did not progress are consistent. The percentage of patients in the study who adhered to the diet and had a recurrence of cardiovascular disease events is less than one percent. This is strong enough data to be used in order to inform people of this cheap and effective option of preventing and reversing cardiovascular

disease. And it supports the hypothesis that a plant-based diet can significantly affect the progression of the cardiovascular epidemic that is rampant in Western countries.

What might explain the impressive results of a plant-based diet? One possibility is that plant polyphenols themselves are heart-protective. Another is that a plant-based diet avoids the negative effects that might result from eating red meat, such as the production trimethylamine-N-oxide, a metabolite of L-carnitine, which has been linked to coronary artery disease (Tuso, et al., 2015). Another possibility is that by restricting a macronutrient (fat), subjects ended up cutting out a huge source of calories, and so perhaps a plant-based diet better cuts calories than does a Mediterranean diet. Another possibility is that fat itself is harmful to the heart and so lowering dietary lipids to under 15 or even 10 percent might be advantageous. Perhaps even a zero-fat diet might not be ill-advised for some. One man cured himself of his migraines, reduced his blood pressure, and reduced his cholesterol from 252 to 206 by switching to a completely fat-free diet. (Brown et al. 1938). This diet, however, was not a plant-based one, but used sugar and defatted milk. If the results of this diet were shown to be useful to more than just one individual, then we might have found the mechanism by which a plant-based diet reverses heart disease: it's the absence of high fat, not the plants themselves that are helpful. This would be a surprising finding but its validity, of course, is a question for further research to determine.

Cancer: Causes and Characteristics

Unlike the causes of heart disease, the causes of cancer are not well understood. They tend to share several basic characteristics: angiogenesis (Baenke, et. al. 2013); altered fat metabolism (McAnderew; Baenke, et.al.2013); a preference for anaeorobic over aerobic metabolism (Warburg, 2009); and perhaps even the capacity for immortality. What might lead an organism to develop these characteristics? One hypothesis, forwarded by Otto Warburg, is that cancers are caused by derangements in respiratory function. Other hypotheses include the theory that cancer is a type of viral infection, that cancer cells are stem cells that (for some reason) have been unable to mature, and that cancer is a product of localized and/or systemic inflammation. But whichever hypothesis we choose, we would have to explain, e.g., the cause of respiratory dysfunction, the inability of stem cell maturation, or the causes of inflammation.

Cancer and a Plant-Based Diet

Seventh Day Adventists generally—not just, as we saw earlier, Danish Adventists—have lower cancer rates compared to the general population (Lemon, et al., 1964). Further, even among Adventists, the type of vegetarian diet adopted affected their cancer rates. Among 69,120 participants of the Adventist Health Study-2, there were 2,939 cases of cancer. While a vegetarian diet is sometimes associated with cancers of the gastrointestinal tract, here, vegetarians were less like to get such cancers, particularly if they were lacto-ovo vegetarians. Likewise, vegetarians—particularly vegan vegetarians—had a lower risk of cancer compared to non-vegetarians (Tantamango-Bartley, et al., 2012).

When we turn to studies assessing the relationship between a plant-based diet and lowered cancer risk, we find that a vegetarian diet, in general, even among the non-religious, appears to have protective effects. A 1990s prospective study of 63,550 people in the United Kingdom found that, compared to people who ate meat, vegetarians were less likely to get cancer (except for colorectal cancer). For vegetarians, "the incidence rate ratio for all malignant neoplasms was 0.89" (Key, et al., 2009).

Further, increasing people's consumption of vegetables and whole grains may be beneficial even among people who have cancer. Case histories of cancer patients have revealed, for example, that pancreatic cancer patients who ate a low fat diet that included moderately high fiber and reduced calorie had a higher 1-year survival rate than patients who did not change their diets. Likewise, patients with stage D2 metastatic prostate cancer who followed such a diet survived longer and with improved quality of life compared to patients who did not change their diets (Carter, et al. 1993). Another study encouraged men with recurrent prostate cancer to eat more vegetables and whole grains. "Median intake of whole grains increased from 1.7 servings/d at baseline to 6.9 and 5.0 servings/d at 3 and 6 months, respectively. Median intake of vegetables increased from 2.8 servings/d at baseline to 5.0 and 4.8 servings/d at 3 and 6 months, respectively. The rate of PSA rise decreased when comparing the prestudy period (0.059) to the period from 0 to 3 months (-0.002, $P < .01$) and increased slightly, though not significantly, when comparing the period from 0 to 3 months to the period from 3 to 6 months (0.029, $P = .4316$). These results provide preliminary evidence that adoption of a plant-based diet is possible to achieve as well as to maintain for several months in patients with recurrent prostate cancer" (Nguyen, et al., 2006).

Additionally, a plant-based low-fat (10-15% kcal), high-fiber (30-40 g per 1,000 kcal/day) diet, combined with daily exercise, in just two weeks reduced overweight women's risk factors for breast cancer. This regimen reduced their serum estradiol, insulin, and IGF-I. Further, serum taken from these women after two weeks proved able to induce apoptosis of several variants of breast cancer cells in vitro (Barnard, et al., 2006).

These are just a few studies, but the evidence is quite suggestive. Plants have a number of protective properties (such as polyphenols) so simply increasing plant foods may be protective.

But part of the protection may be also the result of reducing the consumption of other foods, particularly processed foods, meat, and added fats. It is striking that people such as McDougall, Ornish, and Pritikin emphasize the importance of keeping dietary fat low, not just of increasing the amount of plant matter.

This makes sense to some extent since some fats themselves might contribute to the development of cancer. For example, prostate cancer is detected among men in the United States at a rate that is fifteen times higher than the rate of detection among men in Asian countries, who eat a lower-fat diet than American men (Parkin, et al., 1990). And, as mentioned earlier, when Asian men move to the United States and presumably increase their intake of fat, they develop prostate cancer at a higher rate than their peers at home (Cook, et. al., 1999). Further, compared to subjects who ingested less fat compared to the controls, lowering fat intake led to the development of smaller tumors as well as to tumors that grew more slowly—at least in immunodeficient mice who were able to eat ad libitum (Tung, 2014). It is worth noting that different types of fat may have different effects. In the study just cited, the mice on both the high-fat and low-fat diets ingested omega-6 fatty acids in the form of corn oil. In contrast, among mice whose main source of fat was saturated, there was little difference in the development of tumors in mice fed a diet high in fat (40 percent) versus those fed a diet low in fat (12 percent). This would suggest, as the authors of this study do, that lowering saturated fats does not slow the growth of prostate tumors, but reducing omega-6 fats might be beneficial (Lloyd, et al. 2010).

Cancer and Ketogenic Diets

Before we too hastily conclude that fat is carcinogenic, we should recognize that there is some evidence that ketogenic diets, which have been shown to be protective against epilepsy, might be protective against cancer too. Some scholars hypothesize that since cancer cells thrive on glucose, depriving them of it might, in principle, lead to their eventual death. On the other hand, since normal cells are able to run on ketones, a very high fat, low carb and low protein diet should be harmful only selectively (to cancer cells, not to normal cells) (Allen, 2014). There are very few human studies that demonstrate the purported benefits of a ketogenic diet. One of these studies included two young girls who had advanced-stage brain tumors that remained even after they were subjected to radiation and chemotherapy. Although the chemotherapy and radiation were not successful, the tumors were able to be managed long-term by using a ketogenic diet. (Nebeling, 1995). Thomas Seyfried has replicated these results among mice with brain tumors (Seyfried 2008; Seyfried 2003). Further studies on mice suggest that a ketogenic diet reduces blood glucose and slows the growth of tumors, a result that is compounded when used in conjunction with hyperbaric oxygen therapy (Poff, 2013).

There is not enough evidence to determine which one is more effective, but it seems that a very high fat diet, just as a low fat plant-based diet, might be effective, to some extent, against cancer.

High Fat or Low Fat?

On first glance, this seems to be a surprising result: two diets that could not be more different from each other both seem to exert some protective effects against cancer. Perhaps the same conclusions can be drawn as was earlier when confronted with the fact that non-Western people who consumed radically different diets nevertheless had similar low rates of cancer: the protective effects of different diets may be due less to their component parts than to their shared avoidance of certain foods, such as processed meats or artificial ingredients. Or it might be that a ketogenic diet is protective against certain type of cancers, but not others, while a low-fat, plant-based diet is more protective against other types of cancers. It should be noted that all of this is based on Warburg's considering that all types of cancer have the same type of causes. But it is possible that each cancer has a different cause and that therefore resolving it would require a diet targeted for that particular cancer. It might even be that cancers themselves might evolve in slightly different ways so that someone who has stage-1 breast cancer might respond well to one kind of diet while the same person with a stage-4 breast cancer might respond badly to the same diet.

However, bracketing that possibility for now, another hypothesis might be that certain foods—or food groups—are protective but only when taken alone such that combining them with another type of food might weaken their protective effects, or even cause negative ones. According to this hypothesis, perhaps a very high fat alone may be protective, as is high-carb plant food diet. Combining both fat and plant-based carbs, on the other hand, might have a different effect. This is a topic for future research.

Conclusion

Western countries have the highest incidence of atherosclerosis. Not coincidentally, these countries have easy access to an abundance of foods that are high in fat, sugar, starch, and cholesterol. If North America and Europe claim to have the most advanced medical care, what are these health care systems doing to prevent atherosclerosis, in particular, and heart disease, in general? In 1989, the National Research Council created a report called "Diet and Health," which recommended keeping cholesterol levels under 200 mg/dL and dietary fat to 30 percent of one's caloric intake. However, these guidelines are contestable, since it is possible that "a greater reduction [of cholesterol and dietary fat] would confer additional health benefits."

(Ornish, 1998). The suggestion that cholesterol levels between 150 and 200 might be too high is supported by a study showing that 35 percent of patients with ischemic heart disease had a cholesterol level between 150 and 200 (Castelli, 1996). The National Research Council, the American Heart Association, and the National Cholesterol Education Program recommend a diet that includes oil, low-fat milk, butter, and cheese. But if atherosclerosis and heart disease in general are closely linked to high cholesterol, their recommended diet by may be too lax to lower cholesterol levels. In light of the evidence, it would appear that in order to prevent and reverse heart disease, we would be well advised to adopt a very low fat, plant-based diet.

Adopting a low-fat, plant-based diet might also help reduce our chances of getting cancer, but there is also evidence suggesting that a high-fat ketogenic diet might be advantageous. Which diet should we prefer? Given differences in our genetics and physiology, some people might be better off on a ketogenic diet while others might fare better on a low-fat plant-based diet. Current knowledge does not tell us who would do better on which diet, but it is reasonable to suppose that people whose ancestors ate a certain type of diet might be more adapted to that diet than other people. The Inuit, for example, over the generations might have evolved the capacity to be able to process huge amounts of fat safely. But other than a few groups whose ancestral diet is known, what should the average Indo-European person do?

Given our lack of knowledge about the long-term effects of ketogenic diets, it might be best to adopt a low-fat diet instead. We have no evidence comparable to that of long-term vegetarian diets, that a long-term ketogenic diet is safe or prevents the development cancer (as opposed to attenuating its growth). We cannot point to the Inuits in support of the safety of a ketogenic diet since, although they ate a high-fat diet, it was nevertheless not on a ketogenic one: they ate some carbohydrates and enough protein that could then be converted, via gluconeogenesis, to glucose. Therefore, it is wise for many of us to adopt a low-fat plant-based diet, especially since it appears to be beneficial in reducing heart disease.

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Cognitive Effects of Breastfeeding

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Abstract
This paper explores the cognitive effects of breastfeeding through Intelligence Testing and Imaging Testing that compares IQ, success, and brain structure of individuals that were breastfed, formula fed, and both breastfed and formula fed. Intelligence studies available are widespread for all age groups and signify a causal relationship between breastfeeding and intelligence. However, imaging testing is not as extensive, but shows a strong correlation between cognitive development and breastfeeding. The mechanism of breast milk's impact on cognitive development is at an exploratory phase, with a possibility that docosahexaenoic and arachidonic acids, along with other nutrients found in breast milk, contribute to preferential neural development. Altogether, the biological principle that structure equals function, is supported by evidence from Intelligence and Imaging tests that structural differences in the brains of those who were breastfed, caused by the components of breast milk, results in superior intelligence (than non-breastfed individuals) throughout life.

Introduction

The development of human beings, like any other organisms, is affected by the method and means of feeding. Breastfeeding has many undisputed benefits recognized across the board by the medical community. However, modern life makes it that physical breastfeeding effects are not an issue of life or death, or even keeping serious illnesses away (Christakis, 2013). Consequently, women need an alternative motive for choosing to breastfeed, and continuing to breastfeed. This paper examines some of the cognitive developmental findings related to prolonged breastfeeding. Indeed, intelligence testing and imaging studies show an encouraging correlation between elevated IQ and babies who were breastfed.

With all research indicating that breastfeeding is the superior option when it comes to feeding babies, continued exploration of the benefits may seem superfluous. Discovered benefits of breastfeeding, however, are mostly limited to physical health advantages, which is not enough to motivate women to breastfeed. Reason being, the physical benefits are usually limited to early childhood, and do not have effects beyond that. Some examples of benefits that do not extend past early childhood include reduction of gastroenteritis, otitis media, and atopic eczema (Christakis, 2013,). On the other hand, cognitive effects from breastfeeding have lifelong implications, starting at infancy, and extending way beyond early childhood. This compilation brings together research studies on IQ and brain structure of different age groups to present a significant correlation between breastfeeding and cognitive development.

Methods

The perusal of journal articles and research papers through the Touro College online library system.

Breastfeeding and Infant Intelligence

Intelligence in infants is a difficult factor to determine, as much of the cognitive functions of babies are shrouded in mystery. Yet, we know that a baby's visual acuity gradually increases until it reaches a standstill at around twelve months. Consequently, researchers used visual acuity as a determining factor in intelligence to look for a correlation between breastfeeding and early infant intelligence. Two groups of infants, one consisting of breastfed infants and the other consisting of formula fed infants, were assessed for intellectual ability using visual acuity as an indicator. Behavioral testing analyzed infants' tendencies to look at patterned cards versus blank cards at ages one, two, three, and four months. From the two groups that were assessed, both improved significantly from the first to fourth month. However, the group of breastfed babies significantly surpassed the formula fed babies in visual acuity scoring. These results were parallel to findings of "a decrease in docosahexaenoic acid (DHA) of red blood cells in formula fed infants" (Michaelsen et al, 2003).

DHA is an omega-3 fatty acid found in breast milk. DHA seems to play a role in cognitive development. This is evidenced by the combination of results of the former study and a randomized intervention study that supplemented infant formula with DHA and tested the visual acuity of these infants. When infants took formula supplemented with DHA, their scores on the visual acuity test were better than infants who were drinking non-supplemented formula, but not as well as infants who were breastfeeding (Michaelsen, 2003).

Yet, DHA cannot be the only ingredient in breast milk influencing cognitive development. If it were, the supplement of DHA in infant-formula would be enough to produce the same visual acuity results as breastfed infants. However, DHA along with other nutrients can be responsible for the cognitive benefits of breast milk over formula. These nutrients, which are not found in cow milk or formula, have positive effects on cognitive development, and cannot be duplicated because they are not all known elements. "Nobody has been able to reproduce human breast milk because there are lots of elements in it that we probably don't even know about" (Lisser, 2002). At least until we figure out how to duplicate all the nutrients found in breast milk, breastfeeding will remain the single, most-effective way to ensure the highest level of cognitive development in infants.

Breastfeeding and Toddler Intelligence

Toddlers too, display higher levels of cognitive functioning when they are breastfed for prolonged periods, compared to babies who are breastfed for less than four months, or formula fed. This was determined by assessing the cognitive development of two-year olds via mental and psychomotor development, using the Bayley Infant Developmental Tests. Psychologists administering the tests found that "at 24 months, infants breastfed for longer than 4 months scored higher than those breastfed for 4 months or less and higher than formula fed infants" (Gomez-Sanchiz et al., 2004). In another instance, toddlers who were breastfed up to three months scored 4-5 points higher than those who were formula fed on psychometric tests, determining IQ (Jedrychowski, 2011).

Exclusive breastfeeding, as opposed to complementary breastfeeding also plays a positive role in the IQ of toddlers. The younger the child, the more significant the difference in IQ is between exclusively breastfed toddlers, and complementary fed toddlers. In one year-olds, exclusive breastfeeding resulted in a .0547 disparity between intelligence scores of toddlers that were exclusively breastfed, and toddlers that were complementary breastfed. At two years, a notable disparity between the intelligence scores of the two groups still remained, but shrunk to .0386. Exclusive breastfeeding affects IQ in toddlers positively, even if the time period of exclusive breastfeeding is short (Jedrychowski et al., 2011).

Breastfeeding and Intelligence in School-Aged Children

During the first year of school, children who are breastfed have an academic advantage over children who are formula fed. According to teacher-assessed education assessments of five year old children in England, using FSP (Foundation Staged Profile), duration of breastfeeding influenced the children' academic success. In areas of personal, social, and emotional development, as well as communication, literacy, and language, longer breastfeeding caused higher scores to be more likely (Heikkilä, 2014). Duration of breastfeeding was divided into four categories; never, less than two months, two to four months, and longer than four months. Children breastfed for up to two months were 9% more likely than children never breastfed to receive good overall achievement scores. Additionally, the longer a child was breastfed, the more likely he/she was to receive good overall achievement scores. Putting the three categories of breastfeeding together, and comparing it to children who were never breastfed, shows a 10-16% greater chance of good overall achievement scores for children who were breastfed (Heikkilä, 2014).

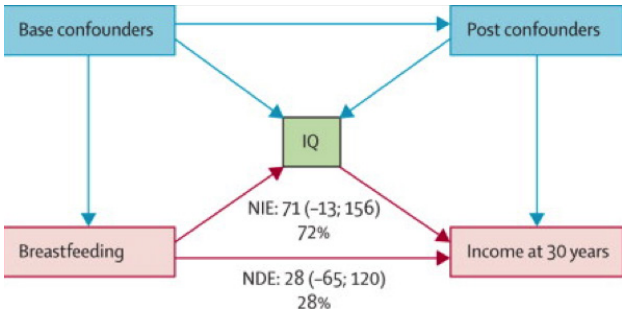
Notably, the relationship between breastfeeding and cognitive development is not exclusive to academic success. Rather, breastfeeding affects cognitive development in areas that include emotional and social intelligence, as well. Emotional and social intelligence first begin to influence success in earlier grades of school, but continue through high school and adulthood to have a more significant impact on success. Consequently, the influences of breastfeeding on emotional and social cognitive development are more prevalent throughout adulthood, compared to early childhood. This directly supports the notion that positive cognitive effects of breastfeeding are long-term, and a better reason than the well-known, short-term physical health effects of breastfeeding, for which women choose to breastfeed (Heikkilä, 2014).

Breastfeeding and Intelligence in Adolescents

The effects of breast-feeding on cognitive development also extend into the realm of adolescent intelligence, which is a long way off from the actual act of breastfeeding. "In New Zealand, breastfeeding duration was positively associated with performance in secondary school tests in students aged 18 years" (Victora et al., 2015). Students who are breastfed are also more likely to finish a higher grade of school. A study of 2,000 male Brazilian adolescents ages 18, found an increasing trend in finishing higher grades

with students who were breastfed. "Those breastfed for 9 months or more were ahead by 0.5-0.8 school grades, relative to those breastfed for less than 1 month" (Victora et al., 2005).

Breastfeeding and Intelligence in Adults



Various studies on the effects of breastfeeding positively influencing adult intelligence found a correlation between the two. In Brazil, intelligence, educational attainment, and income were all higher in 30 year-olds who were breastfed as infants. The table on the left shows that IQ mediated 72% of all incomes, with external factors only mediating 28% of income (Victora et al., 2015).

.This was after base cofounders, including family income at birth, parental education, household score index, genomic ancestry, maternal smoking during pregnancy, and birth weight, were

taken into account. The post-cofounder (educational attainment) was also taken into account. The results of this study strongly supports the notion that breastfeeding influences IQ, which in turn influences income and educational attainment (Victora et al., 2015).

Other studies found a positive correlation between breastfeeding and IQ in adults. For example, an IQ test administered to 27 year old Danish citizens in Copenhagen found a six-point difference in the IQ scores of people breastfed for a while versus people who were not breastfed for a long period of time. "Babies breastfed less than a month had a mean IQ of 99, compared with an average of 106 among infants breastfed seven to nine months" (Lisser, 2002). Similarly, in Belarus, IQ scores were on average 7.5 points higher in 65 year-old adults who were breastfed. Another study also shows that 53 year olds in Britain who were breastfed scored better on the National Adult Reading Test than their non-breastfed counterparts (Victora et al., 2015). Altogether, the studies on adult intelligence and breastfeeding support the thesis that breastfeeding affects intelligence at all ages.

Breastfeeding and Brain Structure

Now that a comprehensive analysis of breastfeeding effects on overall intelligence, from infant through adulthood, is understood, it is important to examine the physical effects of breastfeeding on the brain structure. Since we know structure parallels function, the development of an organ should correlate with the function it carries out. Consequently, if the brains of children who are breastfed have higher IQs, then something in their brains' structure ought to represent the higher functioning intelligence emitted from the brain. Moreover, finding a specific occurrence in the brains of people who are breastfed that cannot be found in the brains of those who are not breastfed would solidify the conclusion that breastfeeding has long lasting cognitive effects.

Breastfeeding and Brain Structure in Toddlers

The brain structure of breastfed toddlers compared to the brain structure of non-breastfed toddlers show increased myelin water fraction (VFM) in the brain structure of the breastfed infants. Myelinated white matter makes up the backbone of the brain's neural systems, and facilitates "rapid and synchronized brain messaging" needed for higher levels of cognitive functions (Deoni, 2013). "Aberrations in myelination, or deficiencies in myelin content or integrity, can have profound deleterious effects on brain function" (Fields, 2008).

A study investigated brain white matter maturation in 133 toddlers, ages 10 months to 4 years. Imaging times lasted 19 minutes in the younger toddlers, and 24 minutes in the 4 year olds. "Each infant was scanned using the mcDESPOT (multicomponent Driven Equilibrium Single Pulse Observation of T1 and T2) white matter imaging technique which provides a quantitative measure of the myelin water fraction (VFM) at each imaging point throughout the brain" (Deoni et al., 2013).

Results found that breastfed toddlers, in comparison to both formula fed, and to those that were breast and formula fed, had more VFM in many regions of the brain associated with visual reception skills, and receptive language scores. "In contrast with children who

received both breast milk and formula, exclusively breastfed children had significantly greater VFM ($p < 0.05$, FER corrected) in brain regions including: left optic radiation adjacent to the angular gyrus; right inferior parietal lobe, near the somatosensory cortex; bilateral premotor cortex; and right prefrontal cortex" (Deoni, 2013). Additional brain regions expected to mature later on had more VFM including the corpus callous, internal capsule, corticospinal tract, cerebellum, and left optic radiation. The results of this study support the researchers' initial hypothesis that docosahexaenoic and arachidonic acids found in breast milk, and not present in formula, causes preferential brain development in breastfed toddlers (Deoni, 2013).

Breastfeeding and Brain Structure in Adolescents

The brain structure of breastfed adolescents compared to the brain structure of non- breastfed adolescents shows a notable difference in cortical thickness. From an MRI analysis of cortical regions associated with cognitive abilities relating to general intelligence, cortical thickness was assessed. Findings suggest that exclusive breastfeeding predicts cortical thickness in the superior and inferior parietal lobules of the parietal cortex (Kafouri et al., 2013). Even after adjustments were made for age and sex, findings show that the longer the duration of breastfeeding, the thicker the cortex (Kafouri et al., 2013). Although the mechanism for a correlation between cortical thickness and breastfeeding is unclear, one possibility was recently suggested: "long-chain polyunsaturated fatty acids (LC-PUFAs), mainly docosahexaenoic acid (DHA), underlie neurodevelopmental benefits of breast milk" (Kafouri et al., 2013). This possibility is supported by Deoni's hypothesis that docosahexaenoic and arachidonic acids found in breast milk causes preferential brain development. Michaelson's finding of "a decrease in DHA of red blood cells in formula fed infants" also supports this possibility (Michaelson, 2003).

Conclusion

Relative to breastfeeding, intelligence testing on all age groups establishes a causal relationship between breastfeeding and IQ. Once imaging testing gets involved, however, the relationship

between cognitive development and breastfeeding is solidified. According to the basic biological principle that structure equals function, the function of greater intelligence, paired with different brain structure in people who are breastfed, confirms, beyond a reasonable doubt, that breastfeeding has widespread and long-term positive effects on cognitive development. Indeed, studies conducted thus far are enough to conclude breastfeeding's effects on cognition. However, more studies on the structure of the brain might help us conclusively determine the mechanism of how breast milk positively influences cognitive development.

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Vaccinations: Weighing the Risks and Benefits

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Abstract

Research has proven that vaccines prevent disease. Important medical organizations conclusively support and advise the administration of vaccinations to prevent diseases that once caused devastating effects both in the individual who contracted, and in communities where these diseases spread. While some groups protest against the constitutionality of vaccinating, others counter that failure to do so subjects the unvaccinated to illness and death by contracting and spreading of the sickness. Despite statistics on reduced incidence of diseases with few consequences as a result of vaccine-induced passive immunity, fears of harm secondary to vaccination loom. In the 21st Century, many people are unaware of diseases that still occur in undeveloped countries due to herd immunity through a majority vaccinated population. The diseases, however, are only a plane ride away. Due to globalization, vaccine preventable diseases can land in anyone's kindergarten. Although the link between vaccination and autism was pinned to research that was subsequently retracted, the rate of not vaccinating children continues to rise and these unvaccinated children pose risk to others who are unable to receive vaccines and compromise the core herd immunity. As modern science strives to demonstrate vaccine safety and efficacy, despite the rare but undisputed adverse effects, individual decisions to vaccinate remain a complex process with differences in perception, beliefs, and values to consider. These tenets will manage to produce research and evidence to support both pro-vaccination and against-vaccination in an attempt to determine if the potential benefits of getting vaccinated outweigh the detrimental side effects that may result. Further, a better understanding of the ramifications secondary to original research that noted negative correlations among vaccinated individuals and the vaccines they received will be understood. These controversies are the ripples experienced as a result of retracted and unethical research.

Introduction

We live in an age of incredible technology, discovery and medicine. Smartphones, spaceships, and bionic eyes are no longer reserved for science fiction. Vaccinations are among the groundbreaking medical discoveries which "allow us to engage our adaptive immune systems to produce highly specific antibodies and immunological memory against a potential future infection" (Federman, 2014). Through exposure to an inactivated pathogen in a safe way, the body's innate immunity learns how to properly adapt to the previously deadly pathogen. Still though, over two hundred years following Edward Jenner's successful use of cowpox material to create immunity to smallpox, in 1796, there are still those hesitant to make use of vaccinations for themselves and their children (Riedel, 2005). Consequently, it is quite concerning that an anti-vaccination movement persists. Particularly, people are concerned over a potential correlation between vaccinations and autism (Federman, 2014). Is vaccination so harmful that it is worth not getting vaccinated? Abstaining can put others at a higher risk of developing the given disease because there will be more disease carriers in the population. Or is vaccination harmless, or at minimum, do the benefits greatly outweigh the potential harmful side effects? Further, is it a matter of science or simply emotion discomforting those against vaccinations?

Methods

The research obtained regarding the potential side effects and benefits of various vaccinations was collected from a variety of sources. Most notably was the use of Touro's online library which provides access to databases such as Pubmed, Proquest, and more. Further, additional articles were found through Google Scholar searches of relevant original and peer reviewed articles on the topic. After reviewing an adequate number of articles on the good and bad of vaccinations, evidence was compiled which attempts to answer the research question.

Potential Benefits of Vaccinations

An important example of an efficacious vaccine is the Smallpox vaccination. In fact, the only way to be protected from Smallpox is through vaccination. Though Smallpox was once a virulent disease taking the lives of over one third of those infected, it has since been eradicated by means of vaccinations (Fenner et al., 1988). The protection from Smallpox, influenza, polio and many other diseases and viruses are already well-known vaccination benefits. Consequently each specific type of vaccination will not be directly expressed in this paper; rather, on the CDC website (2015) the entire list of dozens of possible vaccinations can be located. Instead, further benefits associated with vaccination uptake will be presented.

Are vaccinations effective at eradicating diseases? Regarding the effectiveness to eradicate an epidemic, there is a concept known as "herd immunity." Fine (1993) explains that "If an infection is to persist, each infected individual must, on average, transmit that infection to at least one other individual. If this does not occur, the infection will disappear progressively from the population." Note, there are those who are too young or too sick to become vaccinated, without herd immunity, many people will be at risk. Therefore, the higher percentage of those vaccinated, the higher the efficacy of any given vaccination in

protecting not only those vaccinated, but also the population as a whole.

Are vaccinations worth the expense? Vaccinations are cost effective and also save considerable amounts of money in healthcare costs. Zhou et al. (2014) determined that for children born in the United States in 2009, the nine vaccinations included in their study will prevent 20 million cases of disease and save 42,000 children from early deaths. Further, the same vaccinations will yield a savings of $13.5 billion in direct medical and non medical costs, including factors such as treatments for a primary infection, travel costs, special education and equipment costs for children disabled by disease, as well as further costs in extended hospital stays caused by medical complications. Furthermore, an additional $68.8 billion will be defrayed in total societal costs, most notably lost wages. The net savings would total a staggering $82.3 billion.

Though $82.3 billion is no small price, still this study did not include the Influenza vaccination, in their estimate. Therefore, the determined financial benefits of vaccinations may be understated. For example, during the eleven influenza epidemics in the United States from 1969 until 1994, there were between 130,000 and170,000 influenza-associated hospitalizations per epidemic with more than 20,000 influenza-associated deaths in 5 of epidemics; and over 40,000 influenza-associated deaths occurred during the other 6 epidemics. (Pleis & Gentleman, 1998). Furthermore, this study is limited to the United States, globally there exists a more vast market for saving. The additional money that is saved in healthcare costs, the further resources that can be allocated for vaccine research. For example, it is believed that diseases like cancer, epilepsy, and many more potentially can be prevented with vaccines but more research is needed. Regardless if these cures are a future possibility, there are plenty other worthy uses for the extra money that can be saved through becoming vaccinated.

Today healthcare workers are able to be protected from their sick patients with vaccine preventable viruses. Healthcare workers can be immunized and safely care for sick patients. They need protection not only from airborne viruses but also blood borne viruses contractible via shared needles. In the United States, it is estimated that there are 385 thousand cases of reported needle stick injuries per year. Incidentally, this number is an underestimation because it is expected that a significant number is unreported (Elmiyeh et al., 2004). People need to continue working and do not report needle stick injuries because they fear getting reprimanded for using equipment improperly.

Another potential benefit of vaccinations is to protect unborn children. Mothers who have received vaccinations can protect their unborn children from birth defects caused by certain viruses and further, vaccinated communities can help eradicate diseases to benefit future generations. Between 1963 and 1965, prior to the licensing of the rubella vaccine in 1969, a global rubella outbreak caused the deaths of 11,000 babies, and also birth defects in 20,000 babies in the United States. Therefore when women are vaccinated as children against rubella, they have significantly decreased the chance of passing the virus to their unborn or newborn children. This potentially eliminates the numerous birth defects associated with rubella, such as hearing and or vision loss, heart problems, congenital cataracts, liver and spleen damage, and mental disabilities (CDC, 2011). These birth defects can decrease quality of life and often require resources to help improve outcomes.

The last benefit is that research indicates that some viruses cause cancer. For example, the human papilloma virus (HPV) has been associated with cervical cancer (Marur et al., 2010). Therefore, it is important to realize that vaccinations are important to immunize against diseases to prevent the onset of side effects that can result; some of these may ultimately be lifesaving.

Potential Side Effects of Vaccinations

Regarding the possible negatives of vaccinations, it is important to consider the evidence for a correlation between vaccinations and developing autism. During the late nineties the link dissuaded people from taking vaccinations and now almost twenty years later the fear lingers. A clinical study found that "behavioral problems had been linked, either by the parents or by the child's physician, with measles, mumps, and rubella vaccination" (Wakefield et al., 1998). This early report became widespread and propelled the anti-vaccination movements taking place even today. The study was preformed using twelve children ranging in ages three to ten, with eleven of them male. Prior tests determined that all twelve children showed satisfactory achievement of early milestones. These children all lived normal lives, but then lost certain skills, notably the ability to communicate. Further, they all had gastrointestinal symptoms. e.g. diarrhea and abdominal pain. The children underwent assessment and review of their gastroenterological, neurological, and developmental records. Results indicated all twelve children had intestinal abnormalities, ranging from aphthoid ulceration to lymphoid nodular hyperplasia. Additionally, "onset of behavioral symptoms was associated, by the parents, with measles, mumps, and rubella vaccination in eight of the twelve children with measles infection in one child, and otitis media in another" (Wakefield et al., 1998). They each developed autistic behavioral disorders, nine of which developed autism. Disintegrative psychosis and possible post-viral or vaccinial encephalitis were less prevalent, representing one and two cases respectively.

It is suggested that autistic-spectrum disorders have a direct connection to intestinal dysfunction (Wakefield et al., 1998). In addition to this study, there are previous studies that link the connection between a dysfunctional or inflamed intestine and autism. For example, the "opioid excess" theory of autism, proposed originally by Panksepp (1979) explains that autistic disorders result from "incomplete breakdown and excessive absorption of gut-derived peptides from foods, including barley, rye, oats, and caesin from milk and dairy produce." These remaining peptides can stimulate the formation of harmful peptidase enzymes which break down endogenous central-nervous-system opioids, leading to disruption of neuroregulation and brain development (Shattock, et al., 1991). Interestingly, it has been observed that following removal of a provocative enteric antigen, the children achieved symptomatic behavioral improvement (Lucarelli, et al., 1995).

Still, the debate around the cause of autism remains controversial, and some, like Wakefield, postulate the incidence is correlated with childhood vaccination. To examine this hypothesis a retrospective study of 537,303 randomly selected children's cases were examined to determine association of the Measles, Mumps, Rubella (MMR) vaccine and autistic disorder. Of the group, a total of 440,655 children had received the MMR vaccine, with only 758 children diagnosed with some degree on the autistic spectrum, of which 316 were diagnosed with the most severe on the spectrum, autistic disorder. The incidence rate was 0.17% which is insignificant compared to the 7.7% to 11% range among various unvaccinated groups. Madsen et al, (2002) therefore concluded that there is no scientific causation between vaccination and autistic disorder or other autistic spectrum disorders.

Regarding the two conflicting studies, the size of their corresponding test groups is incomparable. Wakefield (1998) performed the study on just 12 children in one time frame, while Madsen conducted a retrospective study on almost half a million vaccinated children over the course of a few years. Though Wakefield seemingly proves a correlation between developing gastrointestinal problems following an MMR vaccination, more research is required to be conclusive that vaccinations can cause autism. However, in Madsen's (2002) study utilized about half a million children studied over multiple years. Therefore, Madsen's conclusion can be trusted that autism is not a side effect of vaccinations.

In an addendum to reinforce his conclusion, Wakefield added another 40 patients to his study, with 39 having the autistic syndrome. Still, his total of 52 patients is not as significant as the broader study done by Madsen. Another potential issue with Wakefield's study is that he quotes Lucarelli who explains that autism is reversible; this is a powerful claim that has yet to be confirmed. Furthermore, the Lancet released an official statement retracting Wakefield's study. Here is their full statement:

> Following the judgment of the UK General Medical Council's Fitness to Practice Panel on Jan 28, 2010, it has become clear that several elements of the 1998 paper by Wakefield et al are incorrect, contrary to the findings of an earlier investigation. In particular, the claims in the original paper that children were "consecutively referred" and that investigations were "approved" by the local ethics committee have been proven to be false. Therefore we fully retract this paper from the published record (the Editors of the Lancet, 2010).

In spite of this retraction, people clung to this concept that there is a relationship between vaccinations and a development of autistic spectrum disorders. It is astounding that the very Wakefield paper which led people to believe the MMR vaccination causes autism was retracted due to ethical misconduct. In addition the Lancet retracted the Wakefield paper for nondisclosure of financial interests. They reported that their sampling was randomized, however, in fact, it was selective. For example, in order to attract subjects, the researches offered a fee for parents of children who received the MMR vaccine and also had a previous diagnosis within the autism spectrum.(Sathyanaraya Rao & Andrade, 2011).

Not only does Madsen (2002) amply prove there is no correlation between vaccinations and autistic disorders, Wakefield's study has since been completely retracted. Perhaps the most appalling long-term affect is that the myth is so deeply ingrained that parents are refusing to vaccinate their kids out of fear of harm, and, unfortunately, some of these kids will or have already succumbed to the greater danger of contracting a vaccine-preventable illness. Because the official retraction was a mere paragraph in length and lacks detailed explanations, it slipped under the radar of the common folk and now people still believe Wakefield has legitimacy.

There is a potentially serious complication from vaccine administration known as Guillain–Barré Syndrome (GBS), which is a paralysis that begins on the lower extremities and migrates up the body. The legs become numb and as it ascends the body it leaves paralysis of muscles in its wake. An immune response is triggered that directly destroys either the myelin sheath surrounding the peripheral nerves or even the axon itself, leaving scar tissue in its midst. Unfortunately, if not promptly treated it will paralyze the breathing center, which is located at C3 of the cervical spinal column and breathing will require mechanical assistance (Koski, 1994). Dr. Tamar Lasky and her colleagues

studied the 1992-1993 Influenza Seasons and did in fact discover a direct increase in cases of Guillain–Barré Syndrome within the first six weeks following vaccination. At first glance, this discovery should shy people away from getting vaccinated. However, Lasky determined the increased risk to be only an additional 0.61 cases per million vaccinations. Further, even after an adjustment to include four factors that would make the original estimate conservative, their most accurate estimate of the attributable risk would be 1.1 case per every million vaccinations. Therefore Lasky argues that, "Even if Guillain–Barré syndrome were a true side effect in subsequent years, the estimated risk for Guillain–Barré syndrome of 1 to 2 cases per million persons vaccinated is substantially less than that for severe influenza, which could be prevented by vaccination in all age groups, especially persons aged ≥65 years and those who have medical indications for influenza vaccination" (Lasky et al., 1998). Therefore, despite the reality of the GBS complication caused by vaccinations, many still opt to prevent serious diseases by vaccination. Although avoiding vaccinations still remains controversial to many, the relative risk of experiencing a complication is low, and it could be considered neglect by parents to abstain from vaccination of children in an era where vaccines can protect these children from many serious diseases. As was aforementioned in this paper, the disproportionate number of 20,000 lives that would be saved from influenza epidemics alone far outweighs the minimal risk of contracting GBS (Pleis & Gentleman, 1988).

Each year there are 30,000 Vaccine Associated Events (VAE)s reported, with 13% comprising disability, hospitalization, serious illness, or death. While most of the reactions were classified as mild involving fever, irritability, or local reactions such as mild redness at the site of the injection that is sometimes caused by the preservative thimerosal or other innocuous inflammatory response (Vaers.hhs.gov, 2015). Even with the reporting system, many events are thought to occur by coincidence, as other syndromes such as Sudden Infant Death Syndrome (SIDS) occurs without any etiological basis (Vaers.hhs.gov, 2015). As Hardt et. al. (2013) point out, vaccine associated disease incidence is reduced in populations to the point of inability to remember what these diseases are and how they can harm. This phenomenon may cause vaccine adverse reactions to loom as predominant errs in how we provide healthcare and disproportionately place fear of harm above disease prevention in the minds of well-meaning caregivers.

An additional potential side effect from vaccinations can stem from the actual vaccinations. As was explained, a vaccination is effective through exposure to inactivated harmless pathogens. In some cases, though, the pathogen can potentially become harmful in the future. An example is varicella, commonly known as chickenpox, a vaccine-preventable illness which is a very common and usually benign childhood disease. Chickenpox however can cause serious painful complications in those who contract the illness in adulthood, in the form of Zoster, commonly known as shingles. The vaccine immunity wanes as one ages, and the dormant virus introduced with the vaccination can strike. As the body's ability to mount an immune response declines, the people will be more dependent on re-administering of the given vaccine (Shuette & Hethcote, 1999). This side effect may scare off potential patients, though in light of this potential side effect, maybe it is in fact more beneficial to withhold from the chicken pox vaccination. Though, a simple solution would be to receive a new vaccination every number of years. Further, Shuette and Hethcote (1999) discovered that even when those vaccinated for Chickenpox develop Shingles as adults, the symptoms are milder, since they still have some immunity, compared to those never vaccinated. Even if chicken pox vaccinations have a reason to be withheld, this side effect is not a reason to refrain from other types of vaccinations, such as Polio, Smallpox or the MMR vaccinations.

Discussion

In conclusion, research has not been able to produce a correlation between autism spectrum disorder and vaccination. Still, it is important to acknowledge the presence of fears, distrust, and other reasons to oppose vaccines. Although the adverse reactions are minimal, to the person who suffers an adverse event that is one too many. The Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC) and American Academy of Pediatrics (AAP) all support vaccine administration, and have successfully reduced prevalence of vaccine preventable diseases. However, we must ensure that appropriate education is disseminated to caregivers of children to protect the safety and lives of others by supporting vaccine administration so people with inability to receive vaccines will be better protected against vaccine preventable diseases. By ensuring a robust vaccination program, and supporting the expansion of research on vaccine preventable diseases, we can help develop vaccines for illnesses and diseases that currently remain untreatable. Perhaps new vaccine research might dispel vaccine myths which will increase the rates of vaccination, protecting more of the population.

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Effects of Vaccine Preservatives and Adjuvants on Childhood Neurodevelopment

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Abstract
Parental concerns about the safety of childhood vaccinations began in the 1990's and continue until today. A primary concern of many parents is whether the adjuvants and preservatives added to the vaccines have the potential to cause neurodevelopmental disorders in young children. An overview of various studies was done to determine if thimerosal affects childhood neurodevelopment with studies suggesting that thimerosal potentially causes neurodevelopmental disorders. However, some studies suggest the opposite. As a result it is impossible to conclude whether thimerosal affects childhood neurodevelopment. However, measures should be taken to remove thimerosal from the childhood vaccination schedule. Additionally, the studies must be done to determine if other components of vaccines can have adverse effects on the developing nervous system.

Introduction

Thimerosal, a mercury-based vaccine preservative, has been a source of controversy since the 1990's. Many parents worried that the presence of mercury in vaccinations could lead to autism spectrum disorder and other medical problems. The Center for Disease Control and the American Academy of Pediatrics collectively decided, in July 1999, to remove thimerosal containing vaccines (Baker, 2007). According to the Food and Drug Administration thimerosal has undergone various studies and it "has a long record of safe and effective use preventing bacterial and fungal contamination of vaccines, with no ill effects established other than minor local reactions at the site of injection" (FDA, 2015). In the United States, the only vaccines that currently contain thimerosal are MPSV4-Menomune (vaccine for meningitis), Td (booster for tetanus and diptheria) manufactured by Decavac, Td (Mass Biologics), Dt (Sanofi) and three of the influenza vaccines (Hamborsky et al., 2015). Some researchers posit that there may be a link between thimerosal and neurodevelopmental problems. Other studies demonstrate that thimerosal does not have such effects. This study was done to determine if thimerosal poses a risk on childhood neurodevelopment.

Purpose of Preservatives and Adjuvants in Vaccines:

The United States Code of Federal Regulations (CFR) mandates the addition of preservatives to vaccines in vials containing multiple doses to prevent contamination of the vaccine after multiple needle insertions. Previously, some vaccines became contaminated during the manufacturing process. As a result, the CFR required the addition of preservatives to kill the microbes that contaminated the vaccine. With the advancement of aseptic manufacturing of vaccines, the necessity of putting preservatives in vaccines has decreased. As a result, many vaccines do not currently require the addition of preservatives into them (Geier et al., 2010). Adjuvants are added to vaccines to help the body develop immunity after injection of killed vaccines. Adjuvants are added to minimize the amount of antigens the body needs to be exposed to in order to generate an immune response (Petrovsky, Aguilar, 2004).

Methyl Mercury vs. Ethyl Mercury

The main question behind the debate over thimerosal is the comparison between ethyl and methyl mercury. Mercury exposure has been found to cause severe health problems such as damage to the nervous, digestive and immune systems. Methyl mercury specifically has been found to affect the developing brain and nervous system. The most susceptible individuals to methyl mercury are fetuses exposed to methyl mercury in utero (WHO, 2013). Methyl mercury has been proven to cause neurotoxicity since the 1960's. Mothers who ate fish in water contaminated by methyl mercury during their pregnancy, gave birth to children with mental retardation, blindness, and spasticity. However, mothers exposed to methyl mercury were less adversely affected than the infants. A study on New Zealand neonates born from mothers who had hair concentrations of mercury greater than six micrograms per gram found that infants with greater mercury exposure had a lower Intelligence Quotient (IQ) by three points. The United States National Academy of Sciences decided that fetal exposure to methyl mercury can be toxic even at low concentrations (Grandjean, Landrigan, 2006).

The Food and Drug Administration states that the legal guidelines for mercury exposure were decided based on studies on methyl mercury. However, thimerosal's derivative is ethyl mercury which as a result of being slightly chemically different can have different effects on the body than methyl mercury. Additionally, high levels of ethyl mercury were found to have severe neurological effects on both adults and children. However, the effects of low dose ethyl mercury exposure have not been determined (Offit, Jew, 2003). As there is no definitive study with a safe exposure concentration of ethyl mercury and the differences in the effects of methyl and ethyl mercury are not known, the FDA considers ethyl mercury to be as toxic as methyl mercury (FDA, 2015). Ethyl mercury was found to increase the tissue concentration of inorganic mercury more than methyl mercury does in rhesus macaques (Hornig et al., 2004). Ethyl mercury levels in the brain post exposure are lower than those of methyl mercury (Clements, 2004). A main source of the controversy on thimerosal is the question if methyl and ethyl mercury have the same or different effects on the body. Some studies show that thimerosal's ethyl mercury does indeed have neurotoxic effects, while others argue that it does not.

Methods

A variety of research papers on the subject were collected from Google Scholar, EBSCO and ProQuest. Access to EBSCO and ProQuest was given through the Touro College Library System. Key words used, were thimerosal, neurodevelopment, adjuvants, vaccines, adverse effects and immunizations.

Results

Thimerosal and Neurodevelopment:

Thimerosal Causes Neurodevelopmental Disorders

In the 1990's many infants had mercury exposure higher than that recommended by the EPA, FDA, and the United States Agency for Toxic Substances and Disease Registry (ATSDR) for methyl mercury. This was a result of both Thimerosal Containing Vaccine (TCV) and environmental exposure. Methyl mercury is a compound that is closely related to ethyl mercury, one of the derivatives of thimerosal. A study of 278,624 infants born between 1990 and 1996 and recorded in the Vaccine Safety Datalink was done in order to determine if thimerosal indeed has neurotoxic effects on infants and young children. During those years the main vaccines containing thimerosal were the Haemophilus Influenza Type b (Hib), hepatitis B, acellular DTaP (Diptheria, Tetanus, acellular Pertussis), and whole-cell DTP. The amount of mercury exposure was calculated for each individual based on the amount and concentration of mercury in each of the mercury containing vaccines. The mercury exposure was measured between two intervals, birth and 7 months and birth and 13 months. The rate of increase in six neurodevelopmental disorders were calculated for each additional 100 microgram of mercury the children were exposed to. Three control disorders were also observed for each 100 microgram increase in mercury exposure due to thimerosal containing vaccines. (Young et al., 2008)

The six neurodevelopmental disorders observed were Autism, Autism Spectrum Disorders (ASD), Hyperkinetic syndrome of childhood (ADD/ADHD), Developmental/Learning Disorders, Disturbances of emotions specific to childhood and adolescence, and tics. The control disorders observed were pneumonia, congenital anomalies, and failure to thrive. The study determined that the ratios of each of these disorders increased with the increased exposure to Thimerosal containing vaccines. The rate ratios were measured with a confidence interval of 95%. For each 100 microgram increase in mercury exposure the ratio of hyperkinetic syndrome of childhood increased by a ratio of 3.15 and Autism and Autism Spectrum disorders increased by ratios of 2.87 and 2.44 respectively. Developmental and Learning disorders had the lowest ratio of increase, while hyperkinetic syndrome had the highest. The control disorders did not have a relatively high increase as a result of higher mercury exposure (Young et al., 2008).

The levels of autism corresponding to each year in the study directly correlated to the amount of mercury exposure via vaccine. In 1990 and 1991, hepatitis B and Hib had been added to the vaccine schedule. After 1992, the DTP vaccine was combined with Hib vaccine, thus leading to a lower mercury exposure than there had been when the two vaccines were kept separate. The levels of autism in those years fluctuate directly with the fluctuations of mercury administered via vaccine (Young et al., 2008).

Another study compared the toxicities of the four most common preservatives added to vaccines in the United States. These four compounds were 2-phenoxyphenol, phenol, Thimerosal, and benzethonium chloride. These compounds were administered to human neurons in vitro and to bacterial cells. The toxicities to each type of cell were measured. The cytotoxicity towards human neuroblastoma cells was measured after 24 hours of incubation. Thimerosal was found to be the most toxic of the four preservatives added to vaccines. Each of the four compounds was also tested on bacterial cells. A thimerosal concentration of ten times the amount added to vaccines was not able to sufficiently kill bacterial cells within twenty minutes (Geier et al., 2010).

While there is room to question the certainty of the thimerosal's effects on children, it his highly likely that thimerosal does adversely affect the neurodevelopment of susceptible infants. In some of the studies thimerosal was the only measured variable, while in others thimerosal was taken into account with other possible neurotoxic environmental exposures. Using the Gesell Development Score, investigators found no correlation between thimerosal exposure from vaccines and neurodevelopmental disorders, if the child nursed for the first six months of life. The GDS was also measured from birth to five years and once again there was no correlation between thimerosal exposure and neurodevelopmental disorders. The studies that took environmental exposure to neurotoxic elements into consideration as well as thimerosal, found that ethyl mercury, one of the byproducts of thimerosal, has adverse effects on neurodevelopment. The controlled variables were the environmental factors that were known to affect neuroplasticity. For example, secondhand smoke, cord blood Hg, and lead (Dorea, 2015).

The Vaccine Adverse Events Reposting System (VAERS) database was used to determine if indeed the rise in childhood neurodevelopmental disorders was caused by thimerosal exposure due to vaccinations. A comparison was made of the prevalence of neurodevelopmental disorders after exposure to either thimerosal containing or thimerosal free DTaP vaccines. Three neurodevelopmental disorders were observed in this study; autism, personality disorders and mental retardation.

The participating patients were split into two groups, the first group had an average mercury exposure of 37.5 microgram, while the second group had a mercury exposure of 87.5 micrograms. Some of the control adverse events were fever, pain, and vomiting. The United States Department of Education report from 2001 was also evaluated to find out how many children in different age groups suffered from a variety of conditions; autism, speech disorders, orthopedic or visual impairments, and deaf-blindness. The amount of mercury the children were exposed to was also compared to the maximum possible amount of mercury exposure allowed by the FDA (Geier, Geier, 2003).

Comparing the prevalence of autism, personality disorders and mental retardation following exposure to thimerosal from the DTaP vaccine and the thimerosal-free DTaP demonstrated a linear correlation between mercury exposure and neurodevelopmental disorders. The odds ratio of autism increased by .029, personality disorders by .012, and mental retardation by .048 for each microgram of mercury. The odds ratio for each of these disorders was higher for each of them after exposure to the DTaP containing thimerosal than for that without it. The United States Department of Education report also had increasing ratios in autism and speech disorders. The amount of febrile seizure, fever, pain, edema, and vomiting was higher for the thimerosal containing DTaP vaccine group, however the amount it rose and the increasing amount of mercury exposure do not directly relate to one another. The study also demonstrated that the amount of mercury the children were exposed to due to the childhood vaccine schedule in different years had 3.2-32 fold higher amount than that recommended by the FDA for oral methylmercury. Exposure to mercury intravenously has more adverse effects on the body than if it has been orally ingested (Geier, Geier, 2003).

A study surveying British children found there to be a relationship between hyperactivity and behavioral problems, motor development and the necessity for speech therapy. Other studies have found that there were significant differences in the impact of thimerosal on different genders. Male children were found to have a higher IQ upon exposure, but the exposure also hampered their behavioral regulation and motor tics. In a study in Italian children thimerosal was found to detrimentally affect girls in the areas of language and motor function (Curtis et al., 2015)

An overview was done by analyzing the results of in vivo and in vitro studies of the thimerosal's effects. In the in vitro studies thimerosal was found to cause mitochondrial damage, reduce oxidation-reduction activity, cause cell degeneration and cell death, and increase the levels of fragmented DNA in human neuroblastoma cells. In some of the overviewed studies the concentration of thimerosal tested was lower than that found in TCV, while in some the concentration ranged from nano to micromolar concentrations. An In vivo study administered different concentrations of thimerosal to suckling Wistar and Lewis rats at 7, 9, 11 and 15 days. The thimerosal was found to decrease pain sensitivity and to significantly accumulate in rat brains. The concentration of thimerosal found in vaccines had neurotoxic effects on human neurons in vitro and in laboratory animals (Dorea, 2011).

Thimerosal Does Not Cause Neurodevelopmental Disorders

A study was done in Poland to determine if there is a correlation between exposure to Thimerosal containing vaccines and cognitive development in the children's' first nine years. All of these children had been given the DTP and hepatitis B vaccine, some of which contained thimerosal and some which didn't. At different points during the first nine years of life, tests were done to assess the level of each child's cognitive development. All the vaccines that the children were exposed to contained aluminum as an adjuvant. In the first month of life 69.2% of the children were exposed to thimerosal, and between the first month and the 6th month 86.2% of the children were exposed to thimerosal containing vaccines. The results of each psychological test done were similar for both children who were and were not exposed to thimerosal. In fact the IQ levels of those exposed to thimerosal was higher overall than that of the control group. The results of the study displayed no correlation between thimerosal exposure and lower cognitive developmental levels. (Mrozek-Budzyn et al., 2014).

Various studies had been done testing thimerosal containing vaccines on rodents, with results demonstrating that thimerosal had no neurodevelopmental effects. However, on the rare occasion that there was a problem with the vaccine, a very high dosage of thimerosal had been administered, 250 fold higher than contained in vaccines. A study was done to compare the effects of different vaccine schedules on a non-human primate model. These were chosen as their anatomy and length of development stages are very similar to those of humans. Neurobehavioral tests were done on the primates to see the effect of a variety of vaccine schedules on the primates' neurodevelopment (Curtis et al., 2015).

In the study seventy-nine rhesus macaques were split into six groups. The first group, the control, received saline injections. The second group received only the MMR vaccine, the third TCV's excluding the MMR vaccine. The fourth group was administered the vaccine schedule from the 1990's, while the fifth group received the same vaccines with accelerated timing at a ratio of 4:1. The sixth group was administered the vaccine

schedule from 2008. In the 1999 vaccine schedule, the TCV's were Hepatitis B, DTaP, and Hib. In the 2008 vaccine schedule, the multiple-dose influenza and meningitis vaccines contained thimerosal. The pregnant macaques in the 2008 group were given the influenza vaccines a month before the expected delivery date. (Curtis et al., 2015).

Each of the infant macaques was tested on the Neonatal Behavioral Assessment Scale to check for nineteen neonatal reflexes. There was no significant difference in the results of each of the six groups for the NBAS test, except in one of the criteria. The macaques were also tested for their object concept performance, their social behavior and the discrimination/reversal learning and learning set. In each of these tests there were no significant differences in the results among the groups. The animals given TCVs reached their goal in fewer trials than animals in the control group in the reversal learning part of the test. Thereby demonstrating that thimerosal did not hamper this area of neurodevelopment. At the age of 12 months the fifth group had a significantly lower amount of exploring behaviors than did the control macaques. The fifth group also had a lower amount of positive behaviors at 2 months, while at 12 months they had a higher amount of positive behaviors than did the rest at two months of age the fourth and sixth groups had significantly fewer negative behaviors, however at 12 months there were no major differences between the six groups (Curtis et al., 2015).

A study in Denmark, used data from the Danish Psychiatric Central Research Register to determine if thimerosal exposure leads to autism spectrum disorder. This study was done to determine if removing thimerosal from vaccines decreased the incidence of autism. The rates of autism diagnosed from 1971 to 2000 for children in three age groups, ages 2-4, 5-6, and 7-9, were reviewed. From the years 1961 to 1970 the children were administered 400 micrograms of thimerosal. From 1971-1992 children were exposed to 250 micrograms of thimerosal. After 1992, vaccines without thimerosal were administered to Danish children. During this period, 956 children had been diagnosed with autism. From 1971 to about 1990 the rates of autism remained stable. After 1991, the rates of autism began to increase, peaking in the year 1999 with the highest incidences occurring in the first two age groups, between 2 – 6 years of age. Each of these children had been born after thimerosal containing vaccines had been removed from the Danish vaccine schedule. Thereby indicating, that the increase of autism does not relate to thimerosal exposure (Madsen et. al, 2003).

Aluminum and Neurodevelopment

Studies have demonstrated that in vitro aluminum exposure has the potential to cause cell death in human neurons. However, aluminum has been found to be less cytotoxic than thimerosal (Dorea, Marques, 2009). A study was done to determine if aluminum exposure as a result of its use as a vaccine adjuvant is a cause of the rise of autism. Data was taken from the US Department of Educational Annual Reports to Congress to determine the rates of autism from 1991-2008 for individuals age 6 – 21. The rates of autism were also compared to the aluminum exposure in regards to the vaccine schedule in other countries. The data was compared to the concentration of aluminum exposure from pediatric vaccines, below 6 years of age. Results demonstrated that the highest level of aluminum in comparison was at age 2 months in the United States. Interestingly, the countries with higher aluminum adjuvant exposure had higher rate of autism (Tomljenovic, Shaw, 2011).

Discussion

Based on the reported studies there is room to question the effects of the Thimerosal and vaccines containing it on human children. Studies done in vitro demonstrate that thimerosal does cause apoptosis and neurotoxic effects to human neurons (Dorea, 2011). However, results occurring in vitro are not always a directly comparable to those in vivo, as in vivo it is difficult to accurately evaluate the concentration of mercury reaching the individual's neurons. Additionally, studies done on Rhesus macaques seem to point out that thimerosal does not hinder neurodevelopment, in some cases the exposed animals had scored higher than the control (Curtis et al., 2015). However, the primate model is not necessarily an exact indication as to what would happen to humans when they would be exposed to the same concentrations of thimerosal, though the results would be similar as the two bodies function similarly. Only seventy-nine macaques were surveyed and then split into six groups, thereby only a small number of macaques represented each vaccine schedule. Therefore, even though the macaque functions similarly to humans, the validity of the results in comparison to human would need a higher number of macaques in each group to demonstrate the efficacy of the results.

The study using the Vaccine Safety Datalink uses a large sample of children, and demonstrates a direct correlation between thimerosal exposure and neurodevelopmental disorders. However, the study does not take into account environmental exposure to mercury and other neurotoxins, thereby making it harder to say with certainty that thimerosal does indeed cause different neurodevelopmental disorders (Young et al., 2008). The study also does not take into account the genetics of the children and the possible predisposition to neurodevelopmental disorders. However, there does seem to be a strong indication that thimerosal has neurodevelopmental effects, as the prevalence of autism in various years was directly related to the fluctuations of mercury in the childhood vaccine schedule, thus indicating

that children born in years that a vaccine schedule with a higher concentration of mercury had increased rates of autism.

Additionally, the primate model is not a direct representation of what would happen in the human models because studies show that the rate of elimination in the blood and brain differ for methyl and ethyl mercury in primates and human infants (Hornig et al., 2004). As a result, ethyl mercury in humans may cause different mercury levels than it would for the rhesus macaques used in two of the above studies.

Based on the above studies it is hard to conclude whether or not thimerosal containing vaccines cause neurodevelopmental disorders. However, some studies do seem to indicate that thimerosal causes autism among other neurodevelopmental disorders. Additionally, thimerosal containing vaccines were found to cause a significant amount of damage to human neurons in vitro, including cell death. As a result, further study must be done to determine with certainty the true effects of thimerosal on human neurodevelopment.

As one study demonstrated that the concentration of thimerosal found in human vaccines was not sufficient to kill bacterial cells, the question arises as to the necessity of placing thimerosal into vaccines. Additionally, after administration of multiple does DTP and Td vaccines that contained thimerosal as a preservative there were outbreaks of pyogenic infections. Indicating that despite the addition of preservatives to vaccines, microbial contamination can still occur (Ball et all, 2001). One of the main vaccines today currently containing thimerosal is the multiple-dose influenza vaccine. Thimerosal is added as a preservative to prevent bacterial contamination of the vaccine. If the concentration of thimerosal needed to effectively rid the vaccine of bacteria is higher than the concentration of thimerosal in the vaccine, then it is likely that the thimerosal is not doing its job. Steps should be taken to remove thimerosal from vaccines as there is a strong potential that thimerosal does indeed cause neurodevelopmental problems. If thimerosal is kept in vaccines, it should be determined whether the benefits thimerosal brings to the vaccine outweigh the risks brought onto children when they are vaccinated.

Fetuses, neonates, and other small children are the most susceptible to neurodevelopmental damage, as these are the periods of brain growth (WHO, 2013 and Dorea, 2015). The World Health Organizations states a fetus is the most susceptible to neurotoxic factors (WHO, 2016). Additionally, during the prenatal and early postnatal stages the Blood Brain Barrier (BBB) is not fully developed. As a result, the likelihood of neurotoxins crossing the BBB is higher in fetuses and newborns (Tomljenovic and Shaw, 2011). As the risks of thimerosal exist and there is not conclusive proof that there is not neurotoxicity, injecting pregnant woman and babies with vaccines containing thimerosal should be avoided, as these are the ages most critical to childhood neurodevelopment. For example, the Center for Disease Control currently recommends that the influenza vaccine be administered to pregnant woman as a woman suffering from influenza during her pregnancy is at great risk (Center for Disease Control, 2015). Pregnant woman are more likely to have longer stays at the hospital as a result of respiratory illnesses, than when not pregnant (American College of Obstetricians and Gynecologists). There seems to be a clear benefit of a pregnant woman being immunized to influenza. However, based on the information above, there is clearly a potential that thimerosal causes neurodevelopmental disorders, and this stage is where the fetus is the most susceptible to neurotoxins. As a result, measures should be taken to ensure that pregnant woman are given the influenza vaccine that does not contain thimerosal. Additionally, the postnatal stages till adolescence are still stages of neurodevelopment. As thimerosal potentially causes neurodevelopmental disorders, the benefits of thimerosal containing vaccines must be evaluated to see if they actually outweigh the risks presented by thimerosal exposure.

Conclusions

There is no definitive evidence to conclusively determine if thimerosal causes neurodevelopmental disorders in young children. However, the potential for thimerosal to cause neurodevelopmental disorders gives rise to question the potential harm of the other agents used as preservatives and adjuvants in vaccine today. One of the main adjuvants used in vaccines today is Aluminum, which does seems to have the potential to affect neurodevelopment, specifically causing autism spectrum disorder. As vaccines are being administered to susceptible individuals, further studies should be done to all the chemicals added to vaccines to determine if they are really safe, and if the benefits they bring to the vaccine outweigh the risks.

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Oocyte Cryopreservation

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Abstract
Anti-mitotic therapies are a form of therapy used to treat cancer patients. The use of these treatments on females can result in fertility complications. Therefore, prior to treatment, women must seek ways to preserve their ability to conceive children after receiving treatment. This study analyzes the outcomes of oocyte cryopreservation and its many variables. Three important variables that can affect the outcome of oocyte cryopreservation are age, cryopreservation method and cryoprotectants. Evidence indicates that human oocyte cryopreservation can enable a woman to preserve her ability to give birth to a healthy child, following anti-mitotic therapies. Hundreds of babies have been born as a result of oocyte cryopreservation. Oocyte cryopreservation can even enable a woman with ovarian cancer to have a healthy offspring, post treatment.

Introduction

Early detection of cancer along with modern medicine has led to a rise in the survival rates of young cancer patients. This results in many cancer survivors who are capable of childbearing. However, since chemotherapy and radiation given during the cancer curing process can result in various fertility issues, patients must find a method to preserve their ability to give birth to children.

One method of preservation is oocyte cryopreservation. Human oocyte cryopreservation is a procedure in which a woman's oocytes are extracted, frozen and then stored. When the woman desires to become pregnant the eggs are thawed, fertilized and transferred in to the uterus.

This type of preservation is preferred by many for various reasons. Many single cancer patients prefer freezing unfertilized eggs, as opposed to fertilized ones, as they don't need any male donors at the time. Another reason women may want to freeze eggs is due to the fact that oocyte quality and quantity diminishes with age. This can cause a lack of healthy eggs to allow for pregnancy. Considering this, many women may prefer to freeze their healthy young and vital oocytes, which are more likely to produce pregnancies.

Furthermore, people may consider oocyte cryopreservation due to ethical, legal and religious hindrances that prevent them from doing embryo cryopreservation. Couples may not want to cryopreserve embryos as the embryos may have to be disposed if the cancer patient dies. (Noyes, et. al. 2010)

The goal of this study is to determine if oocyte cryopreservation is a means that enables women to have healthy offspring, post anti-mitotic therapy.

Methods

Pub med.gov, google scholar and Touro databases such as, EBSCO host, were used to research relevant studies and reviews for the background, process and results of oocyte cryopreservation. The review paper's references were used to find additional original papers that were relevant to the question proposed above. Key words such as oocyte cryopreservation, oocyte cryopreservation in cancer patients, slow freezing and vitrification were used in order to find articles.

Discussion

Various studies were done on oocyte cryopreservation. In one experiment, twenty-two cancer patients, between ages 21 and 38, underwent cycles of oocyte cryopreservation. After drug stimulation, oocytes were harvested from sixteen of the twenty-two infertile women, subsequently fertilized and implanted in them. The other six infertile patients received donor eggs. Only mature oocytes were preserved. The eggs were preserved by two methods, slow cooling method and vitrification. A total of 295/355 oocytes were recovered with a 92% survival rate.

At the time of publication of the study, fourteen of the patients had become pregnant, one had miscarried, and three pregnancies were still ongoing. A total of thirteen babies were born to the other expectant ten patients. Eleven of these babies were completely healthy, however a set of twins were born prematurely due to premature dilatation of an incompetent cervix. These twins suffered some complications of prematurity, but upon reaching two years of age, the twins were thriving within the average norms. Besides for two cases of gestational diabetes, no other complications were reported (Grifo, Noyes, 2010). Gestational diabetes is common in women during pregnancy. According to the CDC, the ratio of women with gestational diabetes ranges between one in every twenty to one in every fifty of expectant women.

Additionally, Dr. Nicole Noyas and other researchers pooled together data to see how many oocyte cryopreservation's resulted in normal babies. Any incomplete data was left out. A total of six hundred and nine births were reported between the years 1986-2008. All the babies born were a result of oocyte cryopreservation. However, the oocytes in those six hundred and nine births were preserved using different methods of cryopreservation. Three hundred and eight went through the process of slow freezing, two hundred eighty nine were preserved using vitrification and twelve had a combination of slow freezing and vitrification. A total of eight anomalies were reported. There were also three hundred twenty seven cryopreserved oocyte births published, totaling nine hundred thirty

six births. Out of the nine hundred thirty six babies, twelve babies were born with birth defects. Overall the anomaly rate is 1.3%. According to the CDC, three percent of babies are born with major structural or genetic defects. The twelve defects and the total incidents statistically occurring in natural conception babies compared to oocyte cryopreservation babies are listed in table 1. (Noyes, et. al. 2009)

Table1

Birth Anomaly	Approximate incidence in natural conception births	Incidence in total of 936 oocyte cryopreservation births (n)
All	One in 33	12 (one in 78)
Skin hemangioma	One in 50–225	1
Cardiac defects	One in 125	3
Neural tube defects	One in 385	0
Cleft lip and palate	One in 710	1
Clubfoot	One in 735	3
Arnold-Chiari syndrome	One in 1200	1
Choanal atresia	One in 7000	1
Biliary atresia	One in 10,000–15,000	1
Rubinstein-Taybi syndrome	One in 100,000–125,000	1

Birth anomalies in natural conception versus oocyte cryopreservation, listed most common to most rare.
Adapted from N. Noyes, E. Porcu & A. Borini, 2009

From the table one can assess that there were no neural tube defects and that the defect of skin hemangioma is the same range as babies born from natural conception. Additionally, cleft lip and palate as well as cardiac defects occurred less in the babies born as a result of oocyte cryopreservation.

Researchers have tried to improve the process of oocyte cryopreservation. One issue that arose from freezing the eggs was that extensive intracellular ice formed during freezing. Extensive intracellular ice can cause cellular disruption in the oocyte during the oocyte cryopreservation process. This can possibly be improved by using cryoprotectants such as, propanediol and sucrose to increase the extent of the dehydration process. The aim of the study done by researchers in Infertility and IVF Center of Buda was to introduce their preliminary clinical results with oocyte cryopreservation. They used slow cooling as the procedure to freeze the eggs. They specifically used propanediol (1.5M) and sucrose (0.3 M) as the cryoprotectants. After incubating the oocytes for 4-6 hours, the oocytes were thawed, fertilized and embryos were transferred into twenty-nine patients. Out of one hundred ten cryopreserved eggs, eighty-four survived. This is a 76% survival rate, which is high but not optimal. From fifty-two embryo transfers, seven resulted in clinical pregnancies, which is 7.3% implantation rate per egg thawed. Chorion biopsies that were performed indicated that there were no chromosomal abnormalities. Out of the seven pregnancies, five of them resulted in four singletons and one set of twins. One was still ongoing at the time of the study and the seventh spontaneously aborted in the tenth week. No abnormalities were indicated in the study. Additionally, there was only a small difference in the pregnancy rate, 33% versus 24%, between those pregnancies that resulted from frozen oocytes and those that resulted from fresh oocytes. As indicated from published literature at that time of the research, fifteen to thirty oocytes were needed in order to achieve one pregnancy. Previously, one hundred to one hundred fifty were needed to achieve one pregnancy. (Konc, et. al. 2008) The results show that oocyte cryopreservation is improving over time.

Vitrification is also known as ultra-rapid cooling. In recent years, vitrification has proven the superior method. Compared to slow freezing, vitrification results in higher oocyte survival and fertilization. (Cil, et. al. 2013) In a study done to compare the outcome of the two methods, the survival, fertilization, pregnancy and implantation rates were 57.9% versus 78.9%, 64.6% versus 72.8%, 7.6% versus 18.2% and 4.3% versus 9.3% correspondingly. The rates were higher in all steps for the vitrification method. (Fadini, et al., 2009)

The duration of cryostorage doesn't undesirably affect the thawing of frozen oocytes. A study was done to see if there is any influence on the outcome of thawing cryopreserved oocytes. There were three groups in the experiment. Group A's eggs were cryostored for one to three months, group B's eggs were cryostored for four to six months and group C's were cryostored for seven to forty eight months. Group C was further divided into three subgroups. Group C1, was cryostored for seven to nine months, group C2 was cryostored for ten to twelve months and group C3 was cryostored for a total of thirteen to forty eight months. The researchers found no significant difference, from groups A, B and C, in the main outcome measurements, which were oocyte survival after thawing, fertilization, implantation, embryo development and quality and birth. Oocytes can be cryopreserved for numerous years without having an effect on the oocytes quality and performance after thawing. (Parmegiani, et. al. 2009)

One factor that may affect the outcome of oocyte cryopreservation is the age. The value of freezing an older woman's oocytes is controversial. (Zhang, et. al. 2015) The rate of implantation of the fertilized egg that resulted from the slow freezing and vitrification methods declines with age. A study was done to collect data on the probability of live birth as of function of age. The

researchers found that live births occur from the slow freezing method until age forty-two, and until age forty-four from the vitrification method. They limited their results to the age range of twenty five to forty two years old, as there were only few cycles that were above or below the twenty-five through forty-two year old range. This study's data was on patients that were infertile. The study was not specifically performed on post cancer patients. (Cil, et. al. 2013)

Furthermore, a study was implemented in order to report the oocyte cryopreservation experience in women aged forty and older. One hundred fifty eight women, aged forty to forty-nine, underwent minimal ovarian stimulation to retrieve their eggs. A total of five hundred thirty two eggs were retrieved and frozen. Four of the women did not have any oocytes retrieved. A total of four hundred eighty five embryos were formed. Out of the four hundred eighty five embryos, only fifty-seven were relatively healthy. Six clinical pregnancies were achieved. Only three resulted in live births. There was a 5.3 % live birth rate per embryo transfer. The other three pregnancies were spontaneously aborted. As per the data, a woman aged forty and older can give birth to a baby after undergoing the process of freezing her eggs. However, there is a low chance that it will indeed happen, as there is a 5.3% chance that the woman will give birth. (Zhang, et. al. 2015)

A woman who has ovarian cancer may risk surgical menopause. Oocyte cryopreservation can be an option for woman facing ovarian cancer. It could also help patients that need to have a one or both ovaries removed. A twenty six year old woman with borderline ovarian tumors had her oocytes cryopreserved after a right adnexectomy. Seven mature eggs were retrieved and frozen. Thirty-nine months later, the woman underwent a left ovariectomy. Three embryos were transferred into the woman's uterus. Endometrial growth was achieved with the help of hormonal replacement treatment. The woman gave birth to healthy twin babies. (Porcu, et al., 2008)

Oocyte cryopreservation can help women have healthy babies even when they don't have their own healthy eggs. Remaining eggs from oocyte cryopreservation cycles can be saved and donated to another couple that are experiencing fertility complications. A study was done in which twenty-eight infertile women froze their oocytes. Twelve of the twenty-eight women had their frozen oocytes thawed. Three of the women used their own eggs in IVF treatment and the other twelve donated their eggs to other women. Premature ovarian failure, physiological menopause, abnormal karyotype and poor ovarian reserve are the reasons that the twelve women needed to receive oocytes from other women. Seven women became pregnant. Six of the seven used donated oocytes. A total of 6 healthy babies were born including a set of twins. The other 2 pregnancies were aborted due to a blighted ovum. (Li, et al., 2005)

Oocyte cryopreservation may not be for everyone. Women with cancers that need to be treated immediately after diagnosis, may not be a candidate for oocyte cryopreservation. This is because oocyte cryopreservation requires ovarian stimulation and retrieval. This can take an average of twelve days. (Noyes, et al., 2011) Additionally, some women that have breast cancer might run into issues with preserving their eggs. This is because estrogen levels rise during ovarian stimulation. High levels of estrogen might not be safe for women with breast cancer. (Rodriguez-Wallberg, Oktay, 2010)

Furthermore, some women may not want to undergo oocyte cryopreservation as it can cause a woman to have a risk having of intra-abdominal bleeding and ovarian hyper stimulation syndrome. However, there is a very low percentage rate of this risk. (Noyes, et al., 2011) Additionally, women with cancers may not be able to cryopreserve their eggs due to economic issues. It is a very expensive procedure. According to NYU Langone Medical Center's website, oocyte cryopreservation can cost about $16,000- $20,000. This includes initial office consultation, egg cryopreservation cycle, prerequisite blood testing and screening medication. As of 2010, cancer patients are generally not offered insurance coverage for oocyte cryopreservation. (Noyes, et al., 2011) Consequently, cryopreservation may not be an option for people that are struggling financially.

Conclusion

Oocyte cryopreservation is a viable method that enables women post mitotic therapies to have healthy offspring. As per the research discussed above, many women were able to have a healthy baby because they froze their oocytes. Even when abnormalities were reported, they were basically within normal range. Oocyte cryopreservation has even enabled a woman with borderline ovarian cancer to have a healthy offspring. Even though the value of freezing an older woman's oocytes is controversial and the rate of implantation of the fertilized egg declines with age, data has shown that oocyte cryopreservation can enable older women to have healthy babies.

There are different variables that may increase the outcome of oocyte cryopreservation. The vitrification method has shown to be the efficient and more reliable method. Cryoprotectants such as, propanediol and sucrose can increase the extent of the dehydration process and thereby prevent the oocytes from disrupting. Furthermore, the duration of cryostorage doesn't undesirably affect the outcome of oocyte cryopreservation.

Oocyte cryopreservation may not be for everyone due to economic reasons and timing of the anti-mitotic therapies. However,

it is a means that enable women to have healthy children even after their oocyte quality and quantity diminish as result of the cancer treatment.

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Mechanism of Fecal Bacteriotherapy in Treating Clostridium Difficile Infection and GI Tract Disorders

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Abstract
Clostridium difficile infection, an increasingly prevalent and virulent condition, is often resistant to treatment. Standard antibiotic therapy is rarely efficient when used to treat recurrent C. difficile infection. Fecal microbiota transplantation (FMT) is a safe, effective and inexpensive treatment that has a cure rate of about 90%, according to clinical trials and reports. This approach may also be applicable in treating ulcerative colitis, a type of inflammatory bowel disease, on the basis of the restoration of flora imbalances. Additionally, recent data suggests that a disproportion in composition of the gut microbiome may contribute to obesity. FMT, may restore a healthy balance. Using information from Touro College's database, this article discusses the reason behind the success of fecal microbiota transplantation when used to treat Clostridium difficile infection and ulcerative colitis. The effect of diet, environment and geography on the bacterial flora is also explored.

Introduction

The human gut microbiome consists of over 100 trillion microbial cells, exceeding the amount of human cells by a factor of 10. Bacteria are found in various parts of the body including nasal passages, oral cavity, skin, urogenital system and the gastrointestinal tract. The gut microbiota carries out a variety of valuable tasks for their human hosts; it assists in the development of innate and adaptive immunity, serves as an energy source, and kills foreign organisms. (Dave, et al. 2012). The quality and composition of the gut microbiome depends largely on diet, medication and weight of its host. The microbiota is capable of secreting or altering the production of molecules that affect both weight gain or loss and fat mass. (Rosenbaum, et al. 2015)

Colonization of the microbial microflora of the internal and external surfaces in a human begins at birth and is termed indigenous bacterial microflora. (Van der Waaij, 1989) The route of delivery when a baby is born determines which type of bacteria will develop. Infants delivered vaginally have microbiota comparable to their own mother's vagina, whereas infants born via Cesarean section develop microbes similar to the bacteria found on the skin surface. (Dominguez-Bello, et al. 2010) After birth, microbes develop and colonize, benefitting both themselves and their host, each contributing something vital to the other. (Van der Waaij 1989) For example, the anaerobic bacteria in our distal gut breaks down complex polysaccharides and ferments the resulting monosaccharides into short chain fatty acids. This is a mutually beneficial relationship; the microbes live in a sheltered anaerobic environment and receive a rich assortment of glycans while offering the host a source of carbon and energy. (Backhed, et al. 2005)

Of all the microbial communities being studied by human microbiome researchers, the gastrointestinal tract (GI) holds a promising future for the discovery of new concepts and therapies. The large colon holds the most abundant and diverse communities of microbes; it has both local and systemic effects on the body. (Dave et al. 2012) The gut microbiota can be thought of as a "microbial organ placed within a host organ," it plays vital roles in metabolism and immune system function. It is known that the microbial communities have a method of communication; they also can consume, store and redistribute energy. (Bakhed, et al. 2005)

Fecal Microbiota Transplantation (FMT) is an FDA experimental drug targeted to treat Clostridium difficile associated diseases (CDAD). It has been successfully used to treat patients with ulcerative colitis (UC) and different forms of inflammatory bowel diseases (IBD). Additionally, by comparing and analyzing the gut microbiome of lean and obese individuals, researchers have been able to hypothesize a cause for obesity and other weight issues. Using this knowledge, microbiologists can manipulate the microbiome and formulate a therapy to decrease obesity worldwide. This research can result in a new kind of pharmacopeia; implanting a more refined probiotic to combat infection, inflammation and dysbiosis.

Methods and Materials

To investigate the mechanism of fecal bacteriotherapy and how it can be utilized to treat Clostridium difficile associated diseases and other gastrointestinal disorders, many original research articles and reviews have been read. Touro College's online database was employed, particularly EBSCO, PubMed, ProQuest and Google scholar. Further research was done by perusing the footnotes and references in review papers to find original research.

Discussion

Fecal Microbiota Transplantation

The indigenous microbiota is vital to the human host, yet it can be altered by various factors of modern day life, most commonly repeated doses of antibiotics. (Van der Waaij, 1989). Dethlefsen et al. tested the fecal microbiota of adults before and after oral administration of ciprofloxacin for five days using pyrosequencing. A rapid reduction in richness of bacterial phyla was seen, resulting in decreased diversity, fullness and balance in the microbiome. Four weeks following the completion of the antibiotic, the microbiota returned to baseline. However,

in some individuals, it took 6 months for the bacterial phyla to completely return to normal. (Dethlefsen et al., 2008) Infectious diseases of the past were thought to be linked with bacteria capable of causing disease in a susceptible host. However, present day infectious diseases are linked to host interaction with potentially pathogenic bacteria that make up the microflora of the digestive tract (Van der Waaij, 1989). A particular infection that results from a disruption of the gut microbiota is Clostridium difficile infection. Clostridium difficile is an anaerobic, spore forming bacillus that produces 2 exotoxins that damage the intestinal mucosa. It is the most commonly recognized cause of anti-microbial associated diarrhea. Clostridium difficile associated disease mainly affects the large colon, causing diarrhea and pseudomembranous colitis. If progressed, the infection can lead to toxic megacolon, sepsis and death (McDonald et al., 2006). The microbial colonies that regularly control the Clostridium difficile communities are inhibited and blocked by antibiotics. Because C. difficile spores are generally resistant to antibiotics, this allows them to germinate back into their vegetative state after antibiotic treatment has ended. If the remaining microbes in the intestine cannot get rid of the infection, Clostridium difficile can multiply and release the toxins that return the disease symptoms. (Bakken et al., 2011)

There are different treatments that exist for recurrent Clostridium difficile infection, many of which involve the use of stronger antibiotics, such as vancomycin and metronidazole. However, as Dr. Johan Bakken says, "the use of antibiotics represents a double-edged sword by suppressing both the pathogen as well as the protective microbiota." In other words, repeated and extended uses of antibiotics have negative effects on the natural gut flora. It is a possible risk factor for further infection due to the continuously altered state of gut microbes. (Bakken et al. 2011)

Another approach to treat recurrent Clostridium difficile infection is to restore the damaged microbial communities using probiotics. Saccharomyces bouldarii is a yeast that colonizes in the colon within 3 days of ingestion, survives gastric acids of the stomach and is cleared from the colon once treatment is discontinued. The effectiveness of the probiotic as an add-on to antibiotics was tested in two trials. The treatment did not decrease recurrence rates in those with their first episode of Clostridium difficile infection but it did decrease the frequency of relapses in those with the recurrent infection. The second trial showed effectiveness only in patients that were on a high dose of vancomycin. (McFarland et al., 1994) (Bakken et al., 2011) Thus, probiotics may have a role in treatment of Clostridium difficile infection, but their effectiveness is less than ideal. (Kelly, LaMont 2008)

Fecal Bacteriotherapy, or fecal microbiota transplantation (FMT), is an innovative and effective treatment for Clostridium difficile infection and other infections of the colon, including ulcerative colitis. It involves implanting stool from a healthy donor into a recipient's diseased colon, thereby allowing a newer and healthier microbiome to develop and flourish. From the trials done and the case studies reported, it seems that recurrent Clostridium difficile infection can be cured with a single treatment of FMT. The logic behind FMT is straightforward: strong antibiotics can cause an imbalance in the normal intestinal flora and reduce colonization resistance, which allows C. difficile to grow and cause symptoms of infection. With the introduction of healthy donor feces, the imbalance can be corrected, the cycle interrupted and the normal colon function returns. (Bakken, et al. 2011)

Although the first four patients to be treated with FMT in the 20th century was in 1958 for pseudomembranous enterocolitis, (Bakken, et al. 2011) the use of fecal material as a treatment for diseases dates back more than 2500 years ago in ancient China. During the Dong-jin dynasty in the 4th century China, Ge Hong, a well-known doctor described the use of human fecal suspension by mouth for people suffering from severe diarrhea or food poisoning. This treatment was considered a medical miracle that brought patients back from the brink of death. In the 16th century, during the Ming dynasty, Li Shizen prescribed a series of fecal material doses to treat abdominal diseases that caused severe diarrhea, fever, pain, vomiting, and constipation. This treatment was called "yellow soup" for aesthetic reasons. (Zhang, et al. 2012)

Nowadays however, the procedure is done in a more sterilized manner and reported to have been successful via colonoscopy (Persky, Brandt 2010), nasogastric tube, (Aas, et al. 2003) nasoduodenal tube, (van Nood, et al. 2013) and self-administered fecal enemas. (Silverman, et al. 2010) One method of implantation was not found to be superior over the other. (Rohlke, Stollman 2012)

Currently, the only FDA approved drug to treat C. difficile infection is the antibiotic vancomycin, which is inadequate for patients suffering from recurrent CDI. It is estimated that 80% of patients treated with the standard antibiotic therapy are cured, however, the remaining 20% are likely to recur 3-28 days after discontinuation of antibiotics. Disease caused by C. difficile has been shown to increase the length of stay of patients by a mean of 7 days, incurring an additional $10,500 in health care charges for every infected patient. (McFarland, et al. 1994) Alternative treatments, including probiotics, surgery and administration of different antibiotics, have been suggested, but none were effective, safe and inexpensive. Fecal microbiota transplant seems to

fit the criteria of being inexpensive, highly effective and safe. In fact, a study done to compare 4 different treatments for CDAD; metronidazole, vancomycin, fidaxomicin and FMT demonstrated that FMT was the "most cost-effective strategy." The incremental cost-effectiveness ratio (ICER) was $17, 016 relative to oral vancomycin. (Konijeti, et al. 2014) It is "the only therapy that restores the phylogenic richness of the recipient's intestinal microbiota without prolonging the perturbation of the normal microbiotic composition." (Bakken, et al. 2011)

A study comparing the fecal material of a patient before and after FMT illustrates the effectiveness of the transplant. Before FMT, the patient's fecal material was composed of atypical bacterial populations including Veillonella, Clostrudium, Lactobacillus, and Streptococcus. Two weeks after FMT, Bacteroidetes began to appear and four weeks after the transplant, the stool studies were culture negative for C. difficile. The recipient's fecal bacterial composition greatly resembled the donor. Throughout the months following FMT the patient reported issues of loose stool, but those issues were resolved within two weeks without therapy. Six months later, the patient was completely cured from C. difficile infection. (Khoruts, et al. 2010)

Further data is needed to assess the effectiveness of FMT, yet given the promising data to date and anticipated research data on the intestinal microbiome, it seems to be the most reasonable treatment for recurrent C. difficile infection (CDI). Bakken et al. put together a guideline for the proper administration of FMT. Included in the guideline is a detailed description of primary indication, donor selection and directions for preparing and administering the stool. Many trials and studies that involving FMT used this guideline as instructions on how to proceed. In order to be eligible for FMT, a patient either has to have recurrent or relapsing CDI with at least three episodes of mild-moderate CDI and a failure of a 6-8 week taper with vancomycin. Patients on major immunosuppressive agents, decompensated liver cirrhosis or have had a recent bone marrow transplant cannot receive the fecal microbiota transplant as it may cause adverse effects.

Currently, little or no data is available to suggest that any reasons, other than the exclusion criteria based on medical history and laboratory testing, would recommend a specific donor over another. However, using intimate contacts as donors minimizes the risk of transmitting an infectious disease. Donors are interviewed thoroughly and must answer a detailed questionnaire regarding their past medical history. Donors are disqualified if they test positive for HIV, Hepatitis B or C infections or had exposure to HIV or viral hepatitis. Individuals that have high risk sexual behaviors, use illicit drugs, had tattoo or body piercing within 6 months or have a history of incarceration cannot donate stool for FMT. Once a donor passes the medical and laboratory tests, their stool is screened for enteric pathogens such as C. difficile toxin A and B, fecal Giardia and Cryptosporidium antigens and Helicobacter pylori fecal antigen. (Bakken, et al. 2011)

The stool donor may or may not take an osmotic laxative the night before the procedure. The stool should be used as soon as possible, preferably within 6 hours. The choice of diluents may vary among practitioners, but either preservative-free normal saline or 4% milk is combined with the stool in a household blender. It should be mixed until it reaches a homogenous slurry consistency and filtered to remove particulate. The slurry stool mixture is to be used immediately. The optimal volume for transplantation has not been established yet; however if by nasogastric tube or via other routes from above, smaller volumes (25-50 mL) should be used and if by colonoscopy or other routes from below, larger volumes (250-500 mL) may be used. (Bakken et. al. 2011)

This is a basic outline on how a practitioner would administer fecal microbiota transplant. However, "practical and aesthetic barriers" have slowed down the widespread use of FMT, despite its success in dramatically resolving CDI after one treatment. Enrollment and screening of donors is a tedious process linked with substantial costs, thereby inhibiting the use of FMT in severe situations. Youngster et al. suggests that by establishing a repository of prescreened frozen donor stool, this treatment may be more easily obtainable from a broader population. He conducted a trial to evaluate the efficacy of FMT in treating relapsing CDI in a pilot cohort of 20 patients, comparing colonoscopic and nasogastric tube administration.

The donors for the study underwent extensive physical examinations and general laboratory screening tests to ensure that all results were within normal range for age and sex. Renal function and electrolytes, complete blood count with differential, complete liver function tests and fecal occult blood testing were some of the tests that fecal donors underwent. All fecal donations were set aside for four weeks to allow retesting of donors for HIV and hepatitis B and C before the feces was inoculated. To facilitate manipulation of the fecal material, donors were asked to take milk of magnesia the day before the donation. The feces was mixed in a commercial blender with normal saline and then were passed through 4 sieves to get rid of any particulate. The final product was concentrated 3-fold by centrifugation and then resuspended in sterile saline with 10% glycerol added as a cytoprotectant. The resulting mixture was frozen at -80°C. Each filtered sample was calculated to have come from 41g of fecal material. Inocula were stored frozen for up to 156 days and thawed in a 37°C water bath, then kept on ice until delivery.

Patients were ordered to stop taking antibiotics for 2 days before the procedure. Recipients designated for a colonoscopic administration underwent a standard bowel preparation with 4 liters of polyethylene glycol electrolyte solution. Then, 90 cc of the thawed inoculum was placed in the right colon via endoscopy. The fecal material was further diluted to 250 cc for adults and 160 cc for pediatric recipients. Patients were given a single dose of loperamide at the time of the procedure in order to retain the material for as long as possible. The patients assigned to nasogastric tube administration were given omeprazole orally for 2 days before the procedure, to ensure that the fecal bacteria will not be affected by the acids in the stomach. After inserting the nasogastric tube, 90 cc of inoculum was administered. In order to minimize vomiting and aspiration, the inoculum was not diluted. (Youngster, et al. 2014)

The goal of the trial was the clinical resolution of diarrhea off antibiotics for C. difficile infection, without a recurrent episode within 8 weeks. Patients who showed no improvement were offered a second FMT by their preferred route of administration, using the same donor inoculum to minimize exposure to infectious agents.

Of the 20 patients in both study arms, 14 were cured after the first FMT; 8 of them were in the colonoscopy group and 6 in the nasogastric tube group. One patient refused a second transplant but the remaining 5 patients were given a second infusion via NGT using feces from their original donors. Four patients were cured after the second FMT, resulting in an overall cure rate of 90%. No patient relapsed within the 8 week period following the initial cure.

There were some serious adverse effects that were deemed unrelated to the trial by investigators and the Institutional review board, which may have been caused by the poor health of the patients with recurrent CDI. 2 patients died; one from an obstructive pulmonary disease and another from metastatic laryngeal cancer. A third patient was diagnosed with adenocarcinoma of the esophagus and a fourth (treated via NGT) was hospitalized for Fourier gangrene.

This trial demonstrated that the transplantation of unrelated frozen donor stool is effective in treating recurrent CDI with an overall cure rate of 90%. Additionally, NGT appears to be a feasible route of administration for elderly patients with the infection. One 89 year old female patient had 16 documented cases of CDI in the previous 15 months. She was hospitalized 4 times and placed on the intensive care unit twice due to the infection. After two infusions, she was cured and was free from any symptoms of the disease off treatment for 12 months. (Youngster, et. al. 2014)

Seeing the success with frozen inocula, Youngster et al. directed another study evaluating the effectiveness of oral capsulized frozen fecal microbiota transplantation in treating relapsing C. difficile infection. The donors were thoroughly screened, as described in the previous study (and the guideline by Bakken, et al. 2011) and similar processing of the fecal material took place. To ensure that the acidic environment of the stomach will not destroy the capsule, commercially available acid-resistant hypromellose capsules were used. The stability of the capsules was tested by mimicking the acidic environment of the stomach. Trypan blue filled capsules were used, at a temperature of 37°C and a pH of 3 or less, and the capsules were stable for 115 minutes before the dye was released.

From July 2013 through January 2014, a total of 20 patients were treated using stool obtained from four donors. The recipients followed up six months later in July 2014. The goal of the study was the clinical resolution of diarrhea, while not receiving antibiotic for C. difficile, without relapse within 8 weeks.

The procedure went as follows: each patient was given 15 capsules, directly from an investigator on 2 consecutive days. 2 days prior to FMT, patients were ordered to stop all antibiotics. They were asked to fast 4 hours prior to and 1 hour post taking the capsule for both days. If there was no improvement after 72 hours, patients were retested and offered another treatment for positive test results.

From the 20 patients treated, 14 had clinical resolution of diarrhea after the first FMT. The remaining 6 patients were retreated 7 days after the first procedure. 5 of the 6 had resolution of diarrhea after the second FMT, however one patient relapsed within the 8 week follow up time period. Thus the overall rate of diarrheal resolution was 90%. (Youngster et. al. 2014)

Fecal microbiota transplantation used to treat C. difficile infection is a highly effective, safe and inexpensive method as described studies and research. Very few, if not any, complications were caused by FMT. The fecal material can be manipulated and can be transplanted as a liquid via NGT or colonoscopy and can also be ingested as a capsule. FMT is currently an FDA experimental drug and therefore the trials and studies are with small samples and lack of placebo or other trial to compare. Granted, the trials and studies done are encouraging, however this may be due to the small sample size. Furthermore, even with careful screening, there is a potential risk of transmission of infections via microbiota transplantation. Larger studies would be needed to confirm these outcomes and to evaluate long-term safety and effectiveness. (Youngster et. al 2014)

Fecal Microbiota Transplant for Ulcerative Colitis

Encouraged by the success for fecal microbiota transplant in the treatment of C. difficile infection, researchers looked to other conditions related to GI microbial imbalance, such as inflammatory bowel disease (IBD), obesity and diabetes mellitus. (Borody, et al. 2103) Inflammatory bowel diseases are thought to be caused by an overall community dysbiosis rather than a single underlying pathogen. In patients with IBD, an increase in Actinobacteria and Proteobacteria and a decrease of Lachnospiraceae, Bacteroidetes, Clostridium leptum, and Bifidobacteria are observed. (Clemente, et. al. 2012)

Ulcerative colitis (UC), a type of IBD, is a chronic devastating inflammatory bowel disease that affects the large intestine. Unfortunately, the current medications used to treat UC have significant adverse effects; they mainly target the inflammation without focusing on the dysbiosis. (Kunde, et al. 2013) The colonic microbiome in UC patients lacks diversity and is dysfunctional. The cause of inflammation of the colon in UC is hypothesized to be caused by inappropriate stimulation of the innate mucosal immune system because of dysbiosis in a genetically susceptible individual. Borody et al. asks the following question: C. difficile infection causes reduced microbial diversity which results in symptoms of colitis; if FMT can reverse this, perhaps a similar treatment can be used to treat ulcerative colitis? (Borody, et al. 2013)

Borody et. al. conducted a trial assessing the clinical, histological and colonoscopic effects of "human probiotic infusion" (HPI), or fecal material transplant in six selected patients with ulcerative colitis. All patients had suffered from severe recurrent symptoms of UC and each, respectively, were on series of moderate to high dose steroids and anti-inflammatory medications. The donors were chosen by the recipient patients and screened thoroughly to prevent any transmission of parasites or bacterial pathogens. The feces (200–300 g) was diluted in 200 to 300 mL normal saline and administered to the patient via retention enema within 10 minutes of its preparation. This process was repeated daily for 5 consecutive days. One week following HPI, some symptoms of ulcerative colitis had resolved in several patients. A complete reversal of symptoms was achieved in all patients 4 months post HPI, by which all other UC medications had been discontinued. From 1 to 13 years later, patients remained asymptomatic with a healthy colonoscopic appearance and normal histology.

However, the pathogenesis of UC is unknown since it is characterized by persistent inflammation. It is suggested that the disease is driven by a chronic infection caused by the absence of vital luminal anaerobes. If the ability of the normal flora to resist colonization by pathogens is hindered, for example, by previous antibiotic use, the numbers of protective anaerobes in the colon, such as Bacteroides, decrease. This would allow the establishment of other pathogenic anaerobes or spore bearing bacteria and a form of recurrent, chronic colitis. The persistence of UC may be due to the implantation of spores in the mucosal surface, analogous to Clostridium difficile infection. Antibiotics may help briefly but are unlikely to be curative because the spores are not eliminated. (Borody et. al. 2003)

Kunde et. al. conducted a pilot study to evaluate the efficiency and safety of fecal microbiota transplantation in children with ulcerative colitis. Ten children, ages 7 to 21 with mild to moderate UC according to the pediatric ulcerative colitis activity index (PUCAI) were enrolled in the study. Clinical response and improvement was defined as decrease in PUCAI by more than 15 points on the index. A decrease in PUCAI to less than 10 points was considered clinical remission. Each donor produced a 90 gram stool sample every day which was blended with 250 mL normal saline and filtered through 2 gauze pieces to remove sediments. It was then separated into 4 aliquots of 60 mL each and kept in a warm water bath (37°) until administration. Each patient received FMT as retention enema for 1 hour (60-mL enema every 15 minutes) daily for 5 days. One subject however, was not able to tolerate the retention enema for 3 consecutive days and was therefore not included in the results. Within one week following the procedure, 7 of the 9 subjects (78%) showed clinical response. Four weeks after FMT, six of the nine patients (67%) maintained the clinical response. However, 3 of the nine subjects (33%) went into clinical remission one week following FMT. All of the adverse events related to FMT, besides fever, were self-limiting, and did not require intervention from health care providers. The majority of them were similar to subjects' baseline symptoms of UC such as bloating and diarrhea.

The dramatic response to FMT after one week was not achieved in all subjects, perhaps due to the severity or duration of the disease, or the ability to retain larger FMT volumes for longer periods. Using FMT via fecal enema to treat UC in children is feasible, as proved in the trial. However, the sample size was small and therefore limits future indication. In order to improve FMT a standardization procedure would need to be arranged for donor selection and ideal routes of administration. (Kunde, et al. 2013)

The Gut Microbiota and Obesity

The World Health Organization (WHO) reports that in 2014, more than 1.9 billion adults, 18 years and older were overweight and of these, over 600 million were obese. Obesity is a growing, worldwide epidemic that has been accompanied by increases in obesity related disorders, including type II diabetes, hypertension and nonalcoholic fatty liver disease. Much emphasis has been placed on the dietary excess or host genes in obese individuals.

Yet, in addition to increased calories or shifts in host metabolism, the composition of the intestinal microbiota can contribute to the progression of obesity. Both diet induced obesity and gene induced obesity have microbe independent and microbe dependent pathways. (Baackhed et al. 2004, Cox, Blaser 2013)

A study was done to investigate the effect that diet has on shaping the gut microbiome. The fecal material of 15 European children and that of 14 children from a rural African village, Bukina Faso were compared and tested with 16S rRNA sequencing, and biochemical analysis. The goal of the study was to explore three questions: the split of bacterial diversity between the two populations, the connection between the gut microbiota and diet, and the distribution of well-known bacterial pathogens within the two populations given the different hygienic and geographic conditions.

The diet of Bukina Faso children is mostly vegetarian; rich in plant polysaccharides, starch and fiber and low in fat and animal protein. All the food was grown and harvested nearby the village by women. The European children were eating a typical Western diet high in animal protein, sugar, starch and fat and low in fiber. A dataset consisting of 438, 219 of filtered, high quality, classifiable 16S rRNA was generated. Of all of the sequences in all the Bukina Faso and European samples 92.4% were found to belong to the most populated bacterial phyla: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. However, it was the proportions that stood out: Actinobacteria and Bacteroidetes were more abundant in the Bukina Faso children than the European children 10.1% versus 6.7% and 57.7% versus 22.4%, whereas Firmicutes and Proteobacteria were represented more in EUROPEAN than BUKINA FASO children, 63.7% versus 27.3% and 6.7% versus 0.8%. Since Prevotella, Xylanibaeter (Bacteroidetes) and Treponema (Spirochaetes) were exclusively found in the Bukina Faso children. De Filippo et. al. hypothesized that these 3 genera could be a result of high fiber intake due to the environmental factors distinguishing the two populations (diet, hygiene, climate and geography). Perhaps these three groups contribute to the extraction of metabolic energy from ingested plant polysaccharides. It is reasonable to deduce that the increase in the Firmicutes to Bacteroidetes (F/B) ratio in European children possibly triggered by their high-calorie diet might predispose them to future obesity. This Firmicutes/Bacteroidetes ratio may also be considered a useful obesity biomarker. (De Filippo, et al. 2010)

A study done by Turnbaugh et al. further demonstrates the effect of diet on the gut microbiota. Mice raised in a germ free environment without any exposure to microbes, and then colonized at specific life stages with different microbial communities are referred to as gnotobiotic animals. Adult human fecal microbiota was transplanted into the recipient mice to establish a stable gut community similar their donors. An aliquot of the fecal dilution was introduced by gavage to the 15 recipient mice. These mice were maintained on a low fat plant polysaccharide (LF/PP) rich diet and fecal samples were collected 1 day, 1 week and 1 month after colonization. Half of the mice were then switched to a high-sugar, high-fat Western diet. The LF/PP and Western diet were maintained for 2 additional months, after which the mice were killed. Results showed that when the diet of the mice were switched from a LF/PP regimen to a high-fat, high sugar "Western" diet the structure of the microbiota was shifted within a single day. The representation of metabolic pathways in the microbiome were changed and the microbiome gene expression was altered. The mice fed a Western diet had an increased representation of the Erysipelotrichi class of bacteria within the Firmicutes phylum relative to mice fed the LF/PP diet. In mice that consumed a Western diet, the Bacilli, another class of the Firmicutes was abundant and a significant decrease in member of Bacteroidetes was found. These mice increased adiposity, demonstrating that this trait is transmissible via microbiota transplantation. Using germ free mice, researchers were able to see a clear correlation between the foods the mice were fed and its effect on the composition of their gut microbiota. Gnotobiotic technology is a promising tool for discovering new classes of probiotics that affect nutrient harvest in a given diet. (Turnbaugh, et al.2008)

A study comparing the fecal material of 31 adult female monozygotic and 23 dizygotic twin pairs and their mothers, concordant for obesity or leanness, was performed, to address how host genotype, environmental exposures and host adiposity influence the gut microbiome. All twins were 25-32 years old of European or African ancestry. Analysis of 16S rRNA datasets produced by the three PCR-based methods, plus shotgun sequencing of community DNA, revealed a lower proportion of Bacteroidetes and a higher proportion of Actinobacteria in obese versus lean Europeanropean and African individuals. Overall, across many methods, obesity was associated with a decrease in diversity of the gut microbiota. "This reduced diversity suggests an analogy: the obese gut microbiota is not like a rainforest or reef, which are adapted to high energy flux and are highly diverse, but rather may be more like a fertilizer runoff where a reduced diversity microbial community blooms with abnormal energy input." (Turnbaugh, et al. 2009)

There is an interesting case study regarding the transmission of "obese bacteria." A 32 year old female patient with recurrent CDI elected to undergo FMT after many failed antibiotic therapies. Around the same time, she tested positive for Helicobacter pylori.

The patient chose her 16 year old daughter, who was approximately 140 pounds to be her stool donor, and, subsequently, her weight increased to 170 pounds. The patient herself was 136 pounds at the time of the transplant. The patient was retreated for H. pylori with metronidazole, tetracycline, bismuth and proton pump inhibitor, and two weeks later she had the FMT. Six hundred cc. of the suspension of the donor stool in sterile water was infused through a colonoscope. The patient dramatically improved after the procedure and CDI did not recur. However, 16 months later, the patient presented again, and reported an unintentional weight gain of 34 pounds. Despite a medically supervised liquid protein diet and exercise program she had been unable to lose weight. Her weight continued to climb despite the diet and exercise and at 36 months post FMT she weighed 177 pounds.

Alang and Kelly offer a several of hypotheses to explain the rapid weight gain following FMT. There is a known link between H. pylori treatment and weight gain, resulting from the increase in appetite after the suppression of the bacteria. Yet, the patient was never obese before FMT and the fact that the stool donor also gained weight leads to the possibility that her obesity resulted from the transplant (Alang, Kelly 2014).

This hypothesis is supported in a study testing the fecal material of twins discordant for obesity. This experiment offers an opportunity to examine connections between obesity and its associated metabolic disorders, diet, and the gut microbiota. Ridaura et al. conducted a study by transplanting the fecal material of twins discordant for obesity into mice in order to replicate the donors' microbiota community in the colon. The impact of these differences on the body composition and metabolic phenotypes were able to be detected, and the effects of diet-by-microbiota interactions analyzed. The mice harboring the transplanted microbiomes from the obese twins exhibited higher expression of microbial genes involved in detoxification and stress responses, in biosynthesis of cobalamin and metabolism of essential amino acids. In contrast, the transplanted microbiomes from lean co-twins exhibited higher expression of genes involved in digestion of plant-derived polysaccharides, fermentation to butyrate and propionate. The results suggest that the transplanted microbiota of the lean co-twins had greater capacity to breakdown and ferment polysaccharides than the microbiota of their obese co-twins. (Ridaura, et al. 2013)

From these studies, it is evident that the gut microbiota reflects the health, diet and metabolism of its host. It seems that the composition of the phyla that make up the gut microbiome in healthy individuals is properly balanced. However, a disrupted microbiome can contribute to obesity by altering energy extraction from food.

Conclusion

By transplanting stool from a healthy donor into a recipient's diseased colon via fecal bacteriotherapy, a newer and healthier microbiome can flourish and the function of the large intestine can be restored. Fecal microbiota transplant has been proven by many clinical trials to be a safe, effective and inexpensive procedure. FMT has been successful in treating many cases C. difficile infection, as well as ulcerative colitis and has a promising future in the field of diet and obesity. The stool material can be readily administered in different ways, as a slurry consistency administered via colonoscopy or nasogastric tube, or packaged in a pill and taken orally; this therapy is easily accessible to almost all populations.

However, one potential drawback of FMT is the small sample size in many of the clinical trials done. Additionally, fecal bacteriotherapy may not be the ideal treatment for every individual suffering with GI tract disorders, for example, it may not be ideal in immunocompromised patients. However, FMT is an FDA approved drug and will undergo further standardization regarding donors and the preparations and administration of the stool.

The future of the gut microbiota related treatment is an active topic of research; much is anticipated from this exploration into the microbiome.

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Artificial Sweeteners and Weight Gain: Fighting or Feeding the Obesity Epidemic?

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Abstract
Our world has developed an obsession with weight control and, as a result, has begun replacing high calorie foods with low-fat and non-caloric substitutes. Artificial sweeteners are a widely used solution to this growing problem. Though the intention when using artificial sweeteners is to lose weight, studies have shown that the opposite sometimes occurs. Researchers attempt to explain this surprising phenomenon with multiple hypotheses. Lack of appetite suppression and reward response may cause individuals to search for more food and to consume more calories. Artificial sweeteners can also have negative affects on biological mechanisms such as resting metabolic rate, as well as the gut microbial environment. These changes can cause improper energy absorption and storage, which leads to weight gain. Sociological effects of artificial sweeteners have lead consumers to enjoy products that are super sweet. They have also convinced consumers that they can eat more (food) but consume less (calories), a misconception that has left the world with a big "fat problem".

Introduction

In a society plagued by obesity, diabetes and overeating, we search for ways to counteract these negative effects. Early research into these problems led to the assumption that the major cause of these maladies was over consumption of sugar. The food industry was taken by storm with the introduction of non-caloric artificial sweeteners (AS) such as saccharin, sucralose, and aspartame, which are widely used to replace sugar in the average western diet. However, are these sweeteners fighting or feeding the problems they were intended to combat?

Studies conducted in this area of science see a direct dose-response relationship between artificial sweetener use and weight gain. This relationship begs an explanation. Researchers propose that the weight gain caused by artificial sweetener can be due to natural circumstances, flawed gut microbial activity, or altered neurometabolic functions. Artificial sweeteners have been connected to both an increased appetite, and inefficient energy absorption. This combination can lead to weight gain and obesity.

Methods

The studies and information in this paper were acquired through the PubMed government database and the Touro College Library databases such as J Store, Ebsco Host, and Proquest. To answer the present questions the articles and reviews have been read through; only the relevant information has been included.

History of Artificial Sweetners

The first sweetener to be discovered was saccharin. A scientist named Constantine Fahlberg stumbled upon it in 1879 at Johns Hopkins University. For nearly half a century saccharin was the only artificial sweetener on the market. Originally, it was for diabetics only, later it was used for anyone wanting to limit their sugar intake. Fifty years later, in the University of Illinois, Michal Sveda discovered Cyclamate. Combining cyclamate with saccharin improved the taste and soon became common practice. By 1969 the FDA banned cyclamate because of its link to cancer, while they deemed saccharin safe to use. Artificial Sweeteners use went down in the general population until the new products surfaced. Aspartame was found in 1965 by James Schlatter at Searle while researching ulcer drugs. This was the first AS that could be metabolized, and the FDA approved it in 1981. Next came acesulfame potassium in 1967 and sucralose in 1979. Neotame was later approved for use is 2002 by the FDA.

In the years between 1999 and 2004 more than 6,000 new products have been created using artificial sweeteners. These sweeteners are most commonly used in carbonated drinks. Sucralose is the most widely used due to its close mimicry of real sucrose taste. The wide use of these sweeteners in countless products ensures that sweeteners affect most aspects of our dietary life (Yang, 2010).

Discussion

Artificial Sweetener use and Weight Change:
The perpetual debate surrounding the effects of artificial sweeteners on the human body gave rise to a lengthy list of studies that attempt to determine the risks as well as the benefits. A controversial matter that has taken priority in these studies is the question regarding weight change in relation to artificial sweetener use. Due to their lack of calories, sweeteners have been used to control diseases such as diabetes as well as prevent diseases such as obesity. Yet, studies have found a substantial dose-response relationship between artificial sweetener use and weight gain. These studies range from 4 day studies to 10 year epidemiological events.

One of the largest scale studies performed was the San Antonio Heart Study (Fowler et al, 2008). The study included 5,158 Mexican and non-Hispanic white Americans between the ages of 25 and 64. All the members of the study lived in randomly selected homes in the San Antonio area. Consisting of 2 cohort studies, the first of which was from 1979 to 1982, the second was from 1984 to 1988. Of the 4,998 surviving individuals, took part in a follow-up study 7- 8 years later. This study focused on artificial sweetener consumption in beverages. Participants

were asked to answer a series of questions regarding amount of cans, bottles or cups of beverages, such as soft drinks, diet or regular, and coffee, sweetened with sugar or AS, they consumed per week. Based on their answers participants were placed in either a user or nonuser category. Dieting status and exercise frequency were recorded at baseline as well. Each participant was categorized by weight at baseline. A BMI of <25 was categorized as normal weight (NW), _>25 but _<30 was overweight (OW) and _>30 was obese (OB). Incidence of OW/OB was defined as the percentage of originally NW participants who entered the OW/OB category by follow-up.

Results of the study show a strong dose-response relationship between AS beverage consumption and change in BMI. In Cohort 1 AS users had a 78% greater change in BMI than non-users and Cohort 2 experienced 74% and 83% greater change in BMIs in quartiles 3 and 4 respectively. The change in BMI followed a consistent pattern within the user subset. The more artificially sweetened beverages consumed per week the greater the change in BMI. Less than 3 ASBs consumed per week resulted in an average change of 1.2 kg/m2 while 22+ ASB per week resulted in 2.0 kg/m2 change and up. Participants who started out as users then chose to discontinue use experienced 58% lower BMIs than those who continued use. Once gender, ethnicity, weight category at baseline, diabetes, dieting status, exercise and cohort were factored in, change in BMIs were 47% higher in artificial sweetener users than non-users, suggesting greater gains, or smaller loses, for users versus non-users. Limitations of the study include a lack of sweetener specific study ability, fruit juices were not included, neither were artificial sweeteners consumed in products including food, other beverages, cosmetics and pharmaceuticals (which can contain aspartame) (Theodore, 2006).

The San Antonio Heart Study is far from the only one performed on this topic. Many studies have lead to the same conclusion showing a relationship between artificial sweetener consumption and weight gain. The list is never ending. The American Cancer Society Study (Stellman, Garfunkel, 1986), which focused on 78,694 women, was conducted in the early 1980's. At a one-year follow-up 2.7 compared to 7.1% more AS users gained weight than non-users. The average difference in the amount gained was just shy of two pounds, nevertheless it was statistically significant. A third well known study was the Nurse's Health Study (Colditz et al, 1990) conducted between the years of 1976 and 1984. In this study 8 year weight gain was associated with saccharin use in 31,940 women.

Researchers such as Blundell (Blundell, Hill, 1986), Rogers (Rogers et al, 1988)(Rogers, Blundell, 1989), Tordoff (Tordoff, Alleva, 1990)(Tordoff, Friedman, 1989), and Lavin (Lavin et al, 1997), who have conducted studies attempting to find a reason for this phenomenon, have seen increased hunger, appetite, and food consumption as a result of AS users. However, reviews written by Rolls

91991) and Malik et al, (2006), which consist of multiple short term studies, have seen either no weight gain, or even increased weight loss in users. A meta analysis of nine interventional studies summarized by De La Hunty (De La Hunty et al, 2006) saw significantly greater weight loss in aspartame users versus non users.

When researchers saw the incongruent results of these studies they chose to conduct a study of their own. In a 10-week study they tried to prove their claim that "short term studies are not very informative because appetite regulation and macronutrient balance probably do not correct for the missing energy and sucrose until the individual has consumed the diet for several days" (Raben et al, 2002). Their results, however, proved their hypothesis wrong. For 10 weeks overweight individuals were placed into either a sucrose group or an artificial sweetener group. The study found no increased hunger or appetite in the AS group compared to the sucrose group, and weight gain was observed in the sucrose group while weight loss occurred in the artificial sweetener group.

The lack of consistent results in these studies can obviously be due to the human components. The controlled short term studies consisted much more of restricting diets and exercise regiments. The long-term studies, spanning multiple years, thus allowing natural eating habits and appetites to develop, were more likely to see increased BMI amongst artificial sweetener users. There is a possibility that those using AS are those who are more susceptible to weight gain, and therefore, we see these results. But research doesn't stop with speculation. Scientists are now trying to understand how non-caloric sweeteners could lead to weight gain.

Biological Response to Sweet Taste

Natural Sugars:

Many different explanations have been suggested as to why artificial sweeteners would cause an increase in weight. The first is the suggestion of increased appetite or lack of appetite suppression. As stated previously, AS use has been continuously linked to hunger and overeating. The debate lies in how sweeteners can cause these biological reactions. In order to understand the specific way in which the body reacts with artificial sweeteners we must first understand how the body acts with natural sugars.

There are 2 pathways of glucose absorption (Mace et al, 2007). One is active transport through the Na+ glucose co-transporter

SGLT1. This pathway reacts only with glucose and is thus unaffected by artificial sweeteners. The second route is known as the apical GLUT2 pathway. This pathway reacts at high concentrations of glucose and can have 3 to 5 times more rapid and precise absorption than the classic SGLT1. The GLUT2 route is mediated by Ca2+. Depolarization of the apical membrane through glucose transport via SGLT1 allows Ca2+ to enter the L-type channel Cav1.3, this causes the terminal web to contract. This is essential for insertion. Little insertion occurs at low concentrations of glucose, in which case the SGLT1 transporter dominates. However, at 30 mM (millimoles) of glucose or more the GLUT2 pathway takes over as the main absorption pathway for unknown reasons.

The calcium concentration goes up as a result of the G- protein coupling receptor, α-gustducin, activated phospholipase c β2- dependent pathway. The GCPR is coupled with the T1R2 and T1R3 sweet taste receptor heterodimer. When these receptors, found in both lingual cells on the tongue and intestinal brush cells in the duodenum, sense sweet taste they release the α-gustducin and set this reaction in motion.

The α-gustducin also induces the secretion of glucagon-like peptide (GLP)-1 and peptide YY from enteroendocrine L-cells (Ford et al, 2011). Both GLP-1 and PYY have been observed to be satiety factors in humans (Flint et al, 1998, Gutzwiller et al, 1999). GLP-1 is known to raise insulin sensitivity as well as increase leptin levels in the hypothalamus, thus increasing satiety in the brain. The sweet taste path continues eventually terminating in the insula/frontal operculum and the orbitofrontal cortex (Small, 2006). The mesolimbic system sends the feeling of satisfaction received for the good taste (Stice et al, 2008). The metabolic products of the ingested foods determine this post-ingestive effect. Therefore, when sugar, enters the body it stimulates the sweet taste receptors, which activate both the absorption pathway and the satiety pathway, providing both an energy source for the body and a reward for the brain. The combination of these factors means the person is no longer in search for food; he is satisfied.

Biological Response to Sweet Taste Artificial Sweeteners:

In 2010, Ford and Peters conducted an experiment to determine if artificial sweeteners invoke this same response as sugar does in the body (Ford et al, 2011). They conducted a signal blind, randomized, crossover study in eight healthy volunteers over a 4 day period. The volunteers consisted of seven females and one male ages 22-27, all in the normal body weight range.

The subjects were randomly selected to receive one of four solutions: 50ml of either water, sucralose, maltodextrin (a non-sweet caloric substance, matched for the sweetness of sucralose in this experiment) or a modified sham-feeding protocol of sucralose (used to study oral stimulation of sweet taste receptors in the mouth versus those in the gastro-intestinal tract). The dose of sucralose used was based on the observed average intake of sucralose per day. Observations were made on four separate days with a minimum of three days left in between each solution study. Participants initial blood work was taken on arrival, they then ingested one of the first three solutions followed by the MSF of the solution that they had swallowed. Blood samples were taken -15 minutes and 0 minutes prior to ingestion and then 15, 30, 45, 60, 90, and 120 min after ingestion. To analyze cephalic phase insulin response as well as GLP-1 release, samples were taken at 2, 4, 6, 8, and 10 minutes after ingestion. Participants were asked to rate their appetites using visual analogue scores for 120 minutes following ingestion, after which time they sat down to a meal and their food intake was noted.

Researchers found that there was no increase in appetite or energy intake after the 2-hour waiting period, however, what they found in the blood samples, is quite fascinating. The plasma insulin and GLP-1 showed no significant change in the first 10 minutes and GLP-1 and PYY concentration were similar in all groups. The stimulation of T1R receptors did not occur in the case of sucralose ingestion. As a result GLP-1 and PYY were not secreted and appetite suppression did not occur. Perhaps the most interesting part is that in vitro sucralose did stimulate the receptors and, as a result, the L-cell secretions of GLP-1 and PYY occurred. The reason for this disparity is still unknown at this point in time.

Though artificial sweeteners don't stimulate these receptors on their own, a study done on rat intestinal tracts was very informative as to the mechanism that is used. In this study on rat intestinal tracts, it was demonstrated that, when combined with a small amount of glucose, AS stimulate the GLUT2 response in a similar way to that of large amounts of glucose (Mace et al, 2007). The rapid absorption of glucose through this pathway is only first observed at a threshold value of 30 mM of glucose, even then it is a minimal response. However, when 20 mM of glucose were ingested in conjunction with just 1 mM of sucralose the rate of glucose absorption doubled (as compared to just 20 mM of glucose). This effect was equivalent to the effect of 75 mM of glucose ingestion. The rapid absorption may lead to a feeling of satiety, but blood glucose levels sky rocket as a result as well. High glucose levels will lead to fat production as a means of conserving all the extra energy in the body. Therefore, trying to save calories "part of the time" can actually have worse repercussions for weight gain and obesity than natural sugars can.

In another study examining a connected response, Graaf et al, studied the functional magnetic resonance images (fMRIs) of subjects who had recently ingested glucose, water, maltodextrin, or aspartame (Graaf et al, 2005). The objective of the study was to examine the separate effects of energy content and sweet taste on the hypothalamic responses, such as cephalic phase insulin response, and ghrelin (the hunger hormone) response suppression, which contribute to a sensation of satiety.

Five participants were scanned for 37 minutes at a time on 4 separate days. The participants were healthy normal weight males. A questionnaire was used to assess the general level of health in their daily lifestyle. Solutions were randomly assigned to participants by picking lots the day before each visit. The aspartame and maltodextrin solutions were matched for sweetness to that of the glucose solution. The subjects didn't know which solution they were receiving. One blood sample was taken before entering the fMRI machine, others were taken once the subjects were inside. The first was taken -5 minutes and -3 minutes before ingestion and then 1, 3, 5, 7, 10, 20, and 29 minutes after ingestion of the substance. Each subject's hypothalamus was segmented into four regions. The regions of interest (ROI) were the upper anterior hypothalamus (UAH) and upper posterior hypothalamus (UPH) because these are the regions known to respond to glucose (Smeets et al, 2005). At each time slot the mean gray matter value of the hypothalamus was calculated and compared to the 7-minute reference period of each participant.

Results of the study show that glucose was the only one of the four substances that resulted in a prolonged decrease in the hypothalamic hunger signal (ghrelin response). Neither the sweet taste of aspartame alone, or the caloric intake of maltodextrin alone elicited this same response. The results blood samples showed that both glucose and maltodextin ingestion resulted in a cephalic phase insulin response and 6increased blood glucose levels. However, the glucose response was much stronger. Increased glucose levels result in leptin release, which is itself associated with a decrease in ghrelin signals, ultimately giving the person a feeling of satiety. Aspartame and water had no such effects. In a similar study, saccharin was tested in place of aspartame, the saccharin did not result in a CPIR either (Teff et al, 1995).

What these studies suggest is that artificial sweeteners do not send the same signals to our brain as real sugars. As a result one doesn't feel satisfied or rewarded after eating. In the absence of these biological reactions there is typically an increase in fat and protein calorie intake (Benton, 2005, Beaton et al, 1992). It has also been noted that the reward system for food shares the behavioral paradigm with all different forms of addiction (Avena et al, 2008). And like other addictions a period of abstinence can lead to a period of over indulgence. Avena et al. noted that after a period in which rats were denied sucrose, an increase in sucrose self-administration occurred (Avena et al, 2005), quite similar to binge eating in humans. Applying this concept to artificial sweetener use, one can assume that replacing sugar with non-caloric sweeteners can actually result in an increase in caloric intake.

Gut Microbial Adaptation to Artificial Sweeteners:

In the early 1980's it was suggested that there might be a link between the commensal flora of the gut and obesity. This suggestion came about when a noted change occurred in the gut microbiota composition after weight loss (Bjorneklett et al, 1981). In 2005 a well known study stated that obesity can result from a higher Firmicutes : Bacteroidetes ratio (Ley et al, 2005), further studies found that there is definitely an altered biome in the GI tract of obese people (Payne, er al., 2012). The debate on this topic is a cause or consequence question. Is it that individuals who have altered gut microbes become obese, or does the micro-biome only change once the person is already obese? Payne suggests that the cycle begins by not eating properly, thereby destroying the natural gut environment. The new ecosystem reacts differently to the substances that enter the system; this behavior can contribute to obesity.

Payne says that our non-diverse "fructose-and sugar substitute-laden, plant polysaccharide- poor Western diets" force the microbiota to adapt to the new and unknown substrates such as artificial sweeteners while being bombarded by familiar substances like fructose. These conditions force the environment to adapt, changing structure, enzyme production and patterns of energy absorption. This survival mechanism, called adaptive metabolism, was demonstrated in rat and pig models for D-tagatose fermentation (Laerke et al, 2000). At the same time that this diet creates new adaptive forms of bacteria, the normal diversity that exists begins to diminish. The link between obesity and a lack of diversity of gut microbiota is widely accepted (Turnbaugh et al, 2008; Ley et al, 2005; Turnbaugh et al, 2006). Turnbaugh et al's. study suggests that the typical western diet promotes growth of Firmicutes while it depletes Bacteroidetes contributing to the unhealthy ratio. Bacteriodetes are the ones most well equipped for the digestion of starch and sucrose.

This newly formed ecosystem evolved in order to promote efficient energy extraction. While the body can only absorb as much energy as was ingested, increased exposure to unknown substrates can put the body into panic mode. As a result of over exposure to unfamiliar substances the bacteria react by acquiring supplementary metabolic energy sources. For example

short chain fatty acids (SCFAs) taken up by the intestine can be converted to energy via the Krebs cycle (Leng et al, 1963). The idea of efficient energy extractions has been observed in obese individuals (Turnbaugh et al, 2006). The extra absorption creates more energy; energy that is unnecessary. The extra energy then has to be stored as adipose tissue, over time this can lead to a build-up and cause someone to become overweight and possibly obese.

Resting Metabolic Rate Adaptation to Artificial Sweeteners:

The resting metabolic rate (RMR) of a person's body has a large effect on total energy expenditure (Ravussin et al, 1982) and low RMR (calculated by using fat-free mass as a reference point) puts them at a greater risk of obesity (Ravussin et al, 1988). In a study done by Kiortsis et al, obese children were put on a calorie restricting diet for six weeks (Kiortsis et al, 1998). After the six week period, weight loss occurred and lower BMI and FFM were calculated. RMR at this time averaged around 10.1% lower than the starting metabolic rate. As RMR went down so did the Serum tri-iodothyronine (Serum T3) levels. A correlation is not well understood but this may be an adaptive response attempting to conserve energy during a period of caloric deprivation.

The data gathered in this study can be applied to this discussion. Artificial sweetener use is a form of calorie reduction. When depriving the body of proper energy sources the RMR decreases. When a person goes back to eating the way they did before the calorie withdrawal period, their new, lower RMR will not be able monitor proper energy expenditure. The low metabolic rate also greatly increases an individual's risk of obesity, so the short term weight loss may not be all that successful.

"The Sweetening of the World's Diet" (Popkin, Neilsen 2003)

The Sweeteners that have been approved by the FDA are intensely sweet. So much so that very little has to be used to achieve the sweetness of sucrose. Sweetners, from aspartame, which is 180 times sweeter than sucrose, to neotame, which can be 7,000 to 13,000 times sweeter, have desensitized the present day palate. We are so used to products with this uber sweetness that companies that use real sugars are forced to manipulate their products so our trained palates recognize them as sweet. This extreme use of sugar in such large quantities is not healthy for anyone and, of course, contributes to the obesity epidemic.

"Low- Fat" Syndrome:

As the prevalence of obesity rises and people become more and more aware of the dangers of being over weight a new culture has been born. Nearly every product known from chocolate to pasta to alcohol can now be found in a low fat, sugar free, or low carb form. Studies show that these labels can distort a person's perception of serving size, and calories per serving (Wansink, Chandon, 2006). These assumptions then mold his or her anticipated pleasure, from the taste of the food, and/or guilt, from the calorie intake, that they will feel for consuming this product. The guilt experienced by consumers is a product of the conflict of interest that goes on in their heads. On the one hand they want the pleasure for the taste of the food, on the other they know the long-term health risks of eating unhealthy foods. These factors combined then determine how much the person will actually eat. When the guilt is decreased or completely erased from the equation, consumers make decisions that are extremely detrimental to their health.

A study was performed to see if this theoretical phenomenon proved to be real. It took place at a university open house to allow for diversity, 361 participants were included in the study. Upon arrival participants were brought to one of two bowls of regular M&M's. The first bowl was labels "Regular M&M's" while the second was labeled "Low-Fat M&M's". The participants were told to serve themselves what they thought was an average serving size, and their bowls were weighed. They were then asked to estimate how many calories they believed were in their serving size. After eating their M&M's the participants were asked how guilty they felt for eating them.

The results of the study show that low-fat labels can be extremely hazardous. Participants ate 28.4% more M&M's when they were labeled "low-fat", their perceived serving size was 25.1% greater and their calorie estimates were nearly 300 calories lower as compared to regular M&M's. All participants felt guiltier for eating the regular M&M's than for eating the low-fat ones, however, overweight individuals felt less guilty about it than the normal weight individuals. The overweight individuals actually said they felt no guilt at all for eating the low-fat M&M's.

The larger problem that this presents is that low-fat does not necessarily mean low calorie. In a survey done on 17 brands that sold a regular and low fat version of the same product, it was determined that though low-fat products had, on average, 59% less fat than the regular products, they only had 15% less calories. Applying these statistics to the previous study outcomes would mean that though consumers had 48% less fat (based on the average lower fat percentage of low fat products) they would have had 9% more calories.

Conclusion

This review was written as an attempt to understand the correlation between artificial sweetener use and weight gain. Multiple scientific and sociological/ psychological hypotheses were studied. Each one can explain this surprising phenomenon.

However, they don't have to stand alone. Combined the multiple hypothesis can tell the life story of an obese individual. It is a vicious cycle that has no real beginning and it can start at any point. But each leads to the next. If you begin at "The sweetening of the worlds diet" you can see an individual enjoying himself with all his overly sugary snacks, because that is what his palate has become accustomed to. Soon he notices his jeans have gotten a little snug so he begins eating "low-fat" products to reduce the guilt he feels for what he's doing to himself. The low-fat products, which contain artificial sweeteners, increase his appetite because they do not stimulate his taste receptors to provide a reward response in his brain. But, maybe this works for him for a while and he drops a few pounds. By then resting metabolic rate will have lowered putting him at a higher risk for obesity. When he can't deprive himself of sugar any longer he'll binge eat those calories that he's been missing. The lower RMR that was created can no longer handle these massive amounts of sugar and cause fat storage to occur. All the while he's been destroying his gut microbe environment. Now, whatever he ingests his body searches for additional energy sources because it fears the lack of sucrose will deplete its energy storage. Combining this glucose intake with AS use also leads to stimulation of the GLUT2 pathway for rapid unnecessary absorption. And so, more and more adipose tissue accumulates. Slowly, day after day, year after year this cycle occurs. Soon he finds himself obese, with metabolic syndrome, diabetes, and chronic heart disease. Though further research is necessary to understand how this affects different age groups, ethnicities, and genders, this can occur. And it's all a result of an attempt to create a non-caloric, healthier sweetener.

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Vaccines and Autism: Is there a link?

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Abstract
There has been a worldwide increase in autism cases in the past few decades, but the cause of it is unclear. It has been suggested that vaccines may be contributing to the rise in autism rates. One claim is that the MMR vaccine can cause intestinal inflammation that may lead non-permeable peptides to be transferred to the brain where it will affect neurodevelopment. This may lead to autism with symptoms of developmental regression and gastrointestinal problems. Another major hypothesis that has received much attention is that a mercury-containing compound, thimerosal, found in many vaccines, can have toxic effects on the central nervous system. By retrieving studies from databases such as Ebsco, Proquest, and Pubmed found in the Touro College Library, this review investigates if there is any truth to these claims. No evidence of a direct link between vaccines and an increase in autism cases have been found.

Introduction
There has been a worldwide increase in autism cases in the past few decades, but the cause of it is unclear. It has been suggested that vaccines may be contributing to the rise in autism rates. One claim is that the MMR vaccine can cause intestinal inflammation that may lead non-permeable peptides to be transferred to the brain where it will affect neurodevelopment. This may lead to autism with symptoms of developmental regression and gastrointestinal problems. Another major hypothesis that has received much attention is that a mercury-containing compound, thimerosal, found in many vaccines, can have toxic effects on the central nervous system. By retrieving studies from databases such as Ebsco, Proquest, and Pubmed found in the Touro College Library, this review investigates if there is any truth to these claims. No evidence of a direct link between vaccines and an increase in autism cases have been found.

Introduction
Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that impairs a child's ability to interact and communicate with others. It is commonly associated with certain behaviors such as difficulty making conversation, delayed language acquisition, and poor motor skills. Autism seems to originate from very early brain development. The most obvious symptoms of the disorder, however, usually begin to show between the ages of 2 and 3. (Autism Speaks, 2016) Autism diagnoses have greatly increased in the past few decades. According to statistics from the U.S. Centers for Disease Control and Prevention, the prevalence of autism in 1000 children was approximately 6.7 in the year 2000 and 14.7 in 2010, a significant increase. (Data & Statistics, 2015).The cause for this dramatic increase is unknown and has been the subject of much investigation. It is believed that genes play a role in an increasing risk for autism (Mascarelli, 2010). However, environmental factors play a part in most cases of autism too. There has been a growing concern of a possible link between vaccines and autism. Two separate theories have been proposed over the years. One is that the Measles-Mumps-Rubella (MMR) vaccine causes autism. The other is that thimerosal, a mercury containing preservative found in many vaccines can lead to damage in the brain. Researchers have sought to discover if there is any truth to these claims.

Methods
Information and research was obtained through databases such as Ebsco, Proquest, and Pubmed. Access was provided by the Touro College Library. Google Scholar was also used to find research articles. Keywords like vaccines, autism, and thimerosal were used to search for relevant material. In addition, references in these articles were retrieved and used as additional sources for further research.

Discussion
MMR Vaccine
In 1968 a link was suggested between the Mumps, Measles, and Rubella (MMR) vaccine and the onset of ASD. Unusual bowel symptoms were described among 12 children who showed developmental regression like losing acquired skills despite previous normal progress. Eight out of the twelve children who had gastrointestinal signs had reported their first symptoms of autism within a month of receiving the MMR immunization. It was concluded that the MMR vaccine caused intestinal inflammation allowing usually non-permeable peptides to be translocated to the bloodstream and ultimately to the brain, where they affected development. (Wakefield, et al. 1998) Although the sample was small with only a dozen children and the results were never replicated, the study received lots of attention from the public, and served as the foundation for much controversy in the years to come. Parent's anecdotes of their own personal experiences were enough for many to keep this alive. As a result, many refused to vaccinate their children worrying about their safety, and outbreaks of vaccine preventable diseases began reappearing in certain areas. The MMR-autism controversy was responsible for a significant decrease in immunizations following that year. The MMR immunization rates in the United Kingdom dropped from 90% in 1988, when they were first introduced, to 80% in 2004, after the article had been read by the public. (Kolodziejski, 2014)

Possible Developmental Regression and Gastrointestinal Symptoms Associated with MMR
Whether MMR is really associated with autism has been under constant debate. There have been many studies done to see if the link exists. Fombonne and Chakrabarti (2001) tested out

the hypothesis that a form of autism existed involving developmental regression and gastrointestinal symptoms caused by the MMR vaccine. It was proposed that if one or more of the following predictions were proved by the data collected, the hypothesis could be validated. The first prediction was that childhood disintegrative disorder (CDD) had become more common. Another is that the average age of first parental concern in autistic children in those who received the MMR vaccine is closer to the mean age of immunization than in those who were not vaccinated with MMR. The third prediction was that developmental regression in those who are vaccinated with MMR had become more frequent. If the age of onset of autism with regression is closer to MMR-vaccination as opposed to those who did have autism without regression, it could also prove that MMR could have caused autism. Another prediction is that those with regressive autism have different symptoms. Finally, those with regressive autism would have gastrointestinal signs and/or inflammatory bowel disorder.

Three samples, 1 epidemiological (n=96) and 2 clinical samples, a pre-MMR (n=68) and a post-MMR (n=98) were used to gather information. In order to assess age of first onset of autistic signs, the parents were asked how old their child was when they first became concerned with his/her development. Regression was noted when a loss of skill, such as language was confirmed based on certain criteria. Assessment of bowel symptoms was done by retrieving data from both the parents and pediatrician.

The first prediction of childhood disintegrative disorder (CDD) increasing was not supported by the data as only one boy met the criterion for CDD, which seems likely to have originated from brain pathology before the MMR immunization was even given. In this epidemiological sample where nearly all the children were vaccinated, it can be assumed that CDD is not increased with vaccine exposure. Second, there was no difference in age of first parental concern in two of the samples exposed to MMR (19.3 and 19.2) compared to the pre-MMR clinical sample (19.5). Third, the rate of regression for the post-MMR sample (15.6%) was lower than the rate in the pre-MMR sample (18.4%). This rules out MMR-induced regressive autism as a cause for the dramatic increase in autism cases. In the epidemiologic sample, 18.8% had gastrointestinal symptoms. Constipation was the most common symptom (9.4%), and no inflammatory bowel disorder was reported. Only 2.1% of the sample experienced both developmental regression and gastrointestinal symptoms, a rate which shows no association between the two. None of the 6 predictions were supported in this study, and no evidence was found to show a distinct syndrome of MMR-induced autism. (Fombonne E, Chakrabarti S, 2001).

Measles Virus RNA in Patients with Autism

There are studies that have reported Measles Virus (MV) RNA in bowel biopsies in autistic children, showing a possible link between intestinal inflammation and the measles virus. A study done by Kawashima et al (2000), examined children with gastrointestinal problems to see if they have the measles virus and if they did, if it derived from wild strains or vaccine strains. Eight of the patients had Crohn's disease, 3 had ulcerative colitis, and 9 had autistic enterocolitis. One of the patients with Crohn's disease, 1 with ulcerative colitis, and 3 of 9 with autism were positive for the virus, while the controls were all negative. The measles virus found in the patient with Crohn's disease proved to have characteristics of wild-strain virus, while the one with ulcerative colitis and in the autistic patients had vaccine strains.

A study including 125 autistic children and 92 control children. MMR antibodies were first measured by ELISA in sera of 24 randomly selected autistic children, 16 normal children, and 14 children with other developmental problems. Autistic children had a higher number of MMR antibodies compared to the other children. Soon after all of the 217 children were checked for MMR antibodies by immunoblotting assay for serum screening. They found that 75 (n=125) autistic children's sera were positive for an unusual MMR antibody, whereas none of the control sera had the antibody. A protein band was detected in the antibody which was immunopositive for measles HA protein but not for measles nucleoprotein and rubella or mumps viral proteins. The results showed that autistic sera detected measles HA protein in the MMR antibody. It is suggested that an inappropriate antibody response to MMR might be related to the development of autism. (Singh, et al, 2002)

However, despite those few studies, many studies have been done that disprove the link between autism and MMR including a few that failed to detect MV-RNA in cases of autism. A case control study tested for the presence of measles virus (MV) RNA in bowel tissue in children with ASD. The purpose was to see if those with GI disturbances and autism are more likely to have MV RNA in their bowel tissue than those with GI symptoms but no autism. This can determine if MMR is linked to this type of autism. The ileal and cecal tissues of 25 children with GI disturbances and autism and 13 children with GI disturbances but no autism were analyzed for MV RNA in 3 different laboratories. The timing of the autistic and gastrointestinal symptoms in respect to MMR immunization was also documented. The results showed no difference in MV RNA findings in the case group and the control group. The timing of the vaccine in regards to the GI symptoms and autism onset were inconsistent with the theory that MMR triggers either the GI symptoms or autism. (Hornig, et al, 2008)

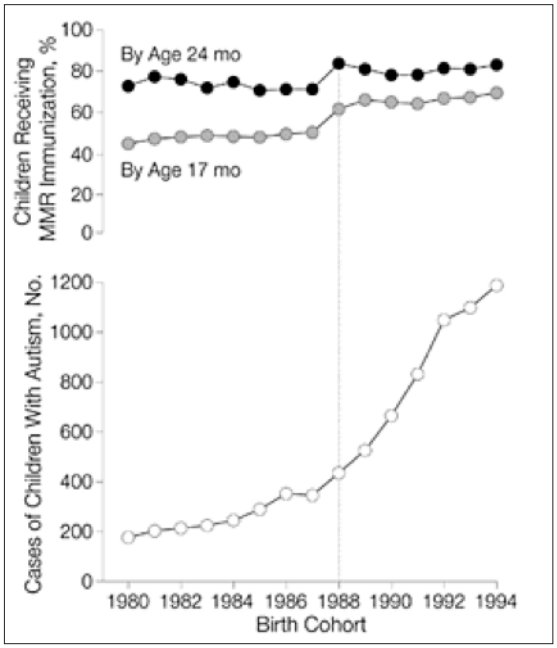
A 14 year prospective study reviewed the number of vaccines that were given from the time The National Board of Health and National Public Health Institute launched their MMR vaccination program in 1982 till the year 1996. 3 million vaccines were given during that period of time and 31 cases of gastrointestinal symptoms were reported after vaccination, ranging from 20 hours to 15 days. Fifty five percent (n=17) had symptoms of diarrhea and vomiting, 23% (n=7) had gingivostomatitis, 16% (n=5) had vomiting only, and (n=2) had abdominal pains. The symptoms usually lasted about a week. None of the children developed autism. In this study, no association was found between MMR and pervasive developmental disorder or inflammatory bowel disease. (Peltola et al, 1998)

Epidemiological Studies

Numerous epidemiologic studies have been done to see if there is an association between MMR and autism. Taylor, et al (1999) did a chart review of cases of autism diagnosed in a region in England, in children born between 1979-1992. They identified 498 cases of ASDs in the population, of which only 293 could be confirmed according to ICD-10 criteria. They analyzed the data in different ways to test if autism was related to the MMR vaccination. Data they collected included the age at which the children were diagnosed, the age of first parental concern, and age when regression became obvious. The trend of autism was examined to see whether or not there had been a sudden step-up in autism diagnoses in children who received the MMR vaccine after it was introduced in England in 1988. They found that the prevalence rates of autism began an exponential rise starting with children born a couple of years before the introduction of the MMR vaccine, and that there had been no sudden step-up after 1988. They concluded that this refuted a temporal relationship.

A study done in California collected data regarding MMR coverage rates and age at the time of immunization for children born between the years 1980-1994 from school immunization records in California Kindergartens. The number of cases of autism diagnosed during those years were retrieved from the California Department of Developmental Services where they were enrolled. The results did not show a correlation between rates of autism and number of immunizations given. As can be seen by Figure 1, autism cases increased from 44 cases per 100,000 live births in the 1980 cohort to 208 cases per 100,000 live birth in the 1994 cohort, a 393% increase. In contrast, MMR immunization rates by the age of 24 months increased from 72% to 82%, only a 10% increase. Since the rate of increasing autism cases does not compare with the only small rise in MMR coverage, a correlation was not observed between the two. (Dales, et al, 2001)

Figure 1



Percentage of Children Receiving Measles-Mumps-Rubella (MMR) Immunization in Second Year of Life and Caseload of Children With Autism, by Year of Birth, California, 1980-1994 (Dales, et al, 2001)

A population study was done in Japan by Honda, et al (2005). It examined incidence of ASD in children born from 1988-1996. Japan's MMR immunization program was launched in 1989 but ended in 1993, due to suspected side effects of the mumps vaccine. This allowed for an opportunity to research the effect of removing the MMR vaccine which was believed to cause a rise in autism. If it was really the cause of the increased autism caseloads when it was introduced, autism would drop after the risk factor (MMR vaccine) was withdrawn. All children who had been diagnosed with ASD were selected from a patient list of the YRC Developmental Psychiatry Unit, which offers diagnostic and intervention services to all those with ASD. The annual trends for both typical autism and all other categories of ASD were examined. According to the statistics, MMR vaccination rates were 69.8%, 42.9%, 33.6%, 24.0%, and 1.8% respectively in the years 1989-1993. As can be seen by this data, the rate decreased significantly during these years, with only a bare minimum of 1.8% of children vaccinated with MMR in the year 1993. The annual trends of ASD were compiled for the years 1988-1996. The ASD incidence rates ranged from 47.6 per 10000 in 1988 to 117.2 per 10000 in those that were born in 1996. The rate of autism incidences continued to rise even after the MMR vaccination program was terminated. This provides evidence that it is highly unlikely that MMR is linked to greater autism incidences. (Table 1) (Honda, et al, 2005)

Table 1

Number and cumulative incidence up to age seven of ASD with developmental regression and total ASD for each year from 1988 to 1996

Year of birth	Birth cohort	ASD with regression: Definite only N	ASD with regression: Definite only Incidence per 10,000 (95% CI)	ASD with regression: Definite + probable N	ASD with regression: Definite + probable Incidence per 10,000 (95% CI)	All ASD N	All ASD Incidence per 10000 (95% CI)
1988	3571	4	11.2 (.2–22.2)	4	11.2 (.2–22.2)	17	47.6 (25.0–70.2)
1989	3246	5	15.4 (1.9–28.9)	5	15.4 (1.9–28.9)	17	52.4 (27.5–77.2)
1990	3492	9	25.8 (9.0–42.6)	10	28.6 (10.9–46.4)	30	85.9 (55.3–116.5)
1991	3763	5	13.3 (1.6–24.9)	8	21.3 (6.5–36.0)	21	55.8 (32.0–79.6)
1992	3632	6	16.5 (3.3–29.7)	8	22.0 (6.8–37.3)	23	63.3 (37.5–89.1)
1993	3618	6	16.6 (3.3–29.8)	8	22.1 (6.8–37.4)	35	96.7 (64.8–128.6)
1994	3905	16	41.0 (20.9–61.0)	16	41.0 (20.9–61.0)	63	161.3 (121.8–200.8)
1995	3128	4	12.8 (.3–25.3)	5	16.0 (2.0–30.0)	36	115.1 (77.7–152.5)
1996	3071	5	16.3 (2.0–30.5)	8	26.1 (8.0–44.1)	36	117.2 (79.2–155.3)
Total	31426	60	19.1 (14.3–23.9)	72	22.9 (17.6–28.2)	278	88.5 (78.1–98.8)

Thimerosal

Since the 1930s, thimerosal, a mercury-containing preservative has been used in many biological and drug products, including multi-dose vaccines, to prevent bacterial and fungal contamination Thimerosal is not found in live vaccines, such as MMR because of the damaging interaction they can have with the active substance. Before 1991, diphtheria-tetanus-pertussis (DTaP) vaccination was the only common vaccine that contained thimerosal. Recently though, more vaccines have been introduced that consist of thimerosal like Hepatitis B and HIB. (Nelson, Bauman, 2003) Too much mercury can have toxic effects especially in little children, where the brain is still developing. As can be seen by Table 2, the FDA estimated that 6-month old babies could have received doses of mercury as high as 187.5 µg in their vaccines and 2 year olds as much as 237.5 µg. (Freed, et al, 2002)

Table 2

Vaccines	1999 Maximum Mercury Dose (µg)	2004 Maximum Mercury Dose (µg)
3 doses of DTaP[†]	75.0	<0.9
3 doses of Hep B[‡]	37.5	<1.5
3 doses of HIB	75.0	0
TOTAL	**187.5**	**<2.4**
Estimated Exposure to Mercury from Vaccines in 1999 and in 2004 (< 2 years of age)		
Vaccines	1999 Maximum Mercury Dose (µg)	2004 Maximum Mercury Dose (µg)
4 doses of DTaP[†]	100	<1.2
3 doses of Hep B	37.5	<1.5
4 doses of HIB	100	0
3 doses of influenza[*]	[**][37.5]	[**]37.5
TOTAL	**237.5 [275]**	**< 40.2**

Because of the increasing amount of this chemical that babies were being exposed to, there was a growing concern that the ethylmercury which is found in thimerosal can cause brain damage and contribute to autism. Fearing this, the FDA removed thimerosal from all vaccines in 1999 even though direct evidence of harm was not found. Since not much was not known about ethylmercury at that point in time, assumptions were made based on knowledge of methylmercury. Methylmercury was known to have toxic effects and many assumed that ethylmercury has similar consequences. Since ethylmercury had not been extensively studied, researchers began to experiment with ethylmercury to see if it can really have those damaging effects in vaccines. (Straton, 2001)

Research was done by analyzing the VAERS database, a database maintained by the CDC which holds a record of all adverse reactions. The VAERS database was used to compare the neurodevelopmental disorders that were found following the administration of thimerosal-containing DTaP vaccines in contrast to thimerosal-free DTaP vaccines. A close linear correlation was found between increasing mercury due to thimerosal vaccines and an increase in the odds ratio of neurodevelopmental disorders. According to the results in the baseline year of 1984, the odds ratio of autism increased by 0.029 per mg of mercury, personality disorders by 0.012 per mg of mercury, and mental retardation by 0.048 per mg of mercury. The total odds of developing autism increased in those immunized with thimerosal-containing vaccines vs those who received thimerosal-free vaccines. This study showed that more research had to be done to see if there is indeed a correlation between mercury levels and higher risks of autism and other developmental disorders. (Geier et al, 2003)

A study done used hair and urine analysis to detect heavy metal exposure in those with autism compared to those who did not have autism. It consisted of 25 autistic children and 25 controls. Metal testing was performed via ICP-MS spectroscopy utilizing cell technique to detect levels of different metals in the hair. The mean mercury level in the hair of autistic children was 0.47±0.42 while the levels in the control children was a lower mean of 0.30±0.3. A similar observation was found of the mercury levels in the urine of autistic children compared to control children, with means of 2.48±2, and 1.10±0, respectively. These results seem to show an association between higher levels of mercury in those with autism. Limitations in the amount of mercury they ingest seems to be important for the safety of these children. However, a larger sample size should be studied on in order to validate these findings. (Blaurock-Busch, 2011)

A study was done by Pichichero et al, (2002) to measure the concentration of mercury found in infants' stool, blood, and urine after they received vaccines. If high levels were found, it would show that thimerosal can be potentially harmful in vaccines. In this experiment, 40 infants who were 6 months or younger received thimerosal containing vaccines while 21 controls received thimerosal free vaccines. Samples of the infants' stool, blood, and urine were obtained 3-28 days after and were

tested for mercury levels. Blood samples contained low levels of mercury with a range of 4.50-20.55 nmol/L. Fourteen out of 15 controls did not have quantifiable levels of mercury. Those with the highest levels of mercury were infants who were measured soon after they were vaccinated. There was no mercury found in urine samples, besides for one of the 2-month olds and three of the 6-month olds who had received thimerosal in their vaccines. The highest amount of mercury in the urine was 6.45 nmol/L. No mercury was found in any of the control infants. Detectable amounts of mercury were found in stool samples in those who were exposed to thimerosal containing vaccines. Those who did received thimerosal free vaccines were not measured for mercury levels in their stool. (Table 3)

Table 3

	Infants aged 2 months		Infants aged 6 months	
	Thiomersal-exposed (n=20)	Controls (n=11)	Thiomersal-exposed (n=20)	Controls (n=10)
Bodyweight (kg)				
Mean (range)	5·3 (4·0–6·4)	NR	8·1 (6·7–10·6)	NR
Total mercury exposure (μg)*				
Mean (range)	45·6 (37·5–62·5)	0	111·3 (87·5–175·0)	0
Blood mercury (nmol/L)				
Number of samples tested	17	8	16	7
Number with mercury in range	12	1	9	0
Mean (SD)†	8·20 (4·85)	4·90	5·15 (1·20)	..
Median (IQR)†	6·15 (4·60–10·85)	4·90	5·30 (4·55–6·10)	..
Range†	4·50–20·55	..	2·85–6·90	..
Urinary mercury (nmol/L)				
Number of samples tested	12	6	15	8
Number with mercury in range	1	0	3	0
Mean (SD)†	3·8‡	..	5·75 (1·05)	..
Median (range)†	3·8‡	..	6·2 (4·55–6·45)	..
Stool mercury (ng/g dry weight)				
Number of samples tested	12	NT	10	NT
Number with mercury in range	12	..	10	..
Mean (SD)†	81·8 (40·3)	..	58·3 (21·2)	..
Median (IQR)†	83·5 (47·0–121·3)	..	58·0 (42·0–68·5)	..
Range†	23·0–141·0	..	29·0–102·0	..

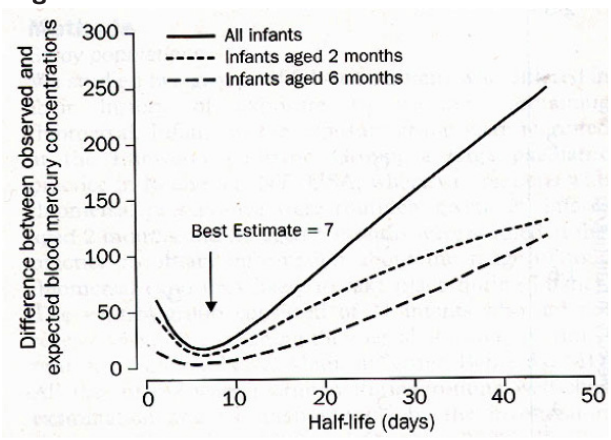
NR=Not recorded. NT=not tested. *Via vaccination. †All calculations done only with samples within range of accurate quantitation. ‡Only one value so SD and range are not applicable.

Concentrations of mercury in blood, urine, and stool of infants who received vaccines containing thiomersal and those who did not

To determine if dietary intake could contribute to mercury levels in the stool, samples were obtained from 9 infants who were age-matched to those who received thimerosal containing vaccines but were not exposed to thimerosal themselves. The mean levels of mercury in these samples was 22 ng/g which was considerably lower than those exposed to thimerosal. Out of all these samples, however, no concentration exceeded 29 nmol/L which is known to be an accepted safe value. The results did show an increased amount of mercury in those who were vaccinated compared to those who did not receive thimerosal, but the quantity was still below the harmful level. Before the samples were measured, a prediction model was made of the expected measurements of mercury corresponding to the possible half-life days ranging from 1-45. The best estimate of the half-life of ethylmercury would be the difference between the predicted value and the observed value.

The concentration of mercury in the blood not long after vaccination suggests that the half-life of ethylmercury is not nearly as long as the half-life of methylmercury. As we can see from Figure 2, the estimated half-life for ethylmercury was 7 days. This contrasts with the half-life of around 40-50 days in methylmercury. The long half-life of methylmercury can allow the organic-mercury to cross over the blood brain barrier where it

Figure 2



can accumulate and get converted to inorganic mercury which can cause neurodevelopmental damage. Ethylmercury, however, which is found in thimerosal is rapidly eliminated through the stool which prevents toxicity from accumulating that could be detrimental to parts of the body including the brain. (Pichichero, et al, 2002)

Since creating controlled experiments is not possible in an existing vaccination program, determining if there is an association between thimerosal and autism is not so simple. Several epidemiological studies have been done to see if there is a causal relationship between receiving thimerosal containing vaccines and an increase in autism cases. A population cohort study of children in Denmark who were born in the years 1990-1996 was done to compare children who received thimerosal in their vaccines and those who did not. Doses that were administered before 1992 were considered to be vaccines containing thimerosal and those doses given after 1992 were thimerosal free vaccines. Information regarding vaccine administrations was obtained from the Danish Board of Health and data regarding autism diagnoses was retrieved from the Danish Psychiatric Central Register. Follow ups were done from one year of age till the year 2000 to see if there would be possible cases of autism disorders in those children. During these years, 440 cases of autism and 787 cases of other autistic-spectrum disorders were diagnosed among those children (table 4).

Table 4

Table. Rate Ratio of Autism and Other Autistic-Spectrum Disorders Comparing Children Vaccinated With a Thimerosal-Containing Vaccine to Children Vaccinated With a Thimerosal-Free Formulation of the Same Vaccine

		Autism			Other Autistic-Spectrum Disorders		
	Person-Years at Risk	No. of Cases	RR (95% CI)*	RR (95% CI)†	No. of Cases	RR (95% CI)*	RR (95% CI)†
Vaccinations							
All thimerosal-free	1 660 159	303	1.00	1.00	430	1.00	1.00
Any containing thimerosal	1 220 006	104	0.85 (0.60-1.20)	0.85 (0.60-1.20)	321	1.12 (0.88-1.43)	1.12 (0.88-1.43)
Doses of thimerosal-containing vaccine							
None	1 660 159	303	1.00	1.00	430	1.00	1.00
1 dose (25 μg eHg)	189 920	18	0.99 (0.59-1.68)	1.01 (0.60-1.71)	40	0.96 (0.67-1.39)	0.95 (0.66-1.37)
2 doses (75 μg eHg)	447 973	33	0.71 (0.46-1.09)	0.70 (0.46-1.09)	130	1.20 (0.92-1.56)	1.20 (0.92-1.56)
3 doses (125 μg eHg)	602 113	53	0.96 (0.63-1.46)	0.96 (0.63-1.47)	151	1.11 (0.83-1.48)	1.13 (0.84-1.51)
Trend (increase in RR per 25 μg eHg)			0.98 (0.90-1.06)	0.98 (0.90-1.06)		1.03 (0.97-1.09)	1.03 (0.98-1.09)

Abbreviations: CI, confidence interval; eHg, ethylmercury; RR, rate ratio.
*Adjusted for confounders: age and calendar period.
†Fully adjusted: age, calendar period, child's sex, child's place of birth, birth weight, 5-minute Apgar score, gestational age, mother's age at birth of child, and mother's country of birth.

We can see from the results shown in table 4, that when comparing those who received at least one dose of thimerosal-containing vaccine and those who only received thimerosal-free vaccinations there was a rate ratio of 0.85% for autism and 1.12% for other autistic spectrum disorders. Also, there was no association between a higher dose of thimerosal-containing vaccine and a higher rate of autism disorders. The increase in rate ratio per 25 ug of mercury was 0.98% in autism and 1.03% in other autistic spectrum disorders. In order to rule out the possibility that some thimerosal-containing vaccines were administered soon after 1992, the vaccinations between June and December of 1992 were omitted from the data and similar results were found. This study showed no association between thimerosal intake and increased autism rates. (Hviid, et al, 2003)

In the United Kingdom, the only thimerosal containing vaccines that have been administered in the past few decades is DTaP and DT. The total amount of thimerosal per dose in these vaccines is 50 ug (25 ug of ethylmercury). In the United Kingdom, the schedule for vaccination called for 3 doses by the age of 4 months, totaling 150 ug of thimerosal at that age. Data regarding date of birth and vaccination dates were obtained for 109,863 children who were born from 1988 to 1997. Some children were excluded from the study including those who had certain medical conditions before the age of 6 months and those who had received the Hep B or flu vaccination before 6 months of age. Hg exposure was calculated based on the number of vaccine doses the children received. A number of developmental disorders were investigated and the number of cases diagnosed, the mean age of diagnoses, and the percentage that was male was assessed. When these children were linked to the dates of their vaccination and any of the neurodevelopmental disorders they had, no evidence of increasing neurodevelopmental disorder rate was found with an increasing dose of ethylmercury. The only disorder that showed a possible link to thimerosal was tics, which had a hazard rate of 1.50 in 4 months of age. The rest of the disorders had a Hazard rate close to 1, showing no link to thimerosal. (Andrews, et al, 2004)

Another study was done in Sweden and Denmark to compare the prevalence of autism cases with the exposure of thimerosal-containing vaccines during the 1980s and 1990s. Data regarding vaccine coverage and autism cases were collected in these countries. The total amount of ethylmercury exposure was calculated by multiplying the amount of thimerosal in vaccines by the number of vaccines with thimerosal administered during that time period. The rate of autism was calculated by dividing the total number of autism cases diagnosed in those years by the number of person-years accumulated during those years.

As shown in figure 3a, the incidences of autism in Sweden began

Figure 3a

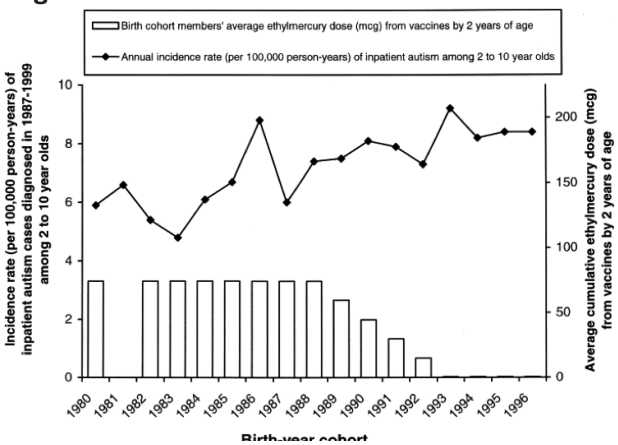
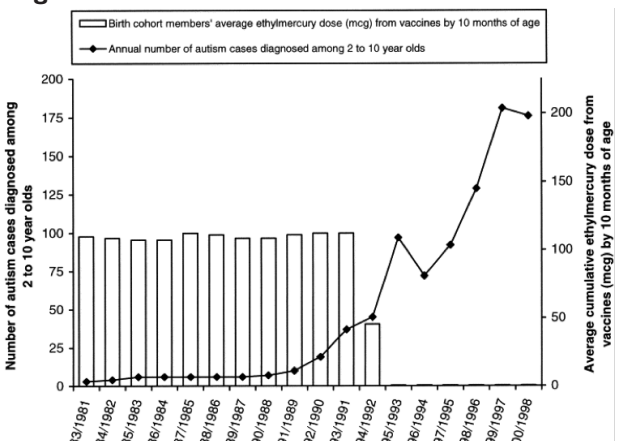


Figure 3b



to rise in the year 1985 to a rate of around 6 per 100,000 person-years and peaked in the year 1993 to a rate of 9.2 per 100,000 person-years. The coverage of thimerosal-containing vaccines, however, have remained steady over most of those years, decreasing over time and eventually being completely removed in 1993. A similar explanation goes for Denmark, shown in figure 3b. Thimerosal-containing vaccination coverage remained constant throughout the years 1981-1991 and ended in 1992, but autism rates continued to increase sharply in the years after that. We can see from these studies that the rise of autism needs a better explanation than the exposure of thimerosal to children.

Conclusion

From the studies that have been done so far, no evidence has been found to show a link between vaccines and autism. Increasing autism rates need to have another explanation. It has been suggested that the broadening of the diagnosis, and the greater awareness of autism and other ASDs have contributed to more diagnoses of autism. The claim that MMR is associated

with a regressive form of autism has been proven to be highly unlikely. The fact that MMR vaccination withdrawal did not lead to a decrease in autism cases indicated that there is not any correlation between the two. The concern that thimerosal-containing vaccines cause autism has also not been validated. However, despite the shorter half-life and less damage that ethylmercury can have in comparison to other mercury compounds like methylmercury, the current literature does not rule out a role for thimerosal in neurodevelopmental damage other than autism to children. Therefore, it is best that thimerosal continue to remain excluded from vaccines.

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How Compatible is Cow's Milk with the Human Immune System?

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Abstract
Cow's milk has been part of the human diet for at least 8,000 years and provides a rich source of proteins, lipids, vitamins, and minerals. Despite its longstanding importance in human nutrition, questions remain about how compatible cow's milk is with our immune system. Cow's milk allergy (CMA) is the most common form of food allergy in infants and children and cow's milk has been implicated in a number of immune-mediated disorders. Reviewing current research obtained through Google Scholar and Touro's library database on CMA and the potential role of cow's milk in systemic inflammation and autoimmunity reveals a tangle of contradictory findings and competing explanations. While current research does not indicate a significant connection between cow's milk and systemic inflammation in healthy adults or to Rheumatoid Arthritis, it does provide significant, if contested evidence for the role of cow's milk in Type 1 diabetes, multiple sclerosis, and Behcet's disease. These evolving findings must be considered when we evaluate the current nutritional guidelines on cow's milk.

Introduction

Cow's Milk in the Human Diet:

Humans are the only animals known to consume the milk of another species, a unique behavior that arose during the Neolithic Revolution. The end of the Younger Dryas coincided with a transformation of human subsistence patterns around the world. Populations of hunter-gatherers became more sedentary and experimented with the domestication of plants and animals. The first cattle were domesticated from wild aurochs (Bos primigenius) over 10,000 years ago, in regions that are part of present-day Turkey and Pakistan (McTavish et al. 2013). There is disagreement about when cattle were first exploited for milk production. The "secondary products revolution" theory, popular during the 1980s and 1990s, held that Neolithic people did not consume dairy products until the fourth millennium BCE, despite much earlier advances in domestication (Sherratt, 1983: Greenfield et al., 1988). Recent evidence has pushed that date back as far as the 9th millennium BCE, when human beings were still "stock-keeping hunter-cultivators" (Vigne and Helmer, 2007). Residue from pottery shards suggests that milk products were widely consumed in Southwestern Asia and Southeastern Europe by the seventh millennium BCE (Evershed et al. 2008).

Since it was first consumed during the Neolithic period, cow's milk has been an essential dietary staple for numerous populations around the globe and has developed an evolving cultural significance. On the Indian subcontinent, cows have been revered for thousands of years and their milk is used for ritual purification as well as nutrition (Simmons, 1974). Ancient Norse mythology tells of how the first creature, Ymir, was sustained by milk from the sacred cow Auðumbla (Haug, Høstmark, and Harstad 2007). In Medieval Europe, cow's milk was venerated as a spiritual substance, embodying the divine rhythms of nature; during the Renaissance it was celebrated for its taste and health giving properties, with folk remedies citing it as a cure for everything from ulcers to epilepsy (Valenze, 2011). Milk consumption was transformed by two factors in the 19th century: an improved - transportation system, which aided in the delivery of fresh milk from local farms to cities, and the development of pasteurization, which helped curb the very serious problem of milk-borne disease (Wilson, 1943: Atkins, 1978). These two advances made cow's milk more safe and convenient than ever before and guaranteed its central place in the Western diet. Today cow's milk and dairy products are an almost ubiquitous component of human nutrition, accounting for 14% of the caloric intake in developed countries (Bordoni et al., 2015). Global milk production amounted to an estimated 784 million tons in 2013, or 100 L of milk per year per person (Bordoni et al., 2015). This massive consumption occurs despite the fact that a substantial majority of the world's adult population is deficient in the lactase enzyme and may experience digestion issues with dairy (Lomer et al. 2008).

The Economics of cow's milk in the United States:

Cow's milk is a major economic commodity in the United States. A 2002 survey estimated that the dairy industry accounts for $140 billion in economic output, $29 billion in household earnings, and more than 900,000 jobs (Cryan, 2004). The U.S. Department of Agriculture estimates that domestic cow's milk production will reach a record 208.7 billion pounds in 2015 ("Dairy Farmers at the Barricades," 2015).

Chemistry and Nutrition of Cow's Milk:

Cow's milk is complex mixture of lipids, proteins, bioactive peptides (e.g. immunoglobulin, cytokines, and enzymes), amino acids, vitamins and minerals. The sugars (primarily lactose) and most minerals are dissolved in solution, the lipids are emulsified in globules, and the proteins are suspended in colloidal dispersions (Huag et al. 2007). About 80% of the proteins in cow's milk are caseins, which form complexes with calcium and phosphate (Huag et al. 2007). Although the composition of cow's milk can vary with the age, breed, nutrition, and stage of lactation of the cow, on average, a cup of milk (244 grams) provides 146 calories, 7.9 grams of fat (4.6 saturated), 7.9 grams of protein, 276 milligrams of calcium, 349 milligrams of potassium, 222 milligrams

of phosphorous, 249 IU of Vitamin A, and 97.6 IU of Vitamin D (USDA, 2015). cow's milk is an important dietary source of the cis9, trans 11 isomer of conjugated linoleic acid and glutathione (Huag et al., 2012).

Allergy, Autoimmunity, and the Hypothetical Role of "Leaky Gut"

The human immune system is an enormously complex collection of structures and processes that protects the body against harm by pathogens. Immunity operates at a number of levels. The skin and mucosal membranes are the first line of protection, acting as a physical and chemical barrier to invaders. The second level of defense is the innate immune system, in which macrophages and neutrophils of the innate immune system provide a robust, but non-specific defense against pathogens, including the cytokine-mediated inflammatory response. Finally, the adaptive immune system provides a targeted response to specific pathogens. It is divided into humoral immunity, mediated by B lymphocytes and their antibodies, and cell-mediated immunity, mediated by T lymphocytes. (Hall, 2016)

Allergy refers to an inappropriate immune response to a harmless substance. Most allergies are mediated by the IgE antibody, which is secreted by B-cells in response to antigens binding mast cells and basophils until a second exposure. Autoimmunity refers to an immune response against the body's own cells and tissues. There are two classical models for the pathogenesis of autoimmune disorders (Fasano, 2012). According to the "molecular mimicry" model, certain microbial antigens resemble self-antigens to a degree that the immune system cross-reacts with the latter and targets them for destruction. In the "bystander effect" model, microbes directly damage tissues, leading to the exposure of internal self-antigens that the immune system interprets as foreign. These two models are continuously being expanded and revised with ongoing research and may represent complementary descriptions of the multifaceted phenomenon of autoimmunity. A third, more tentative hypothesis involving the gut is presented below.

The role of the gut in immune disorders is a fascinating new area of research. While we often think of the skin as great primary barrier of our immune system, the intestines are actually the largest surface in the body, amounting to an area more than 200 times greater than the skin (Hollander 1999). Maintaining a proper balance between immune tolerance and sensitivity over this vast surface area is an exceedingly complicated task, and the failure of such regulation is associated with allergy and various forms of autoimmunity (Dejaco et al. 2006: Vitaliti et al. 2012). cow's milk is often the first foreign substance that an infant's gut will encounter and the first serious challenge to immune homeostasis in the intestines. Some researchers have suggested that there is a group of individuals who are especially prone to immune complications from food antigens, due to excessive permeability of the intestines and abnormal microbiota (Perrier, C and Corthésy, 2010: Fasano 2012). These individuals, the theory goes, are not only at a greater risk for conventional food allergies but may also find that various foods which are well tolerated by the general populations contribute to immune dysfunction. While some promising research has been conducted into the contributions of intestinal barrier dysfunction to immune-mediated disorders (De Kort, et al. 2011), and excessive intestinal permeability is particularly well attested in the pathogenesis of celiac disease (Hollander, 1999), the general role of "leaky gut" in autoimmunity remains ill-defined and controversial. While this paper does not intend to assess the validity of this hypothesis, a growing body of research on diet, allergy, and immune disorders suggests the need for an evolving paradigm of the role of the gut in autoimmunity.

Purpose:

This paper will review current research on cow's milk and the human immune system. It will begin by exploring the prevalence, natural history, and immunopathogenesis of cow milk allergies (CMA). It will then examine research on the possible contributions of cow's milk to systemic inflammation in people without clinically defined CMA. Finally, it will assess the possible link between the consumption of cow's milk and a number of immune-mediated disorders. All research into the role of cow's milk in non-immune-mediated disorders, including lactose intolerance, has been excluded. The discussion will examine the factors affecting research into the health of cow's milk and assess nutritional guidelines in light of current findings.

Methods

All research articles for this paper were obtained by searching Google Scholar and Touro's Library Database.

Literature Review

Definition, Prevalence, and Natural History of Cow's Milk Allergy (CMA):

Cow's milk allergy (CMA) is an adverse immune reaction to one or more components of cow's milk. CMA is the most common food allergy in infancy and childhood, occurring in in 2%-3% of children in the developed world, (Ahrens et al., 2012). It is typically classified into IgE-mediated allergies and non-IgE-mediated allergies; the former are more common and almost always resolve during childhood and the latter persist in a small percentage of adults (Crittenden and Bennett, 2005). One study of over 4,000 children with IgE-mediated CMA found total resolution to be 19% by age 4 years, 42% by age 8 years, 64% by age 12 years, and 79% by 16 years (Skripak, et al., 2007). CMA can cause a range of reactions, including diarrhea and vomiting,

loss of blood into the intestines, respiratory tract infection, and anaphylaxis (Freier and Kletter, 1970). A study of food allergies in Britain during the 1990s found that CMA accounted for greatest number of fatalities (Macdougall et al., 2002). Some of the symptoms of CMA may initially be confused with lactose intolerance or Hirschsprung's disease (Kubota et al. 2006). In order to make the diagnose a physician must observe "a definite disappearance of symptoms after elimination of cow's milk from the diet, recurrence of identical symptoms after one cow's milk challenge, disappearance of symptoms after re-elimination of cow's milk, and exclusion of lactose intolerance and coincidental infections" (Sprikkelman et al. 2000).

Immunopathogenesis of CMA:

Research into the immunopathogenesis of CMA is ongoing and its mechanisms are not fully understood. The major allergic components of milk have been identified as four proteins in the casein fraction (as1-, as2-, b-and k-casein) and two proteins in the whey family, although there is great heterogeneity among the allergenic epitopes of these proteins (Ahrens et al., 2012). The mechanisms of CMA are typically classified into IgE-mediated and non-IgE-mediated.

IgE-mediated CMA occurs in two stages (Vitaliti et al. 2012: Brandtzaeg 2001: Beyer et al. 2002). When an allergic child first consumes cow's milk, the immune system undergoes a process of "sensibilization." First, antigen-presenting cells (APCs) consume milk particles and display allergenic fragments on their surfaces. Then 2 T helper (Th2) cells, which are insufficiently regulated by the immune system in CMA, come into contact with the allergen fragments and become activated. The Th2 cells in turn activate B cells, which produce large amounts of antigen-specific IgE. IgE antibodies against cow's milk proteins are then secreted and bind to the surface of mast cells and basophiles. After this immune arsenal has been built up and the child again consumes cow's milk, the allergy moves to its "activation" phase. IgE associated with mast cells bind allergenic epitopes on milk proteins, triggering an intracellular cascade that culminates in the release of histamine, platelet activating factor, and other inflammatory mediators. Chatchatee et al. (2001) found that the presence or absence of two binding regions IgE (AA 69-78 and AA 173-194) can be used to predict whether an allergy will resolve in early childhood or persist.

Although many cases of CMA involve an IgE-mediated mechanism there are also many cases that do not present circulating IgE specific for cow's milk proteins. The immunopathogenesis of non-IgE- mediated CMA is more obscure and a number of mechanisms have been proposed. One theory suggests the reaction is mediated by Th1 cells, host immunity effectors that typically act against intracellular bacteria and protozoa and have already been implicated in Type-1 Diabetes (Lee et al., 2010: Zhu and Paul, 2008). Another theory points to interactions between T lymphocytes, mast cells, and neurons (Lee et al. 2010). Some individuals with CMA demonstrate both IgE-mediated and non-IgE-mediated reactions.

Research has implicated the dysfunction of Tregs, a subpopulation of T cells that modulate the immune system and maintain tolerance to self-antigens in both IgE-mediated and non-IgE-mediated reactions. The body must maintain a delicate balance between mucosal tolerance and hypersensitivity: too great a tolerance will allow dangerous antigens to accumulate in the body and too great a sensitivity will lead to indiscriminate immunization against harmless foreign particles. Tregs help to regulate this balance by secreting "tolerogenic cytokines" such as TGF-beta 1 and IL-10. The resolution of CMA in children has been associated with the development of Treg cells. For example, Karlsson et al. (2004) gave milk to 21 children who had been following an elimination diet for at least two months and found that those who had outgrown the allergy had higher levels of circulating CD4(+)CD25(+) T cells.

Looking Beyond CMA:

The prevalence of CMA is well established and research has increasingly shed light on its mechanisms, but what about the impact of cow's milk on the immune systems of people without clinically defined CMA? In some quarters dairy has been accused of having "pro-inflammatory" properties and implicated as a cause or aggravating factor in a number of immune-mediated conditions. Some health resources have advised that people cut out dairy entirely. The rest of this paper will examine some of the research on these claims about cow's milk and the immune system.

Cow's milk and Systemic Inflammation:

Systemic inflammation is caused by the release of pro-inflammatory cytokines from immune-related cells and the chronic activation of the innate immune system. It is a risk factor for atherosclerosis, metabolic syndrome, type 2 diabetes, cardiovascular diseases, and other conditions (Labonte et al. 2013). The causes of systemic inflammation are notoriously difficult to isolate, and there has been disagreement over whether cow's milk-based dairy can contribute to systemic inflammation in healthy adults.

Although some epidemiological studies have found a correlation between dairy consumption and biomarkers of inflammation, the overwhelming majority of controlled studies have found a neutral or anti-inflammatory effect (Bordoni et al. 2015). Nestel et al. (2012) looked at four different full-fat dairy foods and found that they did not increase eight circulating biomarkers

related to inflammation. Schmid et al. (2015) found no significant difference in inflammatory markers after subjects ate a high-fat dairy and high-fat non-dairy meal. Several studies have even reported an inverse correlation between dairy consumption and inflammation. Panagiotakos et al. (2010) looked at the concentrations of the inflammatory factors CRP, IL-6, and TNF-α in individuals consuming more than 14 servings of dairy products a week and found them to be 29%, 9%, and 20% lower than those in individuals consuming less than 8 servings a week. Esmaillzadeh and Azadbakht (2010) studied 486 healthy women aged 40-60 years found that subjects on a low-fat dairy diet had lower circulating levels of IL-6 and sVCAM-1 than the control. Labonte, et al. (2013) conducted a meta-analysis of 9 studies and found no significant relationship between dairy consumption and systemic inflammation. Finally, Bordoni et al. (2015) conducted the largest meta-study to date (52 human studies) and found that the consumption of dairy products is generally associated with anti-inflammatory effects in humans. Of particular note was that none of the studies using low-fat dairy products indicated a pro-inflammatory response. Taken together, these findings suggest that the association between cow's milk and systemic inflammation in healthy adults is largely unfounded.

Cow's milk and Immune-Mediated Diabetes:

Diabetes mellitus type 1 (T1D) is a form of diabetes mellitus that results when T-cell-mediated autoimmunity destroys the insulin-producing beta cells of the pancreas. Although this form of diabetes has a strong genetic basis, it is also influenced by environment. The precise environmental triggers of T1D are a matter of ongoing research and debate, with some suggesting the role of particular antigens (Knip and Simell, 2012). The literature appears to be split on the question of whether cow's milk has a role in the pathogenesis of T1D.

The controversy began in 1984, when Borch-Johnsen et al. suggested there was an inverse-correlation between T1D and the duration of breast-feeding. Although some subsequent studies cast doubt on this link, researchers began to explore early exposure to cow's milk (as opposed to discontinued feeding by human milk) as a possible cause. Following this hypothesis, Scott (1990) looked at consumption of milk per-capita and found a significant positive correlation between consumption of unfermented milk protein and incidence of T1D in data from various countries. More precise epidemiological studies soon followed, such as a study of 690 T1D children in Finland, which found that children were 1.5 times more likely to develop T1D if they were exposed cow's milk early in life (Virtanen et al. 1993). The first meta-analyses showed a modest, but significant increase of diabetes in children who were exposed to cow's milk before the age of 3 months (Gerstein, 1994; Norris & Scott, 1996). Some recent studies have expanded on these findings. Kinip et al. (2010) reported on the Trial to Reduce IDDM in the Genetically at Risk (TRIGR), in which Finnish researchers assigned 230 infants to drink either conventional cow's milk-based formula or a dairy-free alternative. Ten years later, children who were fed the non-dairy alternative exhibited 50-60% fewer markers of β-cell autoimmunity. Villagrán-García et al. (2015) studied 150 children and found that those who began drinking cow's milk in early childhood were four times more likely to have T1D, one of the most dramatic results yet reported.

Several mechanisms have been proposed for a possible link between a child's early exposure to cow's milk and T1D. One theory suggests that cow's milk proteins may mimic autoantigens of the pancreas beta cells, leading to the autoimmune destruction of these cells (Kolb & Pozzilli, 1999). Another suggests that early exposure to cow's milk leads to elevated antibodies against bovine insulin and subsequent immunization against human insulin (Vaarala et al., 1999). A more recent proposal involves beta-lactoglobulin, a whey protein found in cow's milk but not in human milk, and glycodelin, a near-homologue that regulates T-cells. According to this theory, some infants produce antibodies against beta-lactoglobulin, which cross-react with glycodelin and allow autoreactive T-cells to proliferate (Goldfarb, 2008).

Although some epidemiological studies have supported a link between cow's milk and T1D, and a number of plausible mechanisms have been proposed, there is also conflicting evidence. For example, Savilahti and Saarinen (2009) found that infants who were exposed to cow's milk very early in life were actually slightly less likely to develop T1D before age 8 (although the discrepancy disappeared by age 11.5). Karlsson et al. (2001) studied 30 children with T1D and 18 healthy, age-matched children and found no difference in their Th1- and Th2-like immune response to cow's milk proteins, suggesting that cow's milk antigens do not have a significant role pathogenesis. Some researchers have argued that the existence of populations in which there is a high level of dairy consumption and a low rate of T1D disproves any connection (e.g. Iceland and Zealand), although these discrepancies have also been explained by the fact that protein content can vary by region (Thorsdottir, 2000). Some have suggested that only cow's milk with A1-type casein is contributing factor to T1D (Laugensen and Elliot, 2003), while others argue that the apparent differences between A1-type and A2-type milk are really due to differences in sun-exposure and vitamin D production (Merriman, 2009). Ultimately, the relationship of cow's milk and T1D remains a matter of debate, despite some epidemiological evidence supporting a causal link and the elaboration of a number of plausible mechanisms.

Cow's milk and Multiple Sclerosis:

Multiple sclerosis (MS) is a chronic inflammatory disease in

which the myelin sheaths that insulate the central nervous system are damaged. A connection between cow's milk and multiple sclerosis (MS) has long been debated. One of the first large scale studies on the question was Malosse et al. (1992), which examined the relationship between MS prevalence and dairy product consumption in 27 countries and 29 populations all over the world. The study found there was strong correlation between MS prevalence and the consumption of liquid cow's milk, but not between MS and more processed forms of dairy, like cheese. Some researchers have proposed that molecular mimicry between CNS myelin antigens and cow's milk proteins could explain the relationship. According to this theory, the IgE targeted to the cow's milk proteins cross-react with the myelin and lead to damage of the nerve cells (Ahrens et al., 2012). It has been suggested that the failure of T cells to regulate auto-reactive CD4+ and CD8+ cells has a role in the disease (Viglietta et al., 2004). Given the possible role of autoreactive T cells in MS, the mechanism proposed by Goldfarb (2008) for T1D may also be plausible here. Particularly interesting in this respect are studies linking the autoimmunity of T1D and MS (Winer et al., 2001).

Some studies have questioned the link between cow's milk and MS. Ramagopalan et al. (2010) examined 6638 cases of MS in Canada and found no significant difference in the percentage who reported childhood CMA, an odd finding if there is a significant cross-reaction between cow's milk proteins and myelin. Ashtari et al. (2012) examined 48 healthy subjects and 48 subjects with MS and found no difference in the detection of cow's milk-specific IgE. Given these contradictory results, the role of cow's milk in MS remains uncertain.

Cow's milk and Betcet's Disease:

Betcet's disease (BD) is characterized by chronic, immune-mediated inflammation of the blood vessels, leading to skin rashes and lesions, optic atrophy, and ulcers of the mouth and genitals. Although the etiology of the disease is not fully known, there is strong evidence for the role of Th17 cells, adaptive immune cells that are also associated with MS and RA (Hatemi et al. 2012).

Research on cow's milk as a factor in Betcet's disease is currently very limited. Triolo et al. (2002) cited promising research on the relationship between cow's milk and immune-mediated conditions like T1D and MS as a basis for examining the role of cow's milk in BD. First, the study cultured lymphocytes from 16 patients with BD and eight normal controls in the presence of ß-casein, ß-lactoglobulin, and a number of controls. ELISA revealed that when cultured with milk proteins lymphocytes from BD subjects produced significantly more IFNγ, a cytokine associated with auto-inflammation, than lymphocytes from the controls. Then the study used ELISA to analyze the serum antibody levels of 46 patients with BD and 37 healthy controls and found significantly higher levels of anti-ß-casein and anti-ß-lactoglobulin antibodies in the subjects with BD.

These results suggest some correlation between BD and sensitivity to milk proteins, but do not define a causal order. The authors suggest that milk proteins may damage the gut and lead to immune dysfunction and offer two possible mechanisms. First, the caseins in cow's milk may give rise to peptides that mimic opiates and bind T cells and macrophages, disrupting their function. Second, molecular mimicry between cow's milk proteins and self-proteins may lead to damaging cross-reactivity. These mechanisms are only speculative however, and there has been little research on the relationship between cow's milk and BD. The rarity of BD (approximately 0.1-7.5 /100,000 in Europe and the USA) makes epidemiological studies difficult to conduct (Zouboulis, 1999).

Cow's milk and Rheumatoid Arthritis:

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that primarily affects the small joints in the hands and feet, leading to swelling, pain, and loss of mobility. cow's milk has sometimes been cited as a contributing factor to RA. Although dairy has been identified as an aggravating factor in individual cases of RA (Panush et al. 1986: Panush, 1990), larger studies have failed to find a connection between the disease and the consumption of cow's milk. Panush et al. (1983), for example, studied 11 subjects on a dairy-free diet and 15 subjects on a placebo diet and found no significant differences in rheumatologic or immunologic findings. The literature on diet-therapy for RA is not extensive and tends to dispute any connection between cow's milk and RA; however, there are indications that dairy may be an aggravating factor in a small percentage of cases.

Discussion

The Challenges of Studying cow's milk:

Determining the impact of cow's milk on the immune system is complicated by a number of factors. Allergic and autoimmune reactions are multifaceted and reflect a confluence of individual biochemistry, genetics, and environmental influences that is not fully understood. Milk itself is a complex mixture with numerous bioactive components, and its composition can vary by region. Moreover, the consumption of cow's milk is always part of a large, multivariable diet, making it hard to rule out confounding factors. Any interpretation of the evidence must be cautious given the enormous complexity of the variables.

Current Nutritional Guidelines for cow's milk:

The 2010 dietary guidelines authored jointly by the Department of Agriculture and the U.S. Department of Health and Human Services stress the importance of cow's milk and cow's milk-products as part of a healthy diet. The guidelines recommend 3 cups

per day of fat-free or low-fat milk and milk products for adults and children over 9, 2.5 cups per day for children ages 4-8, and 2 cups for children ages 2 -3.

Assessment of Results:

Cow's milk is a vital staple of American nutrition, economy, and culture. This does not diminish the importance of continued research into the effects of cow's milk on human health. CMA is the most common form of allergy in childhood and can lead to serious complications. Beyond clinical CMA, cow's milk may impact the immune system in subtle, unexpected ways. Upon reviewing the evidence, there is no strong indication that cow's milk is a contributing factor to systemic inflammation in healthy adults or to RA. However, there is some significant, but contested evidence that it plays a role in the pathogenesis of T1D, MS, and Behcet disease. Currently, there is much we do not know. One theme emerging from the literature is the difficulty of drawing conclusions from epidemiological studies. Some epidemiological studies reveal a striking correlation between cow's milk and various immune disorders while others yield conflicting results. While prospective cohort studies offer obvious advantages over retrospective studies, they can still yield opposing findings, as evidenced by the large disagreement between Savilahti and Saarinen (2009) and Knip et al. (2010) over whether cow's milk is a risk factor for T1D. Such discrepancies suggest the need for further research and meta-analysis.

Additional Considerations and Conclusions:

The results are too messy and speculative to write off cow's milk as a "dangerous" food or to clear it of all suspicion. They do underscore the fact that we do not yet know enough about the bioactivity of cow's milk to rule out the possibility of detrimental effects on immune function. This uncertainty is compounded by recent findings that link cow's milk to prostate cancer and Parkinson's (Chen et al., 2007: Mandair et al., 2014). Numerous studies have questioned the traditional belief that consuming cow's milk improves bone health and some have even found that it increases risk of fracture (Michaëlsson K. et. al. 2014: Feskanich et al. 2014). Although many of these findings are still questionable, taken together they may warrant a reconsideration of current guidelines, especially when people in developed nations generally can obtain calcium and other vital nutrients from alternative sources. Given the important place that cow's milk has held in human nutrition and culture for thousands of years, and the vital role it plays in the contemporary American economy, any shift in thinking about dairy might be difficult to achieve. Nevertheless, it is important to evaluate the evolving body of evidence without cultural or economic bias and arrive at nutritional guidelines on the basis of sound science alone.

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The Psychological and Neurological Effectiveness of Placebo Treatment

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Abstract
Placebo effect is an alternative medical approach that doctors utilize in treating health issues. For years, people thought that placebo pills were inert drugs or medically illegitimate measures that have psychological effects on the patient, therefore alleviating the patient's pain. However, in recent years, with the advent of technology, more studies are involving neurological aspects to the already-proven psychological aspects of the placebo effect. Yet there is still some opposition and much to be proven better on this topic. What is very important is that although there is opposition, there is growing recognition that the placebo effect may actually involve changes in brain chemistry, and that the placebo effect might be a fundamental part of good medical care that will one day be universally embraced by doctors and patients as well.

Acronyms:

CBP	Chronic Back Pain
CCK	Cholecystokinin
CR	Conditioned Response
CS	Conditioned Stimulus/Stimuli
NSAID	Nonsteroidal Anti-Inflammatory Drug
PAG	Periaqueductal Gray
UCR	Unconditioned Response
UCS	Unconditioned Stimulus/Stimuli

Introduction

Since the 18th century, people have believed that placebo pills were inert drugs or medically unqualified measures that have psychological effects on the patient, therefore easing the patient's pain (Beecher 1955). Hence, the term placebo is a Latin derivative for "I shall please" (Moerman 2002). Furthermore, in recent years, many studies are associating neurological aspects to the psychological effects of the placebo effect.

Today there is a range of treatment that people categorize as placebo - as basic as administering a Band-Aid on a wound to inserting needles into a patient receiving acupuncture. Because such procedures do not contain much medical efficiency, their pain-reducing results are termed 'placebo effect.' If these practices do not involve elements or substances that are responsible for altering a health condition then what is it about these techniques that can make one pain free? Because placebo analgesia has proven to be one of the most successful models in the research of the placebo effect, this review will explain the long-known psychological influences of the placebo analgesia, as well as the newer and unconcluded neurological influences of the placebo analgesia.

Several psychological mechanisms contribute to the appearance, enhancement or duration of the placebo effects. Firstly, Pavlov's classical conditioning is a major contributor towards the placebo effect, whereby a patient is trained to respond positively towards a placebo stimulus. Expectations also play a big role - the patient's anticipation of clinical improvement is partially responsible for the onset of the placebo effect. The overall experience that the patient experiences influences the patient's expectations; doctor-patient relationship, the trial site, the appearance of the pill, and the way the treatment is given are all experience related factors that impact one's expectations.

How can it be that just by one merely having belief one can experience less or no pain? The neural mechanisms whereby placebo conditioning leads to placebo analgesia remain unclear. Scientists are attempting to find better explanations for the placebo effect by attributing it to neurological mechanisms of the body. Ascending pain signals travel through one's spinal cord, then to the thalamus, and then to the sensory- processing regions of one's cerebral cortex. In order for one not to experience pain due do to a pain provoking stimulus, there would have to be changes in the sensory network of the brain or in the spinal cord. Recently, researchers are associating opiate analgesics to the placebo effect. They are attempting to prove that placebo treatment engages opioid systems which block pain in the spinal cord. However, studies also show that there in fact is non-opioid related analgesia. Nonetheless, the explanations remain unclear. From the research done, it is evident that the placebo effect has psychological aspects and that there is a difference in brain activity due to placebo treatment which is seemingly responsible for placebo analgesia. Nonetheless, future research is required in order to pinpoint the definite neural activity responsible for the pain reduction.

Methods

In an effort to answer the question asked above, many published articles, clinical trials, and research papers have been examined. Primarily, Touro College's library database as well as Google Scholar were used to search for pertinent material. Original research papers that were referenced were also studied. An

attempt was made to determine if the placebo effect, as a result of the placebo treatment, is psychologically and or neurologically fact or fiction.

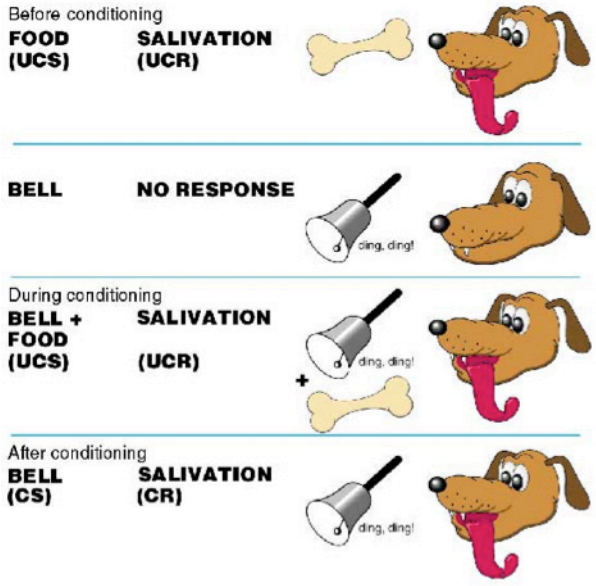
Discussion

Psychological Aspects: Learning and Classical Conditioning

People who suffer from pain, like a headache, and who usually ingest aspirin, learn to associate the color, taste, and shape of the pill with pain-decrease. After recurring associations, if such people are given a placebo pill, such as a sugar pill resembling aspirin, they will feel less pain. Other stimuli, such as medical personnel features, hospitals, and therapeutic equipment can also be associated with clinical improvements and therefore act as conditioned stimuli that impact healing effects (Benedetti, et. al. 2010). A 1960s' experiment found that a scopolamine injection, a medication used to treat motion sickness and other types of nausea, affected motor changes in a rat. Furthermore, identical motor changes also transpired after a placebo injection made of saline solution was administered after the scopolamine injection (Herrnstein 1962). Corresponding occurrences exist in humans too. Notably, if a placebo pill or procedure is given after two preceding administrations of an effective painkiller or practice, the placebo analgesic response is much larger (Amanzio, Benedetti, 1999). That effect emphasizes that the placebo effect is definitely a learning phenomenon.

Like other profound scientific developments, classical conditioning was discovered accidentally. In the 1890s, Russian physiologist Ivan Pavlov was observing dogs salivate in response to being fed. He then noticed that the dogs began salivating whenever he arrived in the room they were in, while he did not always have food with him (McLeod 2007). From this incident, Pavlov believed that some responses are innate, they don't need to be learned. In example, dogs do not learn to salivate whenever they see food as this reflex is inherent. In psychological terms, an unconditioned stimulus leads to an unconditioned response. Furthermore, over time the dogs began salivating as soon as they saw anything that they associated with their food. Therefore, they would salivate in the presence of a lab assistant even when he did not come with food (Windholz 1995). In psychological terms, the phenomenon is due because when an unconditioned stimulus is paired with a neutral stimulus, the latter becomes a conditioned stimulus that propels a conditioned response comparable to the unconditioned response. Pavlov furthered his research by experimenting with a bell; the dog was conditioned to salivate when a bell rang as he associated it with the food.

Numerous clinical trials attribute the positive placebo treatment effects to Pavlov's classical conditioning. Just as a dog salivated in response to a food-associated person or object, people



can experience a reduction in pain due to a painkiller-associated placebo procedure or drug. Indeed, a 1970s' experiment proves that placebo effects are in large due to classical conditioning; saccharin, a flavored drinking solution, was paired with cyclophosphamide, an immunosuppressive drug, and given to rats. After, the rats were immunized with sheep red blood cells. On the seventh day, the rats that had been again exposed to saccharin at the time of antigenic stimulation had a lower concentration of hemagglutinating antibodies in comparison with non-conditioned animals given saccharin, conditioned animals that were not re-exposed to saccharin, and a placebo group. Hence, the experiment proves that after associative learning, simply a liquid has the ability to mimic the effects of an active drug and thus legitimizes the placebo effect (Ader, Cohen 1975).

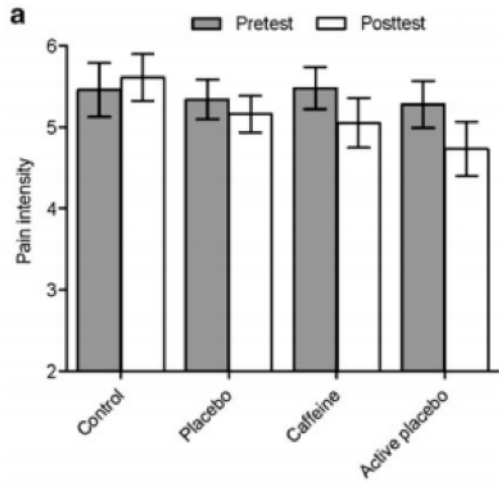
In response to people who argue that such conditioning is not possible to exist amongst human beings, an analogous trial provides substantial evidence that behavioral conditioning of immunosuppression is in fact promising in humans. Recurrent associations between cyclosporine A - an immunosuppressant drug - and a flavored drink incited conditioned immunosuppression in healthy male volunteers. However, more than one associative learning trial was necessary in order to bring about the fascinating results (Goebel, et al 2002).

Psychological Aspects: Expectancy

While Pavlovian conditioning serves as an unconscious influence towards placebo effects, expectancy serves as a conscious one. The expectancy theory hypothesizes that one's expectations impact ones future experience (Kirsch 1999). Therefore, if one expects clinical improvement, his chances of improving are that much greater. This theory is evident in the following 2010

double-blind clinical trial: A painful laser stimulus was delivered individually to twenty healthy participants before and after each was given a 0 or 4 mg/kg cup of caffeine. Some participants were told that their drink contained a painkiller, while the others were told their drink was a placebo solution. After measuring the reported pain and expectancy levels, it was clear that the information that a painkiller was given enhanced the analgesic effect of caffeine compared to caffeine given with no drug information (Bjørkedal, Flaten 2011). Moreover, those that were in the active placebo condition group - were told they were given a painkiller and they received caffeine – overall reported less pain than the subjects in the caffeine condition group – were not told they were given painkiller and they received caffeine. This reiterates the expectancy theory, as the first group was given two qualities (caffeine and drug information) that made them expect an improvement, while the latter group was only given one (caffeine). The expectancy of pain relief modulated the

Figure 2

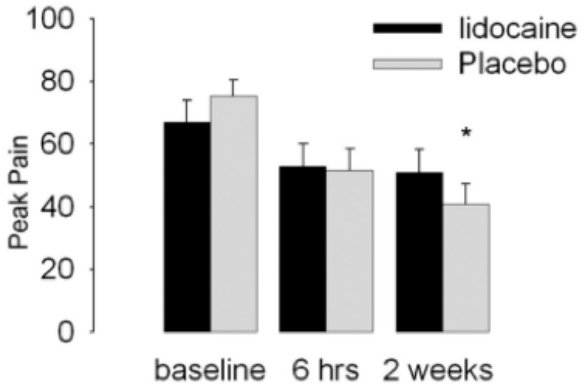


Subjects reported larger reductions in pain after 4mg/kg caffeine (caffeine, active placebo) compared to after 0mg caffeine (control, placebo). (Bjørkedal, Flaten 2011)

decreased pain experienced by the subjects (fig. 2).

From the above trial, it is evident that verbal gestures enhance expectancy in patients. Additionally, the overall experience that the patient experiences influences his/her expectancy of clinical improvement and consequently his/her clinical results. The doctor-patient relationship, the trial site, the appearance of the pill, and the way the treatment is given are all included in one's experience and can enhance the placebo effect. In another study, the effectiveness of 5% Lidocaine patches was compared with the effectiveness of placebo patches in treating chronic back pain. In the randomized double-blind study, 15 patients received 5% Lidocaine patches and 15 received placebo patches. Functional MRI was used to ascertain brain activity for variations of spontaneous pain, at the starting time and at two more points thereafter (6 hours and 2 weeks). As reported, there was no noteworthy difference between the two groups in their pain intensity and in pain related brain activity. And both groups reported more than a 50% pain decrease. Moreover, when compared with an untreated chronic-back-pain-group at similar intervals, the patch treated CBP group experienced a considerably bigger decrease in back pain (Hashmi et al, 2012).

Figure 3



Variation of CBP pain with treatment type and treatment duration. Treatment duration, but not type, significantly decreased CBP pain. (Hashmi et al).

These findings support the expectancy theory, as the patients who experienced a more encouraging experience by being given a patch consequently felt less pain later on (fig. 3).

Another method to reinforce one's expectation of clinical improvement, is to educate the individual about the so-called painkiller one is going to ingest. A random group of fifty university students agreed to either undergo placebo treatment under the appearance of a new analgesic painkiller capsule or to be put in the control group which did not receive placebo treatment. While some participants were handed educational handouts explaining the analgesic effect, others were not. The subjects then received electrically-induced pain at varied intensities, and those that ingested the realistic looking drug capsules reported analgesia. Furthermore, participants who also read the handouts regarding analgesia reported an even higher decrease in pain (Tang, Colagiuri 2013). This experiment highlights that the appearance of the pill and the knowledge of analgesia both contribute towards the patients expectancy and thus towards his or her placebo analgesia.

Psychological Aspects: Conditioning vs. Expectancy Theory

After much research, it is evident that both the conditioning and expectancy theories are driving forces of placebo effects.

However, can they coexist, and is one more potent than the other? In response to the former, both psychological mechanisms can surely coincide. For example, a patient who receives a sugar pill on its own after repetitively receiving the pill together with other analgesic drugs will imply both mechanisms; the sugar pill has become the conditioned stimulus to invoke analgesia, and the fact that the patient received medicine prompts him to expect a recovery.

Regarding which mechanism is more vital, Stewart-Williams and Podd (2004) reviewed many clinical trials and concluded similarly to what many others have suggested: each mechanism plays a different part in the production of placebo effects. When a doctor verbally encourages a patient, when an inert pill or procedure resembles a legitimate pill or procedure, and when other such characteristics mentioned previously exist when a patient receives a placebo, he/she learns to expect an improvement, which is a conscious belief. When one's conscious is involved, he can experience subjective and or physiological placebo effects. On the other hand, when placebos represent CS, the patient can either unconsciously believe in a placebo effect or the patient can consciously expect improvements in his situation. The conscious expectations and the non-conscious learning yields subjective and physiological (objective) placebo effects. Whilst expectancy learning is always a conscious action, conditioning can be conscious or not - depending on whether expectancy coincides with the conditioning. Nonetheless, each form of learning can produce subjective as well as physiological outcomes.

Others describe the correlation between expectations and conditioning differently. A study concluded that placebo responses are facilitated by expectation when conscious physiological processes - for example, pain - exist, notwithstanding, a conditioning procedure may simultaneously be implemented (Benedetti et. al, 2003). Sixty participants were divided into five groups and induced with pain in their forearms once per day for five days. Such ischemic pain increases over time rapidly, and the pain becomes excruciating after approximately 13 minutes. After the painful stimulus was implied, each participant stopped a timer when the pain became unbearable, and thus the average pain tolerance level for each group was recorded. As each group was treated differently throughout the five days, the results of each group greatly varied. Some were told analgesia suggestions or hyperalgesia suggestion, some experienced conditioining via ketorolac anti inflamatory analgesic, and some received a combination. Evidently, verbally induced analgesia expectations raised the pain tolerance; whereas, suggestions of hyperalgesia eradicated pain tolerance. This testifies that not only are positive words empowering, negativity is impactful too. Moreover, this highlights the detrimental effects of unencouraging words in the medical scene. When analgesia suggestions and conditioning were both implemented, the tolerance level rose higher. Yet, when conditioning and hyperalgesia suggestions coincided, the expectations outweighed the preconditioning effects of ketorolac, as the subjects's overall pain tolerance decreased. The study proves that analgesic placebo responses seem to be primarily mediated by expectations, such as verbally induced expectations.

The study of the placebo phenomenon has important implications on non-placebo procedures. The equivalent psychological factors that mediate the placebo effect are likely to be effective when an individual is given an active substance or undergoes an active procedure. Accordingly, a better understanding of the psychological mechanisms essential to the placebo effect is relevant in order to contribute these psychological factors to non-placebo treatments as well. For example, by increasing patients' positive expectancies for the effects of an active drug or procedure, it is conceivable that the drug or procedure will yield stronger drug effects without the use of stronger doses. There are numerous ways that physicians can achieve this: They can inform patients about others for whom the treatment proved successful, and doctors should update patients on the clinical research that portrays the treatment as worthwhile. Additionally, physicians should make people aware of the minor side effects related with the treatment. Then, when people come across any such symptoms in themselves (irrespective of whether these symptoms are a result of the active drug), they are likely to assume that the drug is working well. This will possibly boost their expectations for a positive effect, which will thereafter enhance the placebo element of the active treatment. Using these strategies, physicians can probably achieve stronger drug results without administering alternative stronger drugs or greater doses. This would be a safer solution for the patient, as he or she will not be exposed to unnecessary strong medication, and this will also decrease the costs of medical care. (Podd, Stewart-Williams 2004)

Neurological Aspects

Placebos have no innate power to elicit given results. Yet, they can produce the effects which are anticipated or sought. Placebo pills and procedures do not act on the brain to bring about placebo effects; however, placebos can stimulate expectations and CR which impact pain-reducing neural systems. What is responsible for the relation between the psychological mechanisms and placebo analgesia?

Neurological Aspects: Opioids

The neurology of placebo was discovered when researchers found that placebo analgesia is facilitated by endogenous opioids. They were prompted to make this hypothesis after they observed that naloxone, the opioid antagonist, can oppose the

effects of placebo analgesia (Levine et. al, 1978). Since then, further studies have emphasized that finding. Researchers examined the mechanisms fundamental to the activation of endogenous opioids in placebo analgesia (Benedetti, Amanzio 1999). Once a day, for five days, ischemic arm pain was induced into 229 participants. The subjects were divided into twelve groups, each treated differently. Each group received a combination of two or more of these

treatments throughout the five-day-experiment duration: the opioid agonist morphine hydrochloride, the non-opioid ketorolac (nonsteroidal anti-inflammatory drug; NSAID) trometh-amine, no treatment, an open injection of saline, or open or hidden opioid antagonist naloxone. The order of each group's treatment was made so that the treatment can serve as drug conditioning, expectation cues, or both. When morphine was administered, and when saline was openly injected and was believed to be a morphine-like (expectation and or drug conditioning), participants experienced less pain. Any participant who received naloxone after morphine (ketorolac was not administered), whether open or hidden and whether used as an expectation cue or not, experienced no difference in pain. When ketorolac was issued, there was pain relief. Moreover, when saline was issued, it mimicked the ketorolac effects. What was even more fascinating about the results was that when naloxone was issued after ketorolac, pain was partially diminished. This proves that naloxone only somewhat blocks analgesic effects; therefore, there must be non-opioid factors in addition to opioid factors influencing placebo analgesia. The results of the experiment prove that the opioid system is surely involved in placebo analgesia, yet there are also other aspects sometimes involved.

There is a lack of evidence proving how non-opioid pathways serve in placebo analgesia. However, just as ketorolac is a NSAID, placebos that mimic ketorolac or other NSAIDs' activity, probably behave like such drugs. They act at peripheral and also central places in the spinal cord, hindering the cyclo-oxygenase enzyme that is essential for the transformation of arachidonic acid into prostaglandins and thus preventing a painful experience.

Because studies prove the anti-opioid accomplishments of the neuropeptide cholecystokinin (CCK), further studies were done and demonstrated that the blockade of CCK receptors makes the placebo analgesic response possible, thus suggesting that the CCK role in placebo analgesia is quite impeding and that the opioid system is important in order for placebo analgesia to occur. This finding imparts that by irritating the anti-opioid action of CCK at the time of a placebo practice, the endogenous opioid systems can be activated. (Benedetti, Amanzio 1997) In a more recent study, forty participants were divided into four groups in order for the effects of CCK type-2 receptors to be examined (Benedetti et. al, 2010). Each participant was induced with ischemic arm pain like in previously mentioned experiments, and each group was treated differently. The results portrayed that activation of CCK type-2 receptors, which was achieved by means of administering the agonist pentagastrin, abolished placebo analgesia even after morphine-conditioning (placebo treatment) occurred. Evidently, CCK resembles naloxone. Furthermore, this study suggests that the equilibrium between CCK elements and opioids is critical in placebo treatment. So, when patients do not respond as predicted to placebo treatments, possibly CCK type-2 receptor hyperactivity exists in the patient's body-makeup.

What exactly is the endogenous opioid system? In general, the endogenous opioid system is an inborn pain-relieving method. It consists of dispersed neurons that produce three opioids: beta-endorphin, the met- and leu-enkephalins, and the dynorphins. These opioids behave like neurotransmitters and neuromodulators at three main categories of receptors - mu (μ), Delta (δ), and kappa (κ). Once attached to the receptors, they send signals which in turn block pain, slow down breathing, and have an overall calming effect; therefore, the patient experiences analgesia.

In order to more fully comprehend how opioids modulate the conduction of pain, one needs to understand the pathway by which pain is transmitted - beginning from its origin until its place of interpretation and perception in the brain. There are two types of first order neurons in which pain is transmitted along: In terms of nociceptive pain transmission, acute pain is transmitted mainly by A-delta afferent sensory nerve fibers, while chronic pain is transmitted mainly by unmyelinated C sensory afferent nerve fibers until it reaches the spinal cord. Because these nerve fibers are unmyelinated, chronic pain is transmitted slower than acute pain. Nociceptors, free nerve endings which are found in numerous visceral and somatic tissues all over the body, are stimulated by different sorts of noxious (harmful) stimuli - such as chemical, thermal, and mechanical - and once stimulated there is a release of leukotrienes, bradykinin, histamine, prostaglandins, potassium ions, and substance P. These substances activate the nociceptors which results in the generation of an action potential down the first order neurons and heading towards the spinal cord. When the fibers arrive at the dorsal gray horn of the spinal cord, they release neurotransmitters - glutamate, substance P, and calcitonin gene related peptide - and thus synapse with second order nerve fibers. The released neurotransmitters incite the depolarization of the second order neuron which crosses over to the opposite side of the spinal cord, entering the contralateral spinothalamic tract and from there ascends up the spinal cord and ultimately into the brain.

Here, pain is modified via descending pathways and this results in an individual experiencing pain.

Now, how do opioids hinder the descending pathways so that an individual does not perceive pain? Opioid agonists bind to μ -opioid receptors within the midbrain of the brainstem, on inhibitory interneurons. The opioid agonist action at these neurons results in a lessening in the inhibitory effects on the nerve fibers exiting the periaqueductal gray (PAG). Hence, they lead to disinhibition of the descending nerves from the PAG. In turn, there is an increase in their motion and in their communication to the raphe nuclei. Moreover, there will be a disinhibition of neurons in the raphe nuclei. There will be a general stimulation of the descending nerve fibers which are descending through the lateral funiculus in the spinal cord, and they eventually reach the dorsal horn of the spinal cord. As this is the site in which the original pain stimulus enters the spinal cord in its ascent to the brain, here the nerve fibers influence the activity of the initial pain stimulus.

Opioids impede afferent sensory nerve fibers, as well, that are ascending the spinal cord traveling towards the brain. Opioids directly attach to presynaptic mu-opioid receptors and thus cause an inhibition of pain transmission through the first order afferent sensory nerve fibers which enter the spinal cord. This leads to a lessening in the discharge of substance P that is required for the stimulation of second order neurons. Furthermore, opioids can directly attach to postsynaptic mu-opioid receptors on different ascending nerve fibers, and this adds to the diminished communication to the ventral posterolateral nucleus and consequently to the cerebral cortex. Additionally, opioids can indirectly inhibit second order ascending neurons in the spinal cord; they modify the ejection of substance P and serotonin from the stimulated descending nerve fibers from the raphe nuclei which control endorphin containing neurons inside the dorsal horn. The outcome of the impacts of opioids on the nerves found in the dorsal horn is the pain transmission inhibition.

As one's body does not produce enough natural opioids to alleviate excruciating or prolonged pain, opioid drugs are manufactured. Comparable to their endogenous complements, opioid drugs - opiates - act at the same receptor sites, producing analgesia as well. This wonder is possible, as the drugs mimic the endogenous opioids' chemical makeup. For this reason, patients who experience much pain are prescribed with painkillers –as they are opiates which mediate analgesia. When a placebo is administered, the goal is that the endogenous opioid system is activated as a result of the psychological influence on the patient's mind – the individual is convinced he or she received an opiate, so his body responds respectively.

Ethics of Placebo Treatment

As the physiology caused by placebo treatment is not conclusive, the placebo effect is not universally recommended. In fact, many view placebo treatment as immoral. According to Nikola Biller-Andorno (2004) of University of Goettingen, Germany, placebo treatment is only permissible when used for therapeutic reasons and when no better proven alternative exists. Additionally, the patient cannot be deceived, rather the doctor must acknowledge the patient's empowerment and autonomy. If the physician fails to abide by these standards, rather than practicing ethically he is practicing blunderingly. Because of such guidelines, it is difficult to practice placebo treatment on sick patients. Therefore, researchers are forced to set up trials in order to examine placebo effects.

Ethnic

After much research, it is noticeable that the placebo effect has become more effective. DiSalvo elaborated about the placebo effect in a 2015 edition of the Forbes magazine. He wrote that after reviewing a study of 84 clinical trials which were carried out between the years 1990 and 2013, he concluded that the placebo effect is getting stronger. However, he adds that this is only true of trials in the United States. Possibly because America is more advanced than many countries, the ability to conduct placebo treatments is more feasible. More importantly, he adds that America is the only country aside from New Zealand that allows drug companies to sell their products directly to consumers. This is blatant when one watches TV and sees the numerous drug commercials. The advertising in itself can contribute towards the placebo effect by creating expectation cues.

Conclusion

After examining the articles and experiments regarding placebo treatments and effects, it is apparent that while placebo treatment definitely has psychological influence on patients, the neurological influence is unclear. Many are skeptical of placebo effect all together. They argue that possibly the percentage of patients that report improvement would regardless have improvement – that is to say, often pain reduces over time so even without a placebo treatment one can be somewhat relieved from pain. However, in many trials, there are 'natural history' groups which are used to compare their pain tolerance to the placebo treated group's pain tolerance. And these studies have proven that the placebo treated group's pain tolerance was better than the non-placebo treated group's tolerance. This topic is fascinating as it proves the power of the mind over matter. Placebo management can trigger mechanisms that are equivalent to those triggered by drugs, which points out a parallel between psychological and pharmaceutical effects. With further research, placebo effect may become completely acceptable and instead of administering drugs which often have harmful side

effects or which are sometimes rejected by patients, placebo treatments will serve as alternatives.

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