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Epigenetics and Obesity: A Multi-Disciplinary Approach to Research and Treatment

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Thank You!

Dedication:
To my Dad, for all the love, life and support over all these years,
Thank you

Epigenetics and the Obesity Epidemic

Considering the recent advances in genomics, it can be supposed that we are looking towards a *Gattica*[1]-like future in which disease susceptibility is gauged at birth through high-throughput genotyping. There is talk of the promise of genetics through individualized medicine and Euan Ashely[2]recently set about to apply the current knowledge of genetics to evaluate an individuals health probabilities. The results of this cutting-edge technology, however, were unsurprising- the individual was susceptible to a variety of heart conditions and diabetes, a conclusion that could be determined from a quick look through his family history. Intriguingly though, Ashley concluded the paper by listing the suggestions for daily lifestyle changes that might help alleviate the individuals genetic health problems. But if genes determine health at a basic level, how can changes to lifestyle have a notable effect on health outcomes? The answer lies predominantly in the emerging field of epigenetics.

Epigenetics refers to a new area of science devoted to the study of gene expression. A specific definition of epigenetics differs depending on the scientist questioned. The reason for this is that the meaning has evolved greatly since its first use in 1942 by Conrad Waddington[3]. Waddington had used the term to describe "causal mechanisms" by which "the genes of the genotype bring about a phenotype"[4]. Waddington described an 'epigenetic landscape' (Fig. 1), comprising of environmental, chemical and biological factors that brought about changes in gene expression, eventually deciding a cells' biological fate. However, differing definitions of the term 'epigenetic' may exclude or include certain phenomena. Daniel

Gottschling[5], for example, defines epigenetics as "a change in phenotype that is heritable but does not involve DNA mutation….The change in phenotype must be switch-like, 'ON' or 'OFF', rather than a graded response, and it must be heritable even if the initial conditions that caused the switch disappear." This definition includes phenomenon like prion diseases. Prions are misfolded proteins that induce further misfolding of proteins in the cytoplasm. Scientists who define epigenetics as a chemical code strictly on top of the DNA that controls DNA structure and therefore expression would not consider prions a form of epigenetic control over phenotypes. This thesis will define epigenetics as heritable, reversible changes in gene expression that are independent of genotype. While this definition includes phenomena such as prion diseases and histone chemical modifications, this thesis will focus mainly on the chemical markings on top of DNA, specifically Cytosine-Guanosine dinucleotide base pair (CpG) methylation.

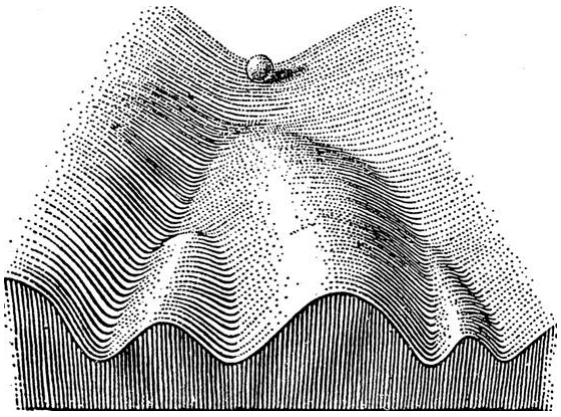

Figure 1: In 1942, Conrad Waddington first used the term 'epigenetics' to describe an epigenetic landscape such as that shown here. The hills and valleys are characteristic of environmental factors that direct the cell towards a specific cell fate[6].

CpG methylation is likely one of the most studied forms of an epigenetic 'code'. There is also more recent study on modification of histone proteins, which also affect gene expression. These 'codes' work function by changing the structure of how the DNA is packaged. Eukaryotic DNA is packaged with histone proteins to form an overlying chromatin structure that eventually forms the chromosome (Fig. 2). The basic structural unit of chromatin is the nucleosome, which is comprised of two wraps of DNA around a central octamer of histone proteins (two dimers, each of four of the five highly conserved histone protein types). The nucleosomes are placed at irregular intervals along the chromosomal DNA to form a "beads-on-a-string" structure. In response to a variety of unknown environmental stimuli, the DNA backbone along cytosine bases that aredirectly adjacent to guanosine bases may become methylated by DNA-methyltransferase enzymes. This degree of methylation subsequently changes the structure of how the DNA is packaged. Highly methylated DNA recruits methyl-specific binding proteins that tend to promote a highly compact chromatin structure termed heterochromatin. In heterochromatin, the underlying DNA is not accessible to molecular machinery and transcriptional proteins that are required for mRNA production and gene expression. Hence, that section of DNA is transcriptionally inactive and the gene is turned 'OFF'. Conversely, methyl groups can be removed from areas of DNA, making it hypomethylated and loosening the chromatin structure to create euchromatin. This allows access of transcriptional proteins and turns the gene 'ON'.

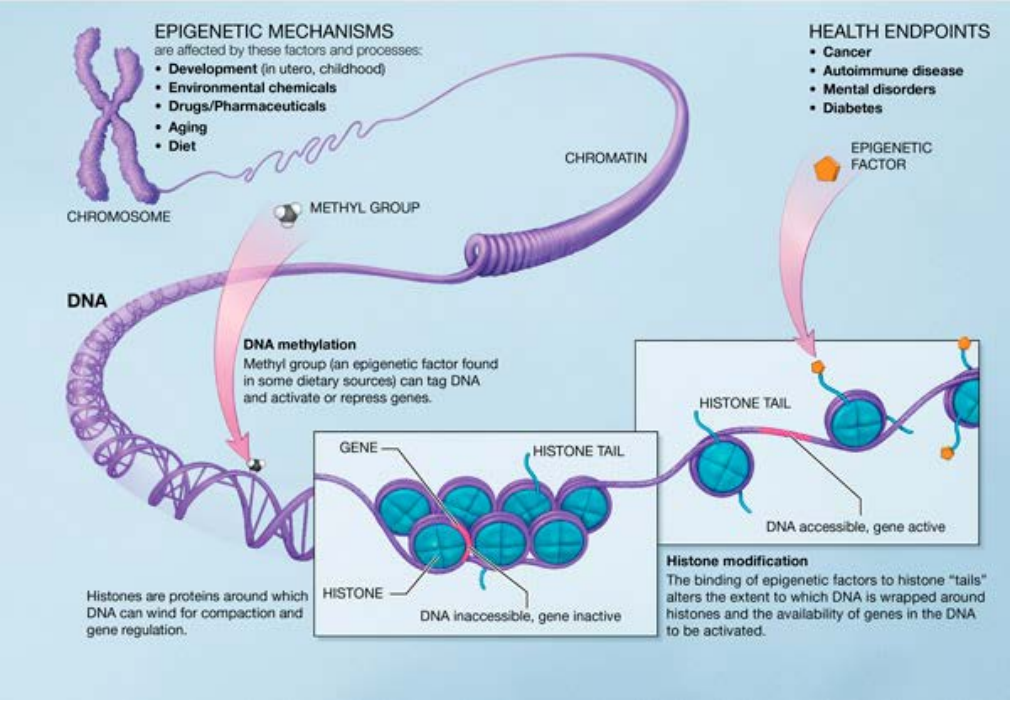

Figure 2: DNA Methylation, one of the most studied forms of epigenetic modification of gene expression. Hypermethylation causes the formation of heterochromatin, causing that piece of DNA to be transcriptionally inactive. Conversely, hypomethylation causes the formation of euchromatin, a loosely wrapped form of DNA that allows the DNA in that space to be transcriptionally active[7].

Changes in chromatin structure due to altered methylation not only induce a variety of phenotypic changes to each individual cell but are implicated in a variety of diseases. Most commonly studied is cancer, in which altered methylation status of oncogenes or tumor suppressor genes can promote or inhibit cancer tumor growth. For example, loss of expression of the tumor suppressor gene CCAAT/enhancer-binding protein-α (C/EBPα), is implicated in acute myelogenous leukemia and in many lung cancers. The cause of this change in expression was found to be hypermethylation of an upstream C/EBPα promoter[8].

The eventual therapeutic appeal of epigenetic studies is two fold: that the code is highly dynamic in response to various environmental stimuli, and that it can also be inherited through multiple generations.

The trans-generational effects of epigenetic phenomena have been demonstrated in lab animals but can be observed also in humans by looking at historical health records. One of the most famous examples of this is the Dutch Hunger Winter. This occurred in October of 1944 when German forces kept food supplies from entering into its western territories. By April of 1945, rations per person had fallen below 500 kcal/day and consisted of bread and potatoes. Food supplies immediately returned to normal after allied liberation in May of 1945, but the impact of the short-term nutritional deficiency and increased stress caused health problems for all individuals involved and for pregnant women and their infants in particular. Mean birth weight (a proxy for adult health) declined by 300g and central neural tube defects were observed with higher frequency in the infants of women who were exposed to the Dutch Hunger Winter in their third trimester. In the longer term it was also noted that the offspring had diabetes at twice the average rate, and experienced increase risk of schizophrenia and other neurological disorders[9, 10].

In another example of trans-generational epigenetic effects, Marcus Pembry[11] and colleagues investigated historical records in Överkalix, Sweden. Pembry was able to make a direct connection between food shortages, specifically during the slow-growth period (between ages 9-12 for boys and 8-10 for girls) and increased diabetes risk in the grandchildren of the same sex.

These transgenerational effects are not only a result of food shortages or under nutrition, but also due to stress. Offsprings of women who were pregnant while imprisoned in Holocaust concentration camps have been found to be more susceptible to Post-traumatic stress disorder (PTSD)[12]. This was first attributed simply to trauma from parent's re-telling of the horrifying stories to their children at young ages. However, further research indicated that the likely pathway for these effects was epigenetic regulation of genes such as c-Fos in the hippocampus[13].

These historical observations indicate that the experiences and environmental stimuli of our ancestors and of ourselves have deep biological effects that can be inherited throughout multiple generations. In the case of Marcus Pembrys studies in Överkalix, the epigenetic marker happened to be sex-chromosome linked, but the important factor is that these changes to expression are not genetic and do not directly cause genetic mutations, allowing for therapeutic changes to occur.

There is some debate as to whether epigenetic markers are inherited directly or if they are induced *de novo* in each successive generation during gestation. Graham Burdge and colleagues[14], studied epigenetic inheritance in rats and found that, by altering the diet of dams during lactation, successive generations would have an altered epigenetic profile than that of the mother. Burdge argued that this indicates *de novo* induction of epigenetic codes. Because there is inheritance seen historically, however, the epigenetic codes of offspring are likely based upon maternal or paternal codes. Alterations to the environment during developmental periods likely alter those codes. This implies that we have some sort of control over

our epigenetic code within one generation; external changes in environment during gestation and other developmental time-periods, such as the slow-growth period, can thus affect methylation patterns and health.

There arenumerous studies on this kind of fetal programming of gene expression during gestation that have eventual impact to later adult health. The studies previously mentioned are examples of this seen historically. More recently, maternal body mass index (BMI) has been correlated with not only high birth weight- a risk factor for cardiac disease in later life- but also with increased methylations of the Peroximsome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A) gene[15]. Folic acid (a known methyl group-donor) supplementation during pregnancy was also correlated with increased methylation of the H19 de-methylated region near the IGF-2 gene[16]. These epigenetic modifications are both also associated with newborn birth weights.

Evidence also suggests though that these programmed epigenotypes can be changed throughout one's post-natal lifetime. This was demonstrated both in newborns and during a lifetime. Further epigenetic programming occurs with maternal behavior to newborns. Weaver and colleagues[17]famously showed that maternal licking of pups was associated with the pups becoming courageous and outgoing later in life while the offspring of dams who didn't lick her pups were often smaller and more cowardly. Weaver then also showed the association of this character phenotype with altered methylation of the gluco-corticoid receptor gene, which regulates metabolism, the immune system and integration of neurotransmitters. In humans, maternal behavior related to breast-feeding has been

shown to have similar biological effects in offspring, including protection against Type 2 Diabetes[18,19].

Epigenetic research described thus far has resulted in the discovery of connections between our external environment, which includes social and cultural lifestyle and stresses, our internal biology and eventual health. Obesity, as a disease of both social and biological parameters, is then very likely mediated by epigenetic mechanisms and the trans-generational effects can help us to understand and hopefully reverse the trend of increasing childhood and adult obesity rates.

Since the early 20th century, obesity has been understood largely to be due to imbalances in energy consumption and usage that stems from a lack of willpower on the part of the individual. While these moral assumptions have since been contested, the basic beginnings of obesity still stem from imbalances in energy and metabolism that can be due to a variety of social, genetic or epigenetic factors.

At the start of the genetic era it was thought that genome-wide association studies would find key genetic polymorphisms that resulted in obesity. While over 50 loci have been associated with obesity, they seem to account for a very small amount of inheritance. The heritability of BMI has been estimated to be around 40-70%[20,21], but association of the Fat Mass and Obesity Associated gene (FTO) explains only about 1% of BMI heritability[22]. Other loci seemed to be mainly associated with only rare extreme forms of obesity[23]. It seems then that a good portion of the inheritance of obesity is likely epigenetic inheritance, and this is significant because this type of biological marker is dynamic and can therefore be subject to therapeutic measures.

Currently, there is significant evidence to suggest that epigenetic phenomena and inheritance mechanisms play a large role in obesity etiology and pathophysiology. A significant connection between epigenetic phenomenon and obesity etiology is the observation that epigenetic imprinting disorders are often characterized by obesity. A frequently stated example of this is Prader-Willi Syndrome (PWS), which is characterized by severe early-onset obesity and an inability to be satiated. PWS is caused by either a parental-specific deletion or inactivation through epigenetic means at the 15q11-q13 location in the individuals' genome[23].

Marcus Pembrys' studies[11] in Överkalix indicate epigenetic inheritance of the obesity-related condition Type 2 Diabetes. Zhang[24]and colleagues showed that maternal over-nutrition and high BMI was associated with up-regulation of Peroxisome proliferator-activated receptor- gamma (PPARγ)-activated genes in visceral fat and an increase in subcutaneous fat in the newborn.

Twin studies have also indicated that the epigenetic code is altered throughout one's adult life[25]. These alterations can affect many different processes along pathways for obesity and adipose tissue growth. Methylation patterns around the *leptin* gene may contribute to leptin resistance, a known cause for obesity[26].

Chronic obesity isassociated with adipose tissue remodeling. As a part of this remodeling, macrophages are recruited to adipose tissue in order to mediate the inflammatory response that characterizes chronic obesity. Biologically, there are two kinds of macrophages- those that are classically activated (M1 form) and exhibit pro-inflammatory features and those that are alternatively activated (M2 form) and

exhibit anti-inflammatory features. Alan Saltiel and colleagues suggested in 2007 that obesity is associated with 'phenotypic switching' of macrophages from the anti-inflammatory form to the pro-inflammatory M1 form[27]. This switch is likely epigenetically controlled, since the genome between M1 and M2 macrophages does not itself change to induce the phenotypic changes.

For the most part, the number of adipocytes in an individual is fixed by early adulthood, a feature that is also likely epigenetically controlled, since epigenetic changes to PPARγ and C/EBPα gene expressions are known to regulate adipogenesis [28-32].

Epigenetics, then, plays a large role in adiposity, obesity and obesity-related conditions such as Type 2 diabetes and heart disease. Because these epigenetic codes can be altered, however, there is indication that the inheritance of the obese phenotype can be hindered as well by changes to lifestyle throughout a lifetime.

This thesis aims to study possible routes for obesity treatment and prevention through both epigenetic and social mediums. To do this, participants were recruited from Wellesley College to donate DNA samples with buccal swabs. DNA was extracted and run through methylation-specific PCR to detect whether genes for adipogenesis such as PPARγ and C/EBPα were methylated or not. The methylation status could then be compared to lifestyles of the participants to discern trends between certain habits and the expression of adiposity-related genes. In this study, lifestyles were self-reported in an interview. This biological study allowed the development of an improved protocol for future investigations into lifestyle correlations with epigenetic codes. Continued study in this area has

potential to identify specific habits and foodsthat may have healthful or harmful effects on gene methylation.

Social and anthropological indications were studied both in the participants from Wellesley College who gave DNA samples, but also through conversations with Kristin Stanford, a postdoctoral researcher at Joslin Diabetes Center, with Dr. Evan Rosen at Beth IsraelDeaconess Hospital in Boston, MA, and through participant observation and conversations with participants in Batavia, NY. Batavia is a small city in Genesee County, NY and represents a unique view into the social factors that contribute to weight management and obesity. The per capita income in Batavia is $20,597 and 21.3% of the population live below the poverty line[33]. This created a stark contrast to the demographics in Wellesley, MA, where per capita income is about $66,800 and only 4.2% of the population lives below the poverty line[33]. This contrast gives a valuable range of social perspectives of weight management but also elucidated many of the political and economic issues that contribute to health both in the current population and, epigenetically, to future generations.

While the biological and social studies in this thesis were conducted separately, the analysis of both with respect to epigenetics gave a unique summation about the issues that hinder initiatives for obesity treatment and illuminated changes that may help reverse the epidemic.

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Studying the Epigenetics of Obesity: A Suggested Protocol

As mentioned earlier in chapter 1, epigenetic inheritance and coding plays an important role in obesity etiology. Connected intimately with this relationship are factors of lifestyle and culture with would affect health via changing epigenetic codes. This study aimed to test and design a scientific investigation of the epigenetics of obesity, including aspects of lifestyle as well as the expression of genes linked to obesity. This was done by interviewing participants about their lifestyles and their understandings of epigenetic and genetic inheritance with relation to health and identity. After this, samples of DNA were taken and analyzed for the expression of obesity-related genes as outlined below. The results of this study revealed opportunities for public health interventions that are discussed in chapter 3, as well as an optimized protocol for future studies that is outlined briefly here and detailed in the Appendix.

Internal Review Board Approval

Due to the close involvement of participants in this study, through personal interviews, participant observation and DNA sampling, Internal Review Board (IRB) approval was required for confirmation of ethical conduct.

In this thesis study: Wellesley College IRB approved this thesis study (See Appendix for a copy of the IRB application). All participants in this study gave informed consent (See Appendix for a copy of the Informed Consent form).

Participant Involvement

The first distinct difference between this biological project and most others is the incorporation of anthropological techniques of participant observation with microbiological procedures. Participant observation takes the place of self-reporting surveys for participants' diets and lifestyle habits. Self-reporting is often inaccurate, and has been historically so for many decades. Not only do individuals often leave out details so as to be judged as 'healthier' on the part of the physician or researcher, there are also details that may be significant but forgotten. A participant may do a fair amount of walking on a daily basis, but will forget to mention it when asked about daily activities because they may not understand that to be apart of a lifestyle choice. Research participants also will often lie, for various reasons, about their diet. In a study done in the 1930s[1], subjects were asked to list their current diet before the doctors fed the participants that exact diet they had self-reported. Of the eight participants, six lost weight, some as much as five pounds in less than a week. The others remained at their starting weight. When interviewing a Post-doctoral researcher and a medical researcher at Beth Israel Deaconess Hospital in Boston, the reliability of self-reported diet was under question as well. [2,3]

Participant observation of individual subjects thus eliminates the error in self-reporting and provides a more accurate picture of the subjects lifestyle, eating habits, diet and stress levels, among other factors for health and social well being. This technique also allows identification of social conflicts to health that may not be immediately obvious, resulting in the illumination of other social factors that might

inhibit weight-loss or the creation of a healthier environment. This supplemental information has the possibility to guide the application of the study's results.

In this thesis study: Participant observation in this thesis study, due to time and resource constraints, involved mainly personal interviews. 16 participants were interviewed from Wellesley, MA about their diet, lifestyle and stress levels (see Appendix for a list of guiding questions used during these interviews). 7 participants were interviewed from Batavia, NY. These two sampling areas gave contrast to the attitudes and lifestyles of persons from difference census demographics. The results of these interviews and observations are discussed in chapter 3.

Tissue Biopsy

The selection of cells to biopsy depends greatly on the gene of interest to the researcher as gene expression very likely differs between cell types. Biopsy technique options may also vary depending on cost and the invasiveness of collection procedures. Considering the relationship of white adipose tissue (WAT) to the development of obesity and the metabolic changes that occur during obesity, WAT cells may be of particular interest. In this case, liposuction is likely a very promising technique for the collection of adipose tissue.

Other types of tissue to investigate, depending on suspicions of gene activity, may also include skeletal muscle, bone or salivary tissue. This is because the production of various cytokines from bone marrow or skeletal muscle has been known to vary with weight loss, gain or exercise. Interleukin-6, for example, is a cytokine involved in the human immune response and has been shown to be produced by skeletal muscle after exercise[4]. Leukocytes are also released from bone marrow. Because obesity is characterized by chronic inflammation in adipose tissue, these immunological cytokines and cellular responses from muscle and bone tissue present a unique area of epigenetic study as well.

In this thesis study: Considering the salivary involvement in digestion, as well as economic constraints, buccal tissue was collected from each participant with swabs using the BuccalAmp DNA Extraction Kit (Epicentre #BQ0901S).

DNA Extraction and Quantitation

Genomic DNA should then be extracted for analysis of DNA methylation and the amount of DNA present in each sample should be quantitated.

In this thesis study: DNA was extracted and purified as per manufacturers instructions for the DNeasy Blood and Tissue Kit (Qiagen #69581). Sampled cells were centrifuged in 4ml Phosphate Buffered Saline (PBS) for 5 minutes at 1800 x g. The supernatant was discarded before the cells were resuspended in 180µl PBS. 25µl of proteinase K and 200µl of Lysis buffer were then added before vortexing and incubation at 56˚C for 10 minutes. 200µl of 96-100% ethanol were then added

before centrifugation for 1 minute at 8,000 rpm and the flow-through was discarded. 500µl of Buffer AW1 (Qiagen #19081) were added before centrifugation at 8,000 rpm for 1 minute. Flow-through was discarded. 500µl of Buffer AW2 (Qiagen #19072) were added before centrifugation at 14,000 rpm for 3 minutes. Flow-through was discarded. Cells were resuspended in 10mM Tris-Cl and 0.5mM EDTA (pH 9.0) before incubation at 21˚C for 1 minute and centrifugation at 8,000 rpm for 1 minute.

DNA was initially attempted to be quantitated using Fluorometry. This was done according to instructions from Sambrook and Russell[5]. Hoechst dye 33342 was originally used until Hoechst dye 33258 could be obtained to be consistent with Sambrook and Russells protocol. Extracted DNA was exposed to Hoechst 33258 dye. Fluorescence was detected using a Spectramax 340PC spectrophotometer (Excitation λ 352nm; Emission λ 460nm). Using stock salmon DNA a baseline scale was to be set up with which to compare and quantify the DNA concentrations in the participant samples. Unofortunately, Spectramax readings were inconsistent. A consistent trend comparing DNA concentration to fluorescence could not be determined. DNA was subsiquently quantitated using a Nanodrop (ND-1000) Spectrometer. Concentrations of DNA within each sample ranged from 5.4- 21.7 (±0.1)ng/µl (Table 1).

Table 1: Concentrations of extracted DNA from participants using the Nanodrop (ND-1000)

DNA Sample #	Concentration (ng/µl)	260/280nm reading
31	14.3	2.18
32	10.2	2.84
34	5.5	2.47
35	21.7	1.74
36	5.4	2.55
38	14.2	2.31
44	18.7	1.70

Gene of interest selection

As there are a number of genes that are thought to be obesity-related, the choice of gene to investigate becomes an important question. Future studies may be able to investigate DNA methylation on a genomic scale, but current limitations in technique and available instrumentation force the researcher to choose only a few genes of interest. It is important also to investigate whether there is a likelihood of differential methylation patterns among individuals of the chosen gene of study. This would eliminate results showing uniform methylation regardless of external factors. Studies should also take into account the variability of methylation profiles at the promoter. The specific DNA sequences chosen are often located in promoter regions for the gene. This is because the promoter regions are primarily methylated into differing chromatin structures that further control gene expression.

In this thesis study: The researcher chose to investigate the genes Peroxisome Proliferator-Activated Receptor gamma (PPARγ) and PPARγ Coactivator 1-alpha (PPARGC1A).

PPARγ gene is of particular interest because it is necessary and sufficient to induce differentiation of mesenchymal stem cells into adipocytes[6,7,8]. This was demonstrated by Dr. Evan Rosen, who researched the roles of the PPARγ and CCAAT Enhancer Binding Protein alpha (C/EBPα) genes in adipogenesis in the late 90s and early 2000s with genetic knockout mice. C/EBPα was sufficient to induce adipogenesis, since it was found that both of these genes induce each other's expression, but not necessary. C/EBPα was found instead to be significant in the development of insulin sensitivity, while PPARγ was identified as the primary effector of adipogenesis.

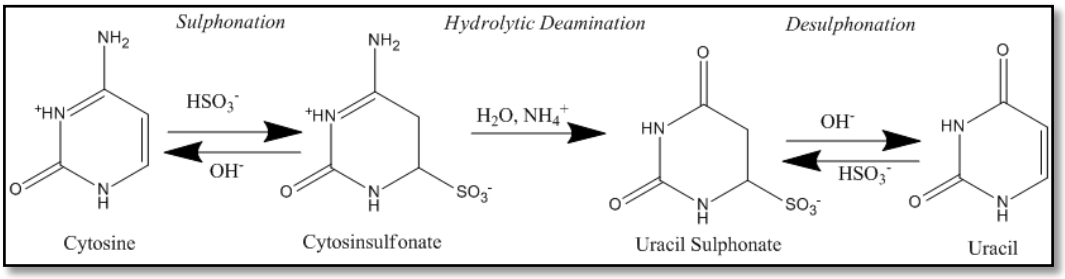
Carolina Gemma and colleagues found that, in newborns, 100% of the PPARγ promoter regions were found to be methylated[9]. While this methylation pattern is likely also to change throughout childhood and later development, this indicated that PPARγ is not a gene of interest in newborns. Assuming that methylation profiles do change throughout one's lifetime, however, PPARγ remains a gene target of interest in adults for this recommended study.

PPARGC1A was also of interest as Gemma and colleagues found correlation between methylation of the PPARGC1A promoter and maternal pregestational Body-Mass Index (BMI)[9]. Gemma and colleagues reported that 45% of PPARGC1A alleles were methylated. While this variation was reported in their newborn

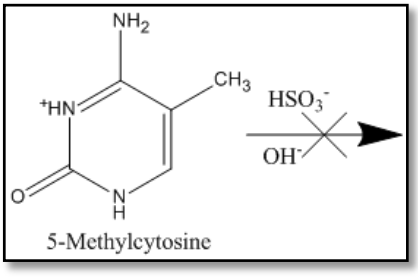
samples, it may correspond also to similar variation among the adult samples collected in this thesis.

Methylation-Specific PCR (MS-PCR)

MS-PCR is a technique developed by James Herman[10] to distinguish whether or not small areas of DNA are methylated. Bisulfite sequencing, a similar procedure, involves the direct sequencing of amplified DNA to distinguish specific methylated CpG nucleotides. Both procedures, however, involve bisulfite modification of the DNA, a treatment of the DNA that converts all unmethylated cytosine bases into uracil bases (Fig. 1a, 1b). PCR using primers specific for the DNA area of interest will amplify the DNA enough to sequence the gene and distinguish methylated bases.



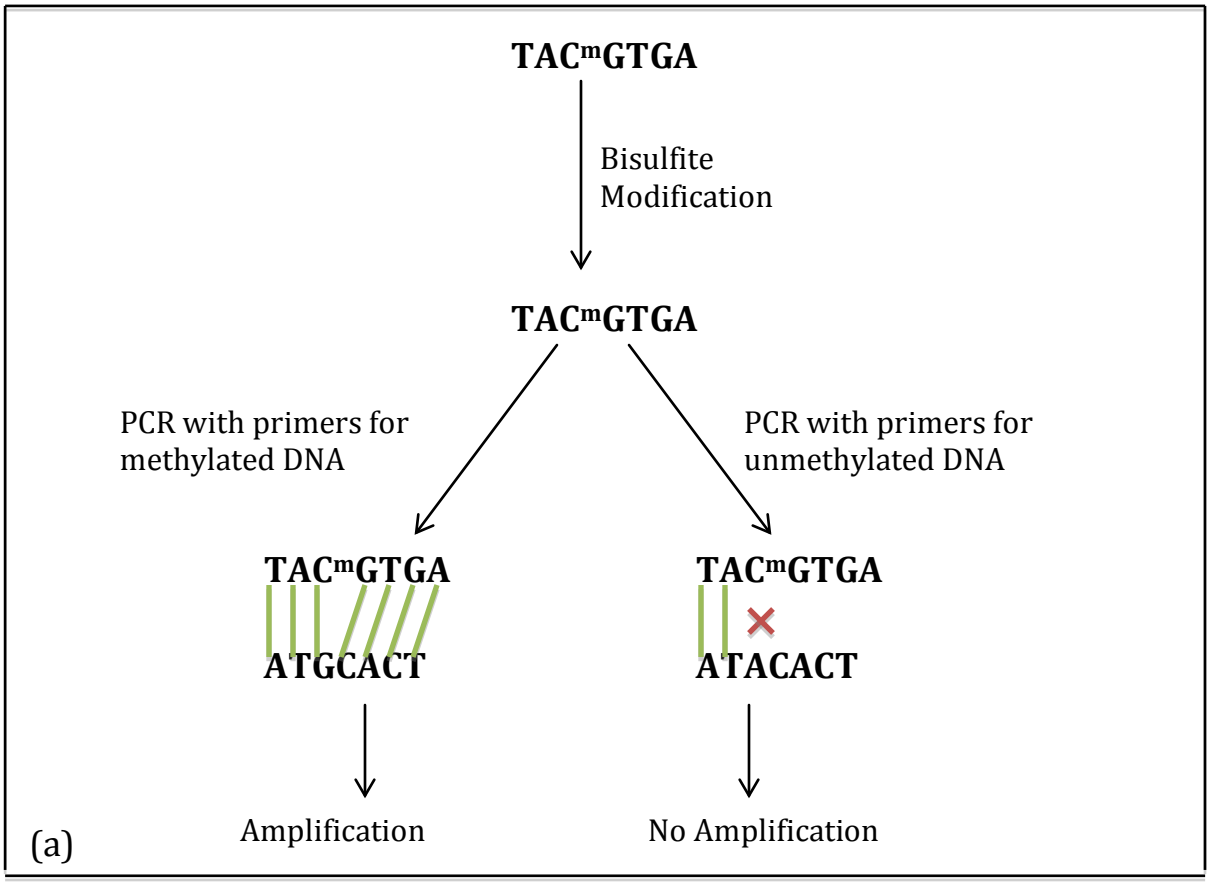
(a)



(b)

Figure 1: The chemical process of bisulfite modification of the DNA nucleotide Cytosine (a) and 5-Methylcytosine (b). In (a), the reaction goes forward, converting cytosine into Uracil. In (b), the methylated cytosine base prevents the reaction from going forward.

MS-PCR also allows supplemental determination of methylation status (Fig. 2a, 2b). This technique uses specific primers that differ for methylated and unmethylated DNA. Once DNA has been bisulfite treated, the sample is split into two different PCR reactions, one with primers for methylated DNA and the other with primers for unmethylated DNA. Upon visualization of amplified DNA with gel electrophoresis, it may be observed if the DNA was methylated or otherwise, which should correspond also to the Bisulfite Sequencing results.



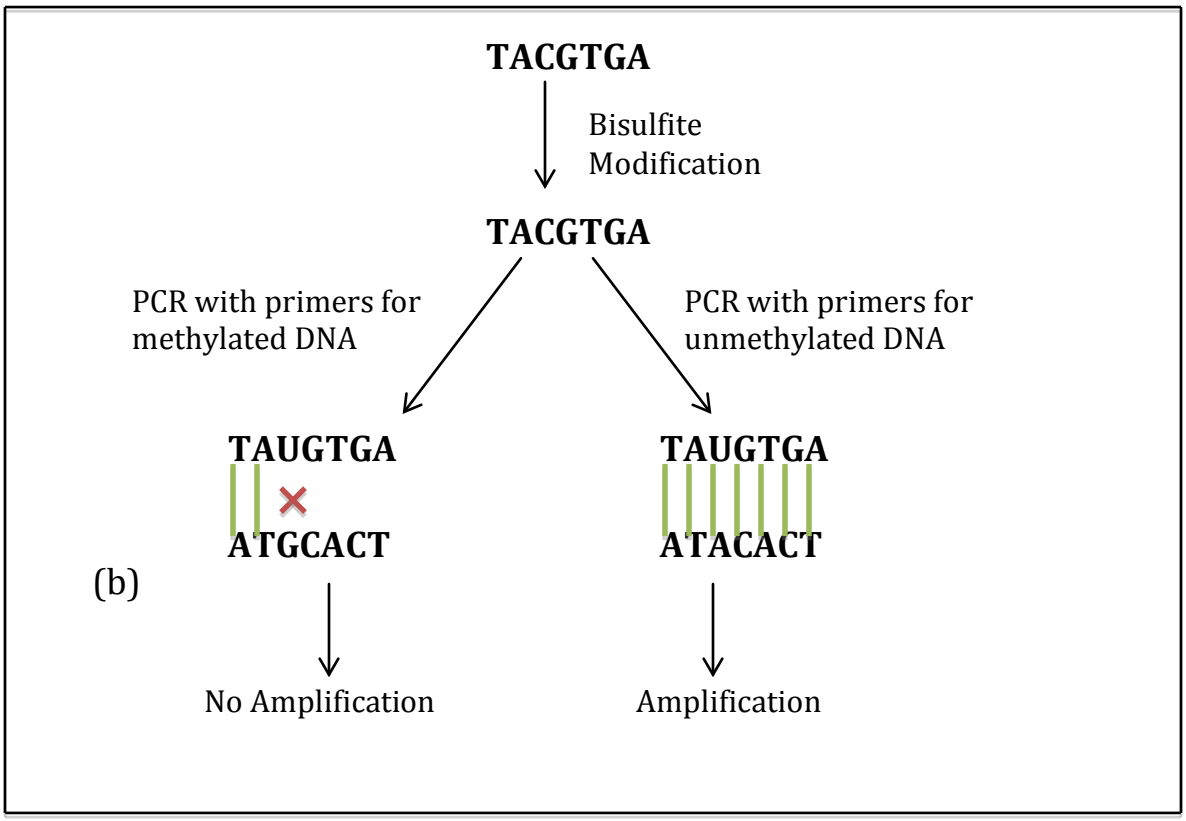


Figure 2: The process of bisulfite modification (a) when the sequence is methylated and (b) when the sequence is unmethylated. A red X shows that binding does not occur between the primer and amplicon sequences, which results in no amplification of the DNA. Amplification of the DNA can be demonstrated by Gel Electrophoresis, a process by which the DNA is separated by weight and stained so that amplified pieces of DNA may be visualized.

In this thesis study:

Bisulfite Modification of purified DNA from participants was performed using the EZ DNA Methylation Gold Kit (Zymo Research D5005S) as per manufacturer's instructions. Briefly, 13.3% DNA in CT Conversion Reagent was incubated in the following thermal cycle (Table 2). DNA and 600 µl of M-Binding Buffer were added to a spin column and centrifuged at <10,000x g for 30 seconds. DNA was washed with 100µl M-Wash Buffer and further centrifugation for 30 seconds at <10,000 x g. The sample was then incubated at 21˚C for 15 minutes in 200µl M-Desulphonation Buffer before further centrifugation at <10,000 x g for 30 seconds. The DNA sample

was washed twice as described above with 200µl M-Wash Buffer. DNA was then eluted with 10µl M-Elution Buffer and 30 seconds of centrifugation at <10,000 x g.

Methylation specific PCR was performed using primers for a PPARGC1A and a PPARγ promoter and corresponding conditions as described by Carolina Gemma and colleagues[9] (Table 2, 3). PCR was run in 20µl final volumes with 10µl PCR Master Mix (Promega #M7501), 100 ng DNA, 300ng forward and reverse primers and DEPC water. Unexpectedly, a DNA amplicon of about 300 bp, larger than the size of the reported PPARGC1A amplicon (141bp), was amplified in all PCR conditions (Fig. 3) with the PPARGC1A promoter. No amplification was seen in all PCR conditions with the PPARγ primers.

Table 2: Temperatures and Times for Bisulfite Modification and MS-PCR thermal cycles.

Cycle:	Thermal Cycle Temperatures (amount of time at temperature):
Bisulfite Modificiation	98˚C (10 minutes) 64˚C (2.5 hours) 4˚C (storage up to 20 hours)
PPARγ MS-PCR	40 cycles: 94˚C (30 seconds) 65˚C (30 seconds) 72˚C (40 seconds) Final Extension at 72˚C (5 minutes)
PPARGC1A MS-PCR	40 cycles: 94˚C (30 seconds) 57˚C (30 seconds) 72˚C (40 seconds) Final Extension at 72˚C (5 minutes)

Table 3: PCR primers used for Methylation-Specific PCR.

Gene	Forward Primer 5'→3'	Reverse Primer 5'→3'	Size, bp
PPARGC1A-M	ATTTTTTATTGTTATGGGGGTAGTC	AAAAATATTTAAAAACGCAAACGAA	143
PPARGC1A-U	TTTTATTGTTATGGGGGTAGTTGA	AAAAAATATTTAAAAACACAAACAAA	141
PPARγ-M	TTGGATAGGTTACGATGGATAGC	AAACGAAATAAAAACGTAAAACACG	101
PPARγ-U	TTGGATAGGTTATGATGGATAGTGT	AACAAAATAAAAACATAAAACACAAA	100

M is methylated-specific primers; U is unmethylated-specific primers.

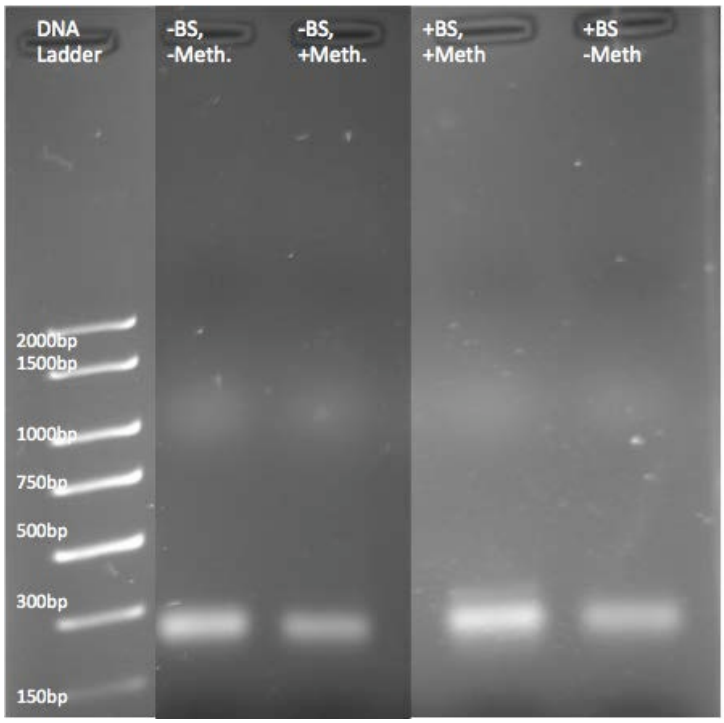


Figure 3: Amplication of PPARGC1A DNA was observed in all PCR conditions, including non-bisulfite modified DNA (-BS) with primers for methylated DNA (+meth). Bisulfite modified DNA (+BS) was also amplified with both primers for both methylated and unmethlated (-meth) DNA.

Investigation into the PPARγ primer code, using comparison to NCBI sequences to the gene were unsuccesful, indicating possible mis-coding of the primers. Research thus primarily continued with PPARGC1A primers. PPARGC1A primer codes were successfully matched to their corresponding NCBI codes.

Troubleshooting then began, starting with investigation of the PPARGC1A primers. PCR conducted with new primer solutions continued to result in amplification in all conditions. (Fig. 4). The amplified DNA was now at about the correct size (141bp) of the expected PPARGC1A amplicon. In conditions with

bisulfite treated DNA and primers for methylated DNA, however, there was now an unexpected second band of DNA amplified at a different size.

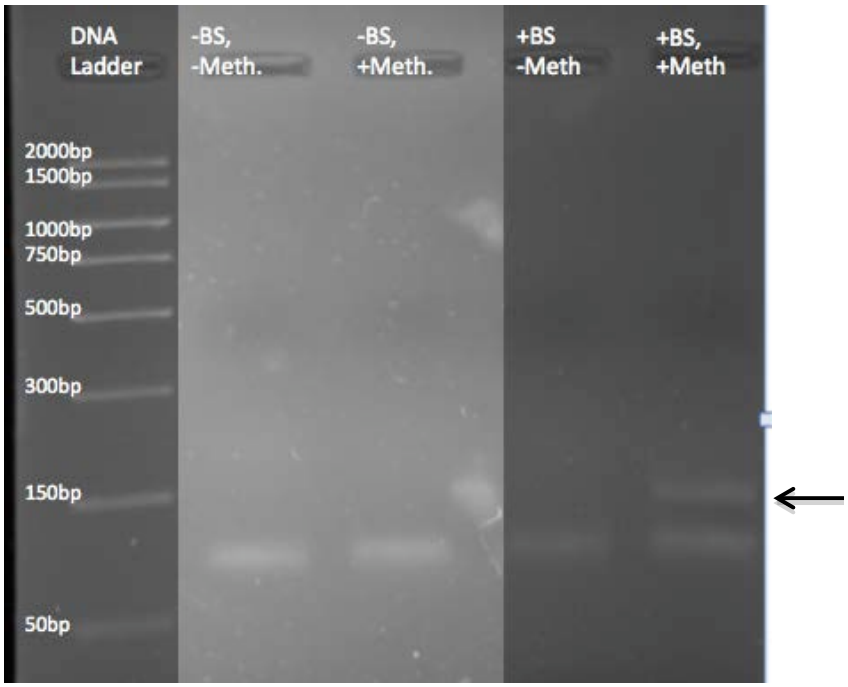


Figure 4: PCR was performed again using new primer solutions. Amplification continued to occur in all conditions but the amplicon was now closer in size to the PPARGC1A promoter. A second piece of unknown DNA (arrow) was also amplified in the bisulfite treated (+BS) DNA with primers for methylated DNA (+Meth).

Troubleshooting then aimed at investigating the DEPC water supply. PCR amplification also occurred in conditions without DNA added and with new sources of DEPC treated water (Fig. 5). The amplicon was again approximately twice its expected size.

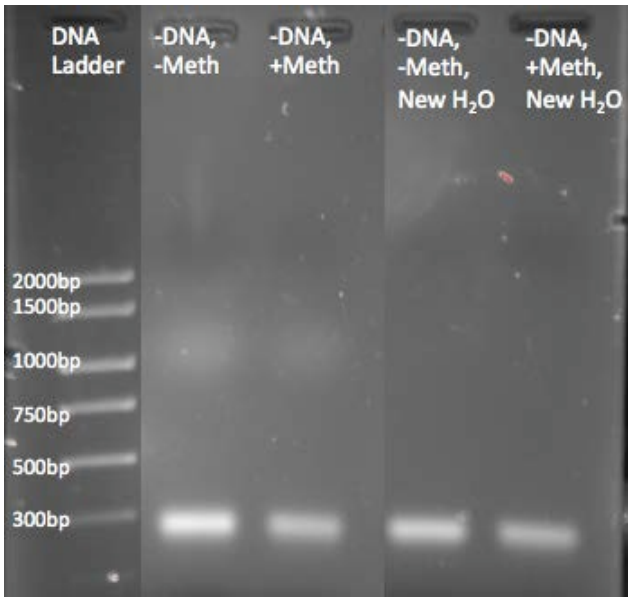


Figure 5: Amplification occurred also in conditions without DNA added (-DNA) and with new sources of DEPC water (New H_2O).

Unfortunately, because of inconsistent PCR amplification, conclusive, replicable results could not be obtained in this particular study. Further study would include more streamlined and sterile procedures and work. Desired results would have shown amplification in PCR containing either sets of primers, but not both, for one sample of DNA. The amplified piece of PPARGC1A promoter would also have been 141 bp in length (Fig. 6).

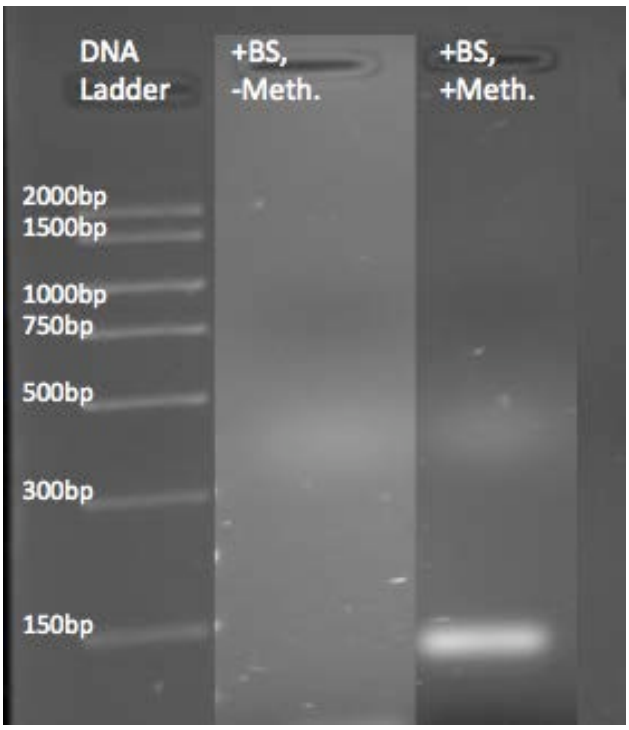


Figure 6: A depiction of anticipated PCR results under conditions in which the PPARGC1A promoter is methylated. Here, the amplicon is about 141 bp, which is the length of the PPARGC1A promoter that was desired for amplification. Amplification of bisulfite treated DNA would be successful in only one of the two conditions. In this example, the PPARGC1A promoter would be methylated, as the DNA was amplified in the presence of primers for methylated DNA only.

Suggested Changes for Future Projects

An optimal investigation into the epigenetics of obesity and obesity-related genes would involve a few large changes to the steps outlined above.

Firstly, the determination of lifestyle and anthropological factors should be a more integral part of the investigation, encompassing not only an interview, but also a more in-depth participant observation lasting for an extended length of time as described by Harvey Bernard[11]. This is for reasons outlined above, specifically for the elucidation of precise diet and lifestyle factors, as well as unexpected social factors inhibiting healthy choices.

Secondly, investigation should optimally include the entire genome. While this may not be possible quite yet, new technology may allow determination of genome-wide methylation. Oxford Nanopore Technologies[12] have developed a fast and portable system in which DNA is fed through a nanopore. Each nucleotide emits a unique electrical signal as it passes through the nanopore, allowing direct sequencing of the DNA, including the distinction of methylated Cytosine bases[11]. Should this novel technology be used, more data could be collected, possibly resulting in elucidation of new genetic and epigenetic correlations to obesity in areas of the genome whose function is not yet understood or fully known.

Thirdly, and most importantly, the determination of methylation status via MS-PCR or other techniques allows researchers to conclude very little about the actual expression of the gene in question. Gene expression comprises a multi-step process, through transcription and translation into a final product. Methylation status only reveals that the gene is able to be transcribed, but in truth the biological cell has other ways of hindering gene expression at other stages in the process towards expression. In order to correlate methylation status with gene expression, therefore, this investigation should also be accompanied by the following techniques to determine whether RNA and protein products are found within the tissue sample as well, revealing the successful translation as well as transcription of the gene of interest.

RT-PCR is a technique allowing detection of RNA that corresponds to the gene of interest, confirming that the gene and its promoter are accessible to transcriptional proteins and are in a euchromatin structure (Fig. 7). It involves the

conversion of RNA samples taken from the biopsied tissues into complimentary DNA strands and the amplification of that DNA. Successful or unsuccessful detection of complimentary RNA should coincide with and therefore confirm DNA methylation profiles determined from bisulfite sequencing and MS-PCR.

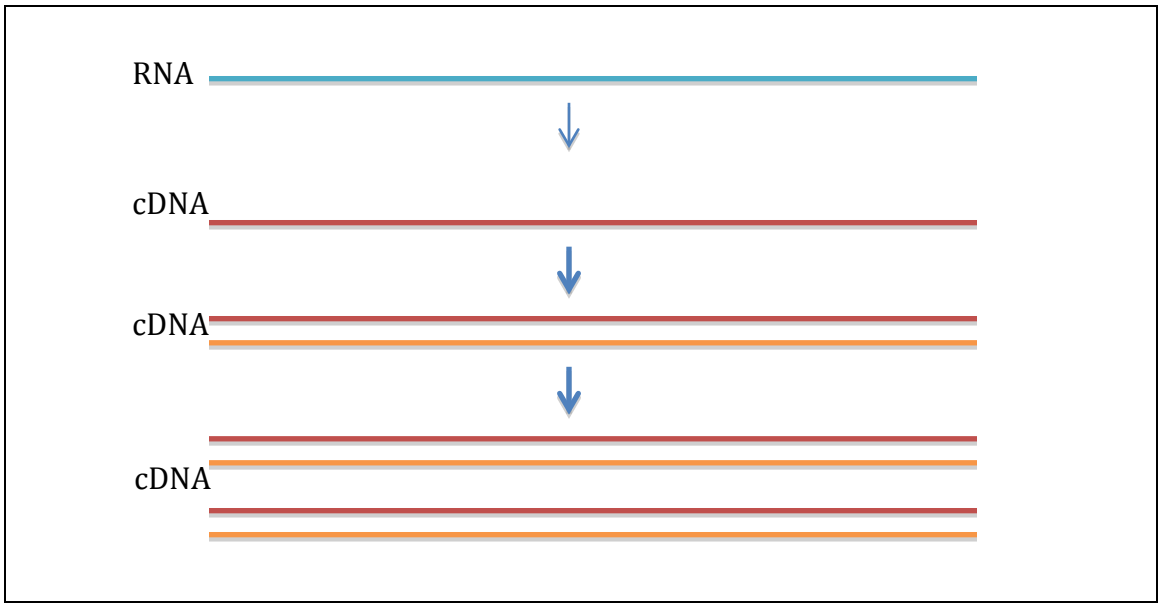


Figure 7: The process of RT-PCR, in which complementary DNA (cDNA) is created from mRNA strands using the enzyme Reverse Transcriptase. A complimentary strand of the cDNA can then be created and both strands will then be amplified through multiple PCR cycles.

In order to confirm that the gene of interest is then not further inhibited through post-transcriptional silencing, the researcher should also scan for active protein products of that gene through a Western Blot procedure.

In summation, a complete investigation into the question of obesity epigenetics would include thorough participant research as well as determination of gene expression (or lack thereof) at all stages of the creation of a product from DNA to protein (Table 4).

Table 4: The suggested protocol determines the CpG methylation code on top of the DNA, while also confirming gene expression at all steps throughout the process of protein production from the genetic code.

Biological Steps to Gene Expression:	Lab Procedure to Assess Expression:
DNA structure	Bisulfite Sequencing MS-PCR Oxford Nanopore Technologies[12]
DNA→RNA	RT-PCR
RNA→Protein	Western Blot

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Social Views of Health: What's wrong and what do we do about it?

Up until now this thesis has focused mainly on biological phenomena that affect the etiology of obesity and diabetes. Research is continuously ongoing in these subjects as the rates of obesity continue to increase in the United States and abroad (Fig. 1).

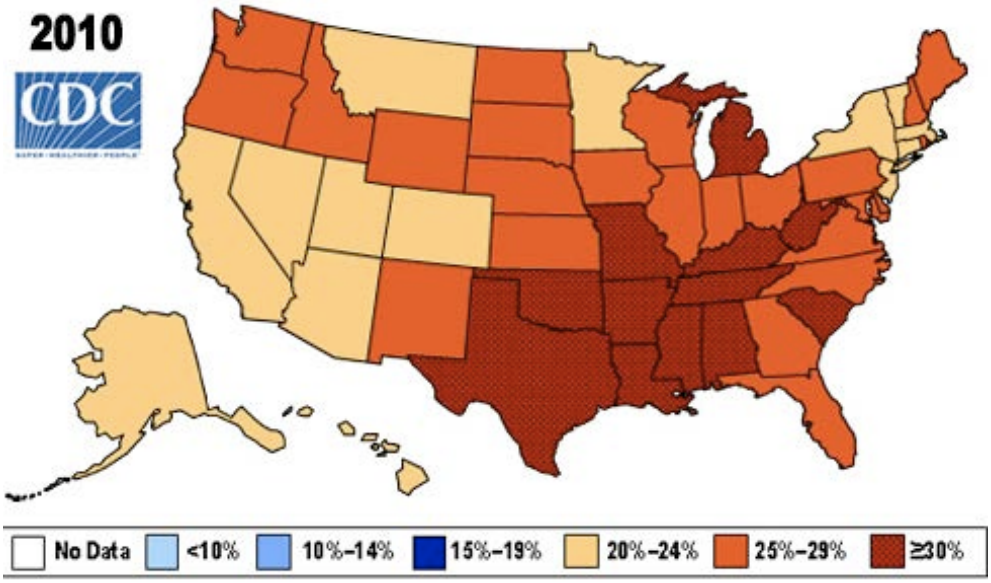

Figure 1: Percentage of obese persons in states across the US. Here, obesity is defined as a BMI > 30 kg/m^2. According to the 2010 census, no state had obesity rates lower than 20%[1].

As research results are released they gradually add to and shape the way we understand these conditions. Breakthroughs in genetics, for example, have brought on the 'genetic era' in which conditions, including obesity, were often reduced to simplistic, deterministic understandings of inheritance. This was a rapid and significant change from the previously supposed understanding of obesity and related conditions as related to some version of moral deficiency. Epigenetic studies

such as that suggested in this thesis have the similar ability to significantly change social dynamics. Results suggesting significant environmental effects on gene expression leading to conditions like obesity and diabetes could affect medical approaches to treatments and personalized nutrition recommendations; these studies could also change views of self and ethnic identification, notions that are no longer static as genes but dynamic as the epigenetic code. And because of the social implications, epigenetic perceptions could permeate into aspects of social interactions, relationships and behavior.

To investigate how this might occur and what kind of social factors would affect the epigenetic code specifically, interviews with participants from a variety of socioeconomic and ethnic backgrounds were conducted. Participants were asked about their understanding of epigenetics, how they create their own identity and if and how that identity relates to food and lifestyle. Interviews were recorded, transcribed and analyzed for consistent themes. Previous interview-based projects on genetics and on the incorporation of nutritional advice were also used to supplement the conclusions made in this chapter.

The results indicated that at face value epigenetic phenomenon were received favorably and coincided with already understood social notions of individualized health. When it comes to incorporating epigenetic results into daily-life and lifestyle changes, however, participants expressed the same kinds of hesitations that they have about current nutritional advice from sources such as the USDA, magazines and the Internet. Despite difficulties in implementing large-scale changes at the population level, epigenomics and nutri-epigenomics have the

potential to empower persons to take an active role in their health at both the preventative and palliative care-levels.

Incorporation of Epigenetic Phenomena into Common Understanding

In the interviews, participants were quite open about their habits that they understood to be healthy. A 17-year-old female explained how she understood the USDA recommendations by saying, "I just eat fruits and veggies and protein everyday. I'm not gonna try to figure it out." Another participant, a 19 year old female, said, "my family is pretty health conscious, my grandparents were as well….If it's a holiday dinner, you're going to have pumpkin pie. But it's not outrageous or anything. It's just one piece of pumpkin pie." One individual also responded proudly that she snacks only on apples and oranges from her college dining halls and only rarely on pretzels. It was also noted that the participants were more open about describing their good habits ("Oh yea, at home I sleep 14 hours a day!"- 20-year-old Female) and were slightly reticent about describing their bad habits ("During the week I guess I average 4 hours of sleep a night- maybe 5. Yea, 5 hours of sleep."- 20-year-old Female). This seemed to be in agreement with findings from Simon Cohn's study on diabetes in which he describes that "For the patient, food provides a subject matter to demonstrate compliance and gain approval"[2]. The participants here, while not diabetic or outwardly sick in anyway, still exhibited this desire for approval of their lifestyle habits.

After briefly describing the basics of epigenetics and epigenetic phenomenon to the participants (see Appendix), the similar desire for approval continued as the

participants molded their own understandings of health and diet to this new phenomenon.

"If that active lifestyle is something that I enjoy, then I'm gonna make it all the better by eating well." (21-year-old Female)

"The way that my family raised me is like, it's not just your genes, you contribute to your body too. It [epigenetics] reinforces that." (17-year-old Female)

"I know a lot of stories about people who are recent immigrants…and they get here and they get fat and are exposed to fatty foods. If you have a sort of recent family history of food deprivation, then your body absorbs nutrients more effectively….My mom is on the Irish side and a lot of women on her side have a problem with weight gain and losing weight. She's convinced that it's because her side of the family lived through the potato famine." (21-year-old Female)

"Of course. If I take care of my body, it'll take care of me." (27-year-old Male)

These quotes collectively show that the participants were reflecting on experiences with family or in their past that would imply epigenetic phenomena and understanding. The researcher explained epigenetic phenomena by describing historical instances in which experiences of ancestors affected health statistics in later generations (such as the Dutch Hunger winter and Marcus Pembrys' study in Överkalix, Sweden[3]). These descriptions were already understood historically, but by describing them in the context of health and inheritance, the researcher may have unintentionally assisted the incorporation of epigenetic inheritance into social understandings of inheritance and health. However, this method of describing

epigenetics, while perhaps artificially assisting in making the phenomena familiar, also accurately described their observable effects.

The participants then readily accepted epigenetic phenomenon and incorporated the ideas into their notions of how the body works. One participant even expressed that one of the attractions of the epigenetic phenomenon specifically was that it emphasized individuality, something that is important in understanding how different bodies work. “I think it’s (tailored diets based on epigenetic data) a great idea, because it’s so specific to you, and I think it’s cool that we can conceive of doing that.” (19 year-old Female).

On its own then, the idea that our lifestyle affects our health and the health of future generations was not immediately shocking to the participants. However, as epigenetic research continues, it can contribute to changing commonplace understandings of health in two different ways- firstly, the fact that our environment affects us at the deepest level of individuality, our DNA, something that we associate with identity and secondly, that our environment affects our offspring for, not only one, but multiple generations. As seen by the participants’ willingness to incorporate new knowledge of epigenetic phenomena into current understandings of health and inheritance- for whatever reason, be it the method or route of introduction- these contributions of epigenetics to health then will supplement, rather than replace, current genetic notions of inherited health.

Food Connections and the "Others"

The concept of identity is unavoidable when discussing social impacts of epigenetic theories of inheritance and health. This is because up until now, static concepts of genetic inheritance have comprised a rather large part of kinship identification and identity. In a discussion with a 21-year-old female, for example, she described this pervasive understanding of genetic determinism. "Yes, I've sort of always been of that mind that, sort of, your genes are what make you, what make everything up about you."

A 20-year-oldfemale also mentioned briefly her previous understandings of genetics but went on to also remark about how epigenetics might affect her understandings of identity and individuality.

Researcher: Do you identify yourself by your genome?
20-year-old Female: "Oh yea! I would. Yes. It's kind of a form of individuality."
Researcher: Could epigenetics change that?
20-year-old Female: "Yea, I guess you can manipulate it a bit. I guess I don't have to be what my genes exactly are, or something like that."

Here, the participant expressed that epigenetic concepts would allow her a level of active control of the genome that she inherited. While her genome may ultimately have a large effect in determining her individuality, the ability to manipulate the gene expression gives additional factors that might determine her identity. In this way, epigenetics may add to social perceptions of the importance of choice in determining identity.

One of the most well-studied and popular parts of a cultural identity, and a part which would also have a large impact on the epigenetic code, is food.

Food is long known to be a form of connection between people and has been exhaustively studied in many cultures as a way to investigate social and economic structure, human behavior and even evolutionary mechanisms of species divergence[4]. Food has been found to explain and correlate to a great extent with systems of value[5,6]- both political and social- as well as systems of memory and memory construction[7].The study of the symbolic nature and meaning of food within society gained popularity with Claude Lévi-Strauss. His famous work on the metaphors of raw and cooked foods as structural dichotomies of 'the natural' and 'the socialized' spurred a great many anthropological works[8]. Levi-Strauss emphasized his structural anthropology theories, hypothesizing that the cooking of foods can be understood through a larger structure of cultural processes.

A more recent review in *Food, Health and Identity*[9], brought together works on changing food practices, food as a marker for identity and links between food and health. These works conclude that "Culture plays a significant role in determining what we classify as food"[9]. The studies in this collection find that food consumption and preparation are closely linked to ethnicity, social status and social relationships. The social habits and rituals that have been created around food then in turn affect health. In turn, health also affects the types of foods eaten, for example in recommended diets for diabetics or those with heart disease. These interactions between food, health and identity are constantly changing, as noted by Caplan, and are likely to be further transformed by epigenetics.

One of the classic examples of cultural food rituals and connections is the family or social dinner, which many of the participants expressed as enjoying. They expressed the ritual of preparing the food together as being almost more integral to community bonding than eating the food, and also expressed that the practice was missed once the participant left the home for college.Family and social dinners contributed greatly to a tighter familial bond.

" I have a social connection to food for sure. It's something that, if I'm going to do something with my social group, it usually revolves around food in some way" (Female, 21-year-old)

"We have family dinners every weekend" (Female, 17-year-old)

"When our family gets together, it's generally oriented around dinners and that kind of stuff. It's generally like a positive." (Female, 19-year-old)

These quotes indicate that the cultural practice of social eating and family dinners is a large part of participants' eating habits and a practice that greatly influences present diet. If culture then determines how we eat and if the foods we eat affect our epigenetic code and therefore our health, it follows logically that culture and cultural identities play a large part in determining our health and physical phenotype.

A practical application of knowledge gained from epigenetic studies such as that suggested in the previous chapter is the availability of tailored diets based on an individual's genotype and epigenotype. Dietary recommendations such as these personalized diets are likely to affectgene expression, eating habits and health but also social and individual identity, since the two are so intimately connected.

Historically, notions of kinship and nationhood have been determined mainly from supposed deep, inherited biological differences between peoples. In the West, this was assumed to be through blood-ties. This gave us idioms such as 'blood-brothers' and 'blood runs thicker than water' that still float among the public discourse today.

With the advent of popular genetics, the shared biological 'blood' that created social ties and bound kin became DNA, a static notion of unwavering links to family. Bob Simpson[10] recalls an example of the notion of kinship and nationhood through shared DNA in the exportation of donated sperm for *in-vitro* fertilization treatments from Denmark to the United Kingdom. News responses spoke of a 'Viking Invasion' of Danish DNA into the English gene pool that initiated an increase in UK sperm donations. "One contributor to the letters page of the *Glasgow Herald* suggested that the origins of the sperm didn't matter as long as it came from a nation that could play football." Simpson goes on to suggest that the notion of 'Danish genes' affecting the 'English' gene pool had enabled a metaphor for a kind of imagined genetic community."With this novel means to essentialization comes the possibility of reworking ethnic identities as imagined genetic communities, that is, communities in which the language, concepts and techniques of modern genetic medicine play their part in shaping identity, its boundaries and what is believed to lie beyond."[10]

And now epigenetics arrives on the scene- a connection between culture, environment and biology that allowsphysical expression of a cultural identity. What then will become of ethnicity and social identity?Genetics is a static inheritance while epigenetics is a dynamic process that can control the genetic code. So we are stuck with genetic inherited identity but what we do with it- our chosen cultural and dynamic identity- can play just as large a part, if not larger because of the interactive and personal nature of cultural identity with health outcomes. Choice of cultural lifestyle and identity then, or even a lack of choice, play a larger part in health and health disparities.

A Social Disease

It's well known that choices for diet and lifestyle habits are culturally and socially determined. People generally eat what their family or close friends eat, and that is usually also determined by what is available, what is popular, what kinds of foods are well advertised or politically subsidized. Obesity then, a condition related to chronic, unhealthy choices can also be understood as a social disease.

In his book *Fat: Fighting the Obesity Epidemic*, Robert Pool[11] describes the importance of understanding obesity as a social phenomenon.

> "If the history of obesity research teaches us anything, it is that obesity-…-is not an individual problem. It is a disease caused by a sick environment to which some of us are more susceptible than others. This is not a way of thinking about obesity that comes naturally to those of us who grew up hearing that weight is a matter of willpower, …, and that if you're fat it's nobody's fault but your own. But it

is a way of thinking about obesity that must become much more common if we, as a society, are to find a reasonable solution to our common problem."[11]

A social disease, affected by social understandings of health, the impact of choices on our future and health and the health of future generations should then also be approached with a broad and social context. Presently and for the past century, the favored approach to fighting obesity has been with nutritional and physical interventions at the individual level, but none of these approaches have been successful at a level needed to reverse the rising rates of obesity. A postdoc researcher at the Joslin Diabetes Center in Boston, who was also interviewed for this thesis, described the problems that occur in these kinds of individual, short-term interventions.

"[The Why WAIT program] is a program at the Joslin [Diabetes Center] where they take Type 2 Diabetics, who are typically relatively obese, and they put them through this kind of 8-week training program. And they look at, you know, serum levels, triglycerides beforehand and then post-[program] and obviously it drops along with body weight and all those things that you would expect to see. And I think that those are really good interventions. The problem with those interventions is that the patient has to continue, and that's typically where you see more of a problem."[12]

One of the reasons that these interventions at the individual level may not work came out in an interview with a 27-year-old female living in Batavia, NY. As per the 2010 census, 21.3% of the population in Batavia live below the poverty line with the per capita income is $20,597[13]. According to the CDC in 2009, 27.8% of

persons in Genesee County are obese[14]. This woman was familiar with the struggles that she and many of her friends had gone through to try and lose weight, but had come to terms with why she was skeptical about making changes.

"What's the point in living forever if you can't do what you enjoy? If all I can eat is tuna and mayonnaise- both things I absolutely hate with the fire of a thousand suns- and I can't hang out with my mom, or Mike [because they smoke] or wrestle around with the pit bulls then I'd rather have 6 months to live." (27-year-old Female).

She expressed here a view that the lifestyle changes suggested by health professionals were often not socially desirable, making it unfavorable to undergo permanent changes. She ended with a joke that received a fair amount of laughter from the surrounding friends.

"An old man goes to his doctor and asks 'Doc, am I gonna live a long life?'. The Doctor replies 'did you eat healthy?'. 'Yep'. 'Did you exercise a lot?' 'Yep.' 'Did you stop having sex with beautiful prostitutes?' 'Yep.' 'Well why the hell would want to live a long life?'" (27-year-old Female).

Collectively, the group in Batavia felt that the nutritional and health advice that would be proliferated by health professionals and interventional programs wasn't desirable or conducive to a happy life, something more important than a longer or healthier life. Lifestyle changes that are necessary for large-scale changes to public health statistics then need to become socially desirable and acceptable in order for obesity rates and health costs to begin to change. This is a daunting task of changing social norms, but should not be considered impossible. Considering that a

new-wave of epigenetic studies will soon arrive with new information for healthy living, there is approaching with it an opportunity to make changes to current systems of health education and public health initiatives.

There are two main issues to current public health efforts to change these social views and combat the obesity epidemic that came up in the interviews conducted in this study. These issues can and should be addressed by public health experts attempting long-term social changes. Firstly, participants expressed the hindrances to making healthy changes in terms of present limitations. This could be something tangible, as in economic strains or time constraints, or intangible, such as the difficulty of constantly thinking into the future and considering the desires of the future-self. Secondly, physician-patient relationships seemed constantly strained and a barrier of mistrust in health workers and their nutritional advice not only didn't encourage participants to follow the advice but left them with less trustworthy sources for advice to fall back on.

Motivation and Temporal Disconnect

The first issue mentioned is a constant struggle with continually thinking about a future self. With relation to health, this means that many people find it difficult or unnecessary to visit a doctor regularly, to seek or make preventative measures, creating a conflict between the desires of the future-self and the needs of the present self. This trend was observed in this study numerous times. A 37-year-old male, for example, expressed that, "I don't run unless I'm being chased". In this case, the future-self may desire training so that, when being chased, they could run

further or faster. But this conflicts with the current understandings of the present-self. In another case, a 39-year-old male was stricken with endocarditis. Only after receiving emergency medical care did he realize the need for a lifestyle change.

This observation is consistent with a previous study done by Anne Keane[15]. Keane interviewed participants in the UK about their sources of nutritional information and the reasons that they would or wouldn't seek out nutritional advice. She concluded, "Their concerns about healthy eating were explained in terms of how they felt now, rather than with reference to their future."[15]

Daniel Goldstein explores this concept of temporal disconnect in as well with respect to financial savings[16], but his conclusions are applicable also to individuals embarking or currently on a weight-loss journey. This disconnect between present and future selves can be related to food or exercise. For example, one participant interviewed in this thesis study expressed the common sentiment that, when dessert comes around, "It's not outrageous or anything, it's just one piece of pumpkin pie" (19-year-old Female). But her future-self may regret that piece of pie, especially if it results in more pieces and gained weight. Goldstein explains that this disconnect is made even more difficult by the fact that there is no physical manifestation of the future-self that might physically act to, for example, force the individuals hand to not take that piece of pie.

This disconnect may even be related to health-related expenses. Healthy food is often expressed as being more expensive than unhealthy foods or fast foods. Participants interviewed in Batavia, especially, found this to be a constant hindrance to health ("When you have to decide between organic food or a roof over your head,

the answer is gonna be the roof." -27-year-old Female). And yet this contradicts the overwhelming expenses of obesity and its related defects. Obesity costs an estimated $147 billion in added health care costs yearly[17]. This battle is then between present cost versus future cost and the disconnect with relation to obesity costs is rather large. But in lower socio-economic circumstances, there often isn't a choice between paying now or later because the present circumstances are so harsh. The obesity epidemic is thus also a political and an economic problem that should be mediated through supplemental and appropriate economic reforms. These reforms should aim to make preventative health decisions for the present-self easier.

In the case that circumstances allow a choice for healthier options, individuals who have successfully made a lifestyle change or embarked on a 'weight-loss journey' employ a number of devices to bridge further, more psychological disconnects. Participants who did so admitted to using what are called 'commitment devices'. This describes a number of precautionary actions in foresight of future temptations. Participants admitted to having used many of these, including sleeping in gym clothes so as to not have excuses to avoid exercise in the morning. A 21-year-old female also affirmed that she would sometimes tape a picture of a skinny woman from a magazine on the treadmill screen to help her run further. These commitment devices can employ guilt or motivation, but their purpose is to ultimately remind the individual of the desires of the future self.

In an effort to further motivate these kinds of journeys or lifestyle changes, epigenetics has some notable contributions to make by adding to the various reasons that one might begin a change or motivate oneself.

Firstly, the trans-generational effects or 'memory' of a lifestyle is an example of a deeper biological connection not only to a future self, but also a future child or grandchild. It should be noted that the image of a future child or grandchild is likely more difficult to visualize than a future self. An impact on the future self can also be more motivating to the present ego than on a future imaginary offspring. However, there is likely to be increased social pressure to create a healthy biological and epigenetic environment for future offspring.

Obese persons are currently already subject to increased social stigma because of their physical appearance. In a study in North Carolina, BMI was found to be directly associated with perceived psychosocial stress[18]. The physical manifestation of obesity is publicly understood as a result of poor lifestyle choices of the individual, resulting in social stigma, however true or false this public understanding is. But possible popular knowledge that obesity may affect children or grandchildren will likely impact views of filial responsibility towards future generations, creating increased stigma and social pressures. The impact of filial responsibility, however, may also serve as an individual and personal motivation to bear healthy children.

The difficulty in incorporating epigenetics into motivation for a journey or lifestyle change, however, is in its non-visible nature. While widespread education of epigenetic mechanisms, nutri-epigenomics and health would help add

to the growing list of reasons for obese persons to lose weight, it is difficult to incorporate it into daily living because it's largely invisible to us. Incorporation of epigenetic understanding then requires the use of imagination or visualization tools.

Increase in motivation through educational proliferation of epigenetic phenomenon, along with political changes to economic policies that make it difficult for persons of lower economic status to make healthy choices to begin with, would then make substantial steps in combating the current obesity epidemic.

Trust Issues of Nutritional Information Sources

Once persons embark on a lifestyle change or commit to making healthier choices, the question turns to the source of information and what is deemed as 'healthy' vs. 'unhealthy'. Presently, the primary source of information is the Internet, which can present a variety of facts and falsifications based on which website is found and trusted. But even seemingly trust-worthy sources, like the USDA food recommendations website[19], nutritionists or other professionals aren't trusted. A participant in this thesis study also from Bataviaexpressed this sentiment:

"I think most people that have to struggle for what they have- relatively uneducated, middle income people, my kind of people- tend to disbelieve that things can be as simple as they appear." (27-year-old Female).

The feeling of mistrust seemed to continue throughout conversations with not only this woman but also others in that area and in similar health situations.

This woman, for example, had gone to a doctor after spinal disc hernias and was told to loose 20-30 pounds in order to help with pain management. The woman described her encounter with her doctor as follows.

> "I had Doctor O, who's this tall, skinny, white chickand she had um…she was like…every time I went in there, if I went in for anything, she was like 'you're overweight, you're overweight, you're overweight, you're overweight'. And it was like the only thing she would tell me. It's like, you know, I'm in here because my back hurts. Yes I'm overweight, but I also have a legitimate back injury. Can you just prescribe me my pain meds and go away? Like, I know I'm overweight! I can't really exercise right now. I just had surgery three months ago. Like, what would you like from me? You know what I mean? ….Yea, and I had actually asked her once. I'm like, well I have a back injury, I can't do much. You know, what do you want me to do. She's like, 'well you have to find exercises that don't impact your back.' Bulls***! Really? That's what I have to do? And she said things like, 'well, you know, it's different for everybody.' And I did find a couple. I mean I know I can bike without it affecting my back. But I can't run. Because that's the first thing she said! 'Well try jogging'. I'm like, F*** you! I can't even walk without pain, go f*** yourself. Jogging!"

Her frustration with the encounter was obvious, and likely contributes at least partially to mistrust in other health-related professions. 'Doctor-bashing' was a favorable conversation for many of the people interviewed in Batavia. The origin of the mistrust seemed to stem from this same kind of frustration with knowledge gaps continuing to grow between the patient and doctors. The 39-year-old male afflicted with endocarditis expressed similar frustrations when he had asked his doctor

about the kinds of lifestyle changes he should make to avoid further complications. "He said, 'nothing, just keep taking this drug.'" The common sentiment among all of those interviewed in Batavia was a frustration with medical professionals who didn't actively try to close the educational gap between patient and physician and who would just 'push pills' without explanation. The notion of creating individualized lifestyle plans for health without the need for drugs seemed a positive alternative, but at the same time an unattainable future in a place where present economic conditions take precedent. Accurate education, then, of both medical professionals and the public are thus increasingly necessary. Closing educational gaps between the physician and the patient would make the patient more comfortable and more likely to take in the nutritional advice, encouraging trust and also confidence in the patient.

Unfortunately, and likely due to this mistrust in medical professionals, persons who were interviewed and reported as having actively sought out nutritional advice, most often received nutritional advice that they trusted from friends, television, magazines and, overwhelmingly, the internet. This social spread of nutritional advice not only perpetuates the notion of obesity as a social epidemic, but also allows public health experts a medium for social intervention- simply by spreading the word, the more accurate word, about epigenetic phenomenon and about healthier lifestyles for one's epigenotype. Proper public education of epigenetic phenomenon and research is critical to any public health initiatives attempting to tackle the obesity epidemic. An understanding of cultural contributions to the pathogenesis as well as the treatment of obesity allows

complementation between biological and social understandings of the condition. Understanding the deep impact of our environment on our health and the health of our descendants also helps to avoid the pitfalls of fatalistic genetic determinism in favor of a more active approach to preventative health and an empowered public.

In conclusion, the participants received the concepts of personalized diets and lifestyles based on epigenotypes positively. This may be because of the familiar way in which the researcher introduced the concepts or because epigenetic concepts correspond already to popular notions that individual bodies require individualized care. But the idea's popularity may also have been due to an ideal sense of empowerment and control over one's body without the need for medical professionals. In essence, the medicalization of diet and lifestyle may lead to the sensation- real or not- of a de-medicalized body.

The will to take control or take back control over one's body and health is not unusual and was frequent throughout the interviews for this thesis. One participant responded, for example, that epigenetics would give more motivation to continue being healthy and that she would feel empowered. Another responded, "If you could give food a solid foundation, like ultimately determining genomic expression and a determinant of health, I do believe the idea of 'food' would become something magical….I find it extraordinarily exciting and fascinating!" (22-year-old male). And a 19-year-old female implied the active role one can play by saying, "If you can modify the expression, then [your health] is up to you."

Unfortunately, many of those participants interviewed seemed as though control of their bodies was lost to genes, medical providers, fate, or otherwise. While epigenetic phenomena and nutritional epigenomics have the ability to let these people regain control over their health and bodies, public health initiatives and political policy changes should simultaneously act to give people the option for that control. These initiatives could target economic issues, possibly giving incentives for food stamps or similar programs to go towards healthier options. Educational programs should target not only individuals, but also communities as a whole, including health providers. These programs may also utilize social systems for nutritional information dissemination already in place- such as popular magazines and the internet- but should work to also make these sources more reliable. Initiatives should also plan to be implemented on a long-term basis rather than short-term basis, since the effect of epigenetic inheritance on obesity etiology is multi-generational.

To conclude this chapter broadly, the addition of epigenetic information into social notions of health should involve a careful supplementation rather than a complete replacement of genetic knowledge. These changes should also consider the social nature of obesity, including the association of factors affecting health with identity and the many social constraints to health such as mistrust of health professionals and temporal disconnection. Tackling the social epidemic that is obesity is a daunting task, but all hope is not yet lost.

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Changes Going Forward

Obesity is a condition that is unique to society in many ways. It's very visible, lending individuals to outward public scrutiny and stigmatization. It's also a condition that has for so long been thought of as a simple energy imbalance that has an equally simple cure- eat healthy and exercise. But because obesity has plagued us for so many decades- since the 1980s at the very latest- the lack of success in stopping this epidemic has put this simplistic and reductionist notion into question. More recently now it's being understood that obesity is a condition that affects so many aspects of our lives and biology. Obesity is a multi-disciplinary condition. For this reason, obesity research and treatment should be approached in a similarly broad and multi-factorial fashion. Epigenetics, as an area of research that begins to allude to an interplay between social lifestyle and biological health, has potential to give a unique view into obesity causation and treatment.

In a basic sense, obesity is affected very clearly by biology. The latest trend is to study the genetics of obesity, which has yielded some very important results in the past decade. Fat Mass and Obesity- Associated Protein (FTO) gene was one of the first genes to have a clear correlation with obesity[1]. But the condition goes beyond Mendelian genetics. Neurologists have found, for example, that some foods, especially those high in sugars or fat, share the same neuronal pathways and symptomology of addictions to scheduled drugs such as cocaine or heroine[2,3]. This has prompted the formation of support groups for those addicted to foods, such as Overeaters Anonymous.

Biological research on obesity began in the early 20th century with a focus on digestion, hunger and the stomach. Later, metabolism became the popular focus of research, prompting researchers at the Rockefeller Institute to keep participants in a clinic constantly, feeding them a controlled diet of calories or nutrients mixed in a sort of smoothie concoction. By carefully controlling calories consumed and measuring the metabolic energy expenditure of the participants, researchers Jules Hirsch and Rudolph Leibel found that body weight tends to remain at a certain 'set-point', and any deviations from that point in efforts to lose or gain weight cause the body to decrease or increase metabolism respectively in order to bring the body back to that 'set-point'[4,5] . The next questions are, however, 'what creates that 'set-point'?' and 'how can we change the 'set-point'?'. Leibel and Hirsch also neglected to investigate effects of exercise on metabolism, a point of research that is much more common today. Epigenetics may likely play a part in determining this biological 'set-point' for weight, but that has yet to be confirmed.

Obesity has also been investigated through psychological perspectives. This began in the mid-20th century when Freudian researchers were apt to conclude that, to the obese person,

> "Food could symbolize 'the mother, with eating being an attempt to orally incorporate the mother.' It could also signify the phallus, so that eating was an 'expression of penis envy and a wish to deprive the male of his penis.' The act of overeating could be an 'expression of an unsatisfied sexual craving.' It could serve as 'a means of expressing hostility.' It was sometimes intended as 'self-punishment and self-degradation, oft-times in response to

guilt.' Or it could be 'a substitute for love, affection, and friendliness' or 'a substitute for pregnancy.'"[6]

Whether these conclusions were accurate or otherwise has become an old argument, but these first psychological analyses into eating habits foreshadowed the most successful weight-loss program types known today- behavioral therapy. These interventions take roots in Pavlovian behavior modifications, but involve the re-programming of eating habits that lead to binge eating or the desire for unhealthy foods. In this way, the obese individuals can gain a sense of control over what they eat. These interventions involve the recording of foods eaten and why they were eaten, the acknowledgement of emotions experienced while eating and an effort to change those emotions. The interventions also urge participants to avoid emotional or social situations in which they might overeat. The success of these behavior modifications demonstrates a key point in obesity etiology- that the condition is not completely biological but involves psychosocial factors as well.

This movement towards the social concept of obesity has been gradual and significant, but not comprehensive. Behavioral therapies have been successful, but not successful enough to halt the growing rates of obesity. And perhaps this is because social factors for obesity may not paint a complete picture. Some people have blamed a toxic environment of pathogens in the western world for the obesity epidemic or other environmental changes that have occurred within the past century, such as

the frequency of chemical additives in packaged and fast foods. But these factors- environmental, social and historical- would all impact health likely through epigenetic processes. This third factor in obesity development makes the condition, and many other related conditions such as diabetes and metabolic syndrome, a three-tiered condition, encompassing socio-cultural, genetic and epigenetic factors (Fig. 1).

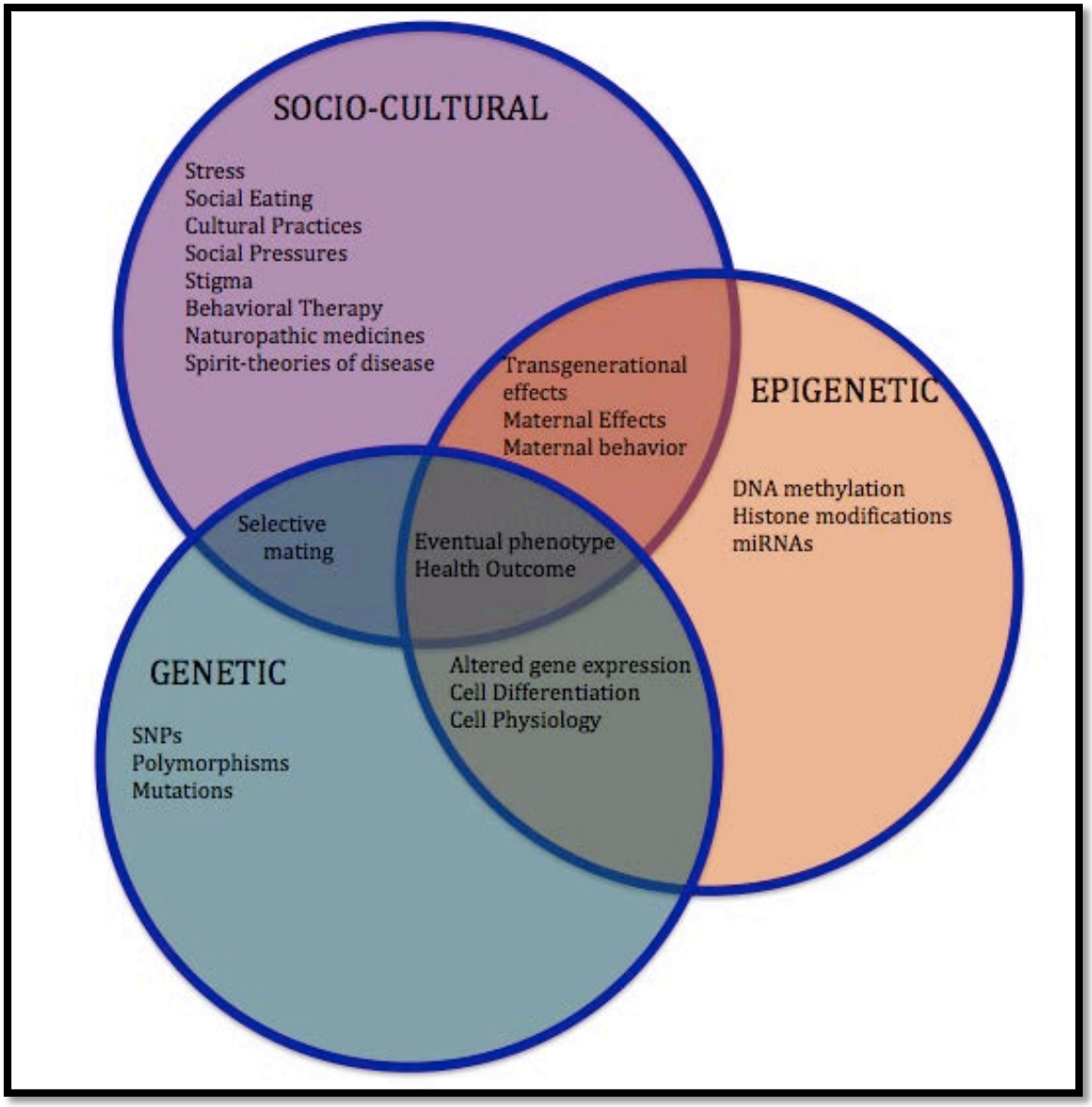

Figure 1: A three-tiered approach to factors that influence health outcome and disease. In this case, health outcome is obesity and other weight-related conditions such as Type 2 Diabetes and Metabolic Syndrome.

The genetic contribution to obesity is considerable, but because genes are the most static of the three factors of health, research into treatment and public health changes to obesity should begin to focus on epigenetic and social phenomena instead. Genetic factors are not to be ignored, and the

future may hold promise for gene therapies, but lifestyle and social changes that affect health are likely to be more affordable and less invasive measures for treatment. The ability to actively change gene expression and take an active role in health provides also a sense of empowerment over one's body and one's self, a notion that did come through in interviews with participants in this thesis.

The multidisciplinary approach to obesity through socio-epigenetic research such as that attempted and described in this thesis is therefore highly likely to provide the most immediate health benefits to obese persons. These research topics include investigations into the kinds of lifestyles that correlate with distinctive epigenetic markings. As suggested in this thesis, a study in this direction might begin with the anthropological technique of participant observation. By living with and observing a participant for a certain amount of time, the researcher will gain specific insights into the individuals' lifestyle and diet, resulting in not only more accurate data (avoiding the pitfalls of self-reporting), but also the elucidation of other social factors that might hinder the individuals health and weight management. The researcher would then take a tissue sample. This could be from adipose tissue, skeletal tissue or otherwise. The methylation code for a specific gene related to obesity can then be deduced via methylation-specific PCR, Bisulfite Sequencing, accompanied by RT-PCR and protein assays. Correlations between lifestyle and certain epigenetic profiles then allow

further studies to investigate a causal relationship that might imply an important focal-point of obesity treatment.

The goal of these studies will be to understand barriers to obesity treatment as well as to identify specific healthful lifestyle changes for individuals. The social portion of this thesis revealed significant socio-economic barriers to lifestyle changes, including a lack of faith and education in the ever-changing nutritional recommendations, frustrations in the physician-patient relationship and temporal-economic constraints. Results of epigenetic studies like that suggested here would allow increasingly specific lifestyle advice at an individual level, appealing to a common sense of unique self.

At a broader social level, education of epigenetic phenomena may also provide further motivation for lifestyle changes as well as a deeper connection to past and future generations that may also encourage a sense of increased filial responsibility.

One of the largest benefits to merging disciplinary studies on obesity, however, is the creation of a broad-view on the condition as a social disease. Being able to see obesity as a condition of society and not just the individual, as a condition of lifestyle and not just diet, as a condition of familial history that we are apart and not a victim of, will be the benefits of future epigenetic and multi-disciplinary research. These immense changes are not impossible- similar changes have been ongoing concerning the 'tobacco epidemic'- but will require the support of many. It will be a universal change of lifestyle that

will likely take many people out of their comfort-zones and into a new territory of active, preventative health, putting a majority of control in the hands of the individual. It's a scary thought that would also put much of blame in our own hands should ones health fail. But it's also a necessity that can appeal to a sense of freedom and empowerment. As epigenetics grows as a field, reconnecting biology with culture, a larger perspective of the obesity epidemic may be created, shedding light on healing mechanisms and public health, political and social interventions that will hopefully recreate a healthier environment and a healthier people.

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Appendix

Contents:

I. Glossary of Terms

Adipocyte: (n.) a fat cell.

Adipogenesis: (n.) the development of a fat cell from a mesenchymal stem cell.
Bisulfite Modification: (n.) the process of modifying the DNA sequence chemically. This process changes unmethylated Cytosine nucleotides into Uracil nucleotides.

Bisulfite Sequencing: (n.) The process by which the DNA code is elucidated after bisulfite modification.

Brown Adipose Tissue: (n.) one of two types of fat tissue found in mammals, characterized by smaller fat droplets and a high amount of mitochondria. The major function of this type of fat is temperature regulation.

C/EBPα: (n.) CCAAT-enhancer binding protein alpha is a gene that codes for the corresponding protein. The C/EBPα protein is sufficient to induce adipogenesis and its dysfunction is implicated in the development of insulin sensitivity.

Chromatin: (n.) The complex of histone proteins, DNA nucleic acids and proteins which bind together and package the DNA to form the chromosome. An octamer of histones surrounded by two coils of DNA form a nucleosome. Nucleosomes arrange together to form 30nm fibers that are further wrapped to form the chromosome.

Commitment Device: (n.) a mechanism by which one might act in foresight of a future misfortune.

Cytokine: (n.) small signaling protein molecules that are secreted by cells in order to communicate with other cells.

DNA Methylation: (n.) The methylation of Cytosine nucleic acids which are directly adjacent to Guanosine nucleotides. This results in an epigenetic code on top of the DNA that controls gene expression.

Euchromatin: (n.) A form of chromatin in which nucleosomes are spaced further apart, exposing template DNA and thus allowing access of transcriptional machinery and the formation of complimentary RNA.

Epigenetics: (n.) A 'code' on top of the DNA which is heritable, reversible and dictates gene expression.

Fetal Programming: (n.) The epigenetic programming of adult health or other phenotypes that are dictated by in utero environmental stimuli.

Genetic Determinism: (n.) The belief that genes directly determine physical attributes, health and behavioral phenotypes.

Heterochromatin: (n.) A form of chromatin in which nucleosomes are spaced closely together and associated with other DNA binding proteins, prohibiting access of transcriptional machinery and the formation of complimentary RNA.

Mesenchymal Stem Cell: (n.) A multipotent stem cell found in bone marrow which has the ability to differentiate into a variety of cell types, including adipocytes, osteoblasts (bone cells) and cartilaginous cells.

Methylation: (n.) The process of adding a methyl group ($-CH_3$) to a molecule

MS-PCR: (n.) Methylation-specific polymerase chain reaction. A process by which DNA may be rapidly copied (amplified). In MS-PCR, the reaction proceeds only when the appropriate methylation-specific primers are added to the reaction tube.

Nucleosome: (n.) A basic unit of chromatin structure, comprising of an octamer of histone proteins that is surrounded by two coils of the DNA chain.

Nutri-Epigenomics: (n.) The study of food and nutrient effects on epigenetic phenomena.

PCR: (n.) Polymerase Chain Reaction. A thermo-cyclic procedure which acts to copy and multiply (amplify) a segment of DNA.

PPARγ: (n.) Peroxisome proliferator-activated receptor gamma. A gene which codes for the PPARγ protein. This protein is necessary and sufficient to induce adipogenesis from mesenchymal stem cells.

PPARGC1A: (n.) Peroxisome proliferator-activated gamma coactivator 1-alpha. A gene which encodes a corresponding protein that has a variety of functions. PPARGC1A is known to interact with PPARγ.

Prader-Willi Syndrome: (n.) A congenital disease that results from a paternal deletion or silencing of the 15q11-q13 area of the genome. The syndrome manifests physically as obesity, reduced muscle tone and mental ability, insatiable appetite and hypogonadism.

RNAi: (n.) a mechanism of post-transcriptional gene-silencing in which mRNA with a particular sequence is degraded or otherwise prevented from translation to form a protein.

Single Nucleotide Polymorphism: (n.) Variation in the DNA sequence occurring at one nucleotide.

White Adipose Tissue: (n.) one of two types of fat tissue found in mammals, characterized by large fat droplets. The major function of this type of fat is energy storage but it also functions as an endocrine organ.

II. IRB Application and Approval

The following was submitted to the Wellesley College IRB in September of 2011.

Wellesley College IRB
Application for the Use of Human Subjects Research

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Project Title:You Are What You Eat (And Do): An Epigenetic Study on Diet, Exercise and Obesity Genes
Anticipated Funding Source: Wellesley College Biological Sciences Department
Duration of Project: Sept. 2011 to April 2012
Approval Requested for: Sept. 2011 to April 2012

1. Purpose

The Purpose of this study is to connect lifestyle factors such as diet and exercise to the expression of genes that have been previously linked to obesity, a condition that currently affects about one-third of the US population. Concurrently, the study will also look at the epidemiological effects of these kinds of epigenetic studies on individual and social perceptions of health and self.
Epigenetics is an exciting new biological field that focuses on the chemical markings on top of DNA that are known to control gene expression. These are often in the form of methyl groups that are attached to the backbone of Cytosine residues in DNA. When an area of DNA has many methyl groups, it can get tightly bound up by Histone proteins causing it to be less accessible to proteins required for the formation of RNA. This then, causes the gene to be silenced.
Importantly, the amount of methylation can change throughout one's lifetime. This can be due to developmental, dietary and environmental factors and its means that what we do can change our gene expression and therefore our biology and health. This thesis study aims to find out what exact factors can affect the expression of obesity genes and therefore has the potential to change paradigms in the treatment of obesity, possibly leading to individualized weight-management and gene-management programs.
These kinds of changes to how we view disease like obesity, as changing and dependant on our choices gives the individual an increased control over their health outcome, can cause huge changes in the construction of our identity as social

creatures and as individuals. It is thus important to conduct a parallel anthropological study on the epidemiological concerns for studies such as this.
The study will require DNA donations from willing participants as well as a short investigation into their lifestyle factors and how the individuals understand the field of epigenetics. To do this, an estimated 100 participants will give a buccal swab sample by rubbing a swab up and down their cheeks. Afterwards, the researcher will conduct a brief interview, asking about dietary habits, childhood health, general health, fitness and about their understanding of epigenetics.
DNA will then be extracted from the samples and run through a methylation-specific PCR, which will amplify the DNA and allow the discernation of methylated bases.

The specific genes amplified and analyzed are the PPARG and PPARGC1A genes. These have both been connected to obesity and diabetes. These genes code for transcription factor proteins that go on to activate other genes implicated in inflammatory and metabolism pathways. These two genes are therefore important as they are connected to many pathways that have the potential to influence health.

2. Participation

Recruitment:

Participants will be found mainly through the Wellesley network via email. This will allow us to reach a large number of people within the desired group and within the short amount of time available for this study. Specifically, emails will be sent to class conferences on the new Wellesley gmail system and to the community conference so that staff will be able to participate as well if they wish. These emails will consist of a summary of the project and any risks or benefits. The informed consent form will be attached so that they will be able to make an informed decision about participation in this study before replying to the email.
Once participants reply, an arrangement will be made for the researcher to meet with them. During the meeting, the researcher will go over the project again and make sure the participant understands fully any risks, benefits and what will be expected of them. Should they still wish to participate, they will sign the consent form. The researcher will then help take a buccal swab sample of cheek cells, and then interview them with the attached list of questions. The interview may be recorded for ease of note-taking, however no names will be mentioned in the recording and it will be labeled with a coded number.

Inducement:
Because of the low-budget for this study and the minimal amount of timeand effort required from participants, no inducement will be given to participants other then the beneficial feeling of altruism towards progress in the health field.

Demographics and affiliations:
Participants will be mainly young or middle-aged caucasian-american adults. Studies have shown that there are differences in the epigenetic markings of different

ethnic groups. To control for this, only Caucasian-american participants will be recruited. Caucasian-americans make up 46% of the Wellesley College student body and will be most easily accessible, but a major reason to conduct this study mainly on Caucasians is for the abundance of epigenetic and genetic studies done on Caucasians. This will help give this study sufficient background material on what kinds of genetic polymorphisms are most common in this chosen population of participants. It would be interesting for future studies with greater funding and sample-size to compare the epigenetic status of obesity genes across ethnicities, but for the scale and purpose of this 100-participant study, the narrowing of participants to young and middle-aged Caucasian-americans will allow for the elimination of ethnic variables, making straight-forward connections between diet, exercise and obesity more easily attained.

Permission

Participants will be above the age of 18. This is because many epigenetic changes are due to horomonal and developmental changes within the body. The exclusion of this group will allow the researcher to specifically look at only diet and lifestyle factors that would affect epigenetic marking. Other persons, such as prisoners or those with diminished autonomy will not likely be included in the study simply due to the population requirements and feasibility.

What participants do:

Participants will be asked to meet once with the researcher to help go over the study, sign the consent form, donate a DNA buccal swab sample from their cheek and to participate in a brief interview about their dietary and lifestyle habits, health and understanding of epigenetic principles.

Length of Participation: Participation should require a maximum of a half hour of time.

3. Investigator Experience

Constance Ohlinger is a Wellesley College senior, majoring in Health and Society, an individual major which takes largely from the biology and anthropology departments. She has laboratory experience, having worked with Professor Hearn for a year on novel compounds with potential use in treating tuberculosis. Although this was an organic-chemistry experience, Constance learned basic techniques for safety in a lab. Constance has also worked at an out-patient surgery center as a nurses aide for two summers. This experience helped to enforce safety when dealing with potential hazardous material or contagions such a blood-bourne pathogens. Educationally, Constance has taken medical ethics as well as numerous physical anthropology courses, all of which emphasized the importance of respect for persons in human research. Medical Ethics (PHIL 249), gave a great overview of history and theory in medical ethics. Constance's final paper was a study on the banking of human genetic material and the risks involved in studies using genetic material such as this thesis. Anthropological Genetics (ANTH 274) and Race and

Human Variation (ANTH 214) were similar courses, looking partly at anthropological studies, with risks similar to this, that disregarded respect for persons and communities in genetic research. With this background, Constance feels she has the knowledge and background to conduct this study with full respect for persons, individuals and ethnic groups as well as the technical background to conduct this study with correct and health laboratory precautions.

Professor Andrew Webb graduated from the University of Southampton, England and is currently Professor of Genetics, Developmental and Molecular Biology at Wellesley College. His classes emphasize experimental approaches to understanding the role of gene expression in the normal and aberrant functioning of cells. His research is currently focused on the development of targeted nanoparticles for the treatment and imaging of pancreatic cancer.

4. Consent

Informed Consent

Participants will be recruited by email as described above (please see Section 2, Recruitment). Once a participant replies to the email showing interest in the study, the researcher will arrange to meet with the participant. During the meeting, the researcher will go over the informed consent form (attached) and the project in general to make sure the participant is truly fully informed about the entire project, risks, benefits and terms of involvement. If the person still wishes to participate after the discussion about the project, they will sign the consent form and the swab sampling and interview will commence. Should they wish to back out of the study after learning more, the persons will be informed that they may wish not to participate and they will not be penalized in any way by that decision. In that case, the sampling and interview will not occur.

Parental Consent

Parental Consent must be given for any participant under the age of 18 to participate. Although this is unlikely due to the desired population, in the rare case that this happens the parent or guardian will be present during the conversation and interview with the researcher. They will also sign the informed consent form should they approve of the participants involvement. In the case that the parent or guardian does not wish the child to participate and will not sign the consent form, that person will not be able to participate.

Right to Terminate

Participants will be made aware that they may terminate their participation at anytime. Should this occur, their DNA sample and recorded interview will be deleted and destroyed. They will be informed at the start of the meeting that no penalty will come of termination should they wish to do so. They will also be informed that no penalty will come to them should they wish not to answer any questions in the interview as well.

Deception
Participants of this study will in no way be deceived.

5. Risks

Potential Risks
Physically, there might be risk in attaining the swab sample as participants might rub too hard, causing mild yet temporary discomfort on the inside of their cheeks. Also, because this study uses genetic material and involved the recording of various health information, there are risks to the privacy of the individual. Analysis of the DNA could show risk in the participants for obesity, diabetes and other medical conditions that are associated or might be associated in the future with these genes. Should any outside body such as an employer or insurance agency get ahold of this material they may use it to influence participant employment or insurance coverage.

Protection against Risks
Protection against irritation from the swabbing will be minimized by guidance of the research during the sampling.
The risk of genetic material and information being attained by outside agencies is minimal. Samples will be coded and no names will be connected to any sample. No names will be mentioned in the interview as well. DNA and any genetic material will also be destroyed after use in this study so that no further research will be conducted against the will of the individual on their donated material and so that no outside agency will be able to get ahold of the material.
Participants will also be reminded that they will be able to withdraw from participation at any time.

6. Benefits
This study has no direct benefit to the participants themselves other than altruistic feelings of having aided in research for the understanding of obesity, a disease that has become a huge part of American health concerns in the past two decades. While the risk of privacy to the individual participants is a worry, the benefits of the study do outweigh the risks. This study has the potential to ask questions of and give answers to changes in modern health care as well as to help identify lifestyle factors that could affect our weight and health outcome genetically. While 100 samples for an epigenetic study of this magnitude is small, the results have the potential to open new areas of research as well as instill interest in other scientists to continue and to broaden this particular study.

7. Confidentiality
Confidentiality will be maintained primarily by eliminating names from the study. Genetic samples and interviews will be coded. No names will be mentioned in the interviews as well. Should quotes be taken directly from the interviews in the final thesis, pseudonyms will be used to mask the individuals identity. Any information that does contain named will be either in a locked binder or password protected, with only researcher knowing the password or having the lock. To

prevent the exposure of applicants after the project has concluded, genetic samples and any documents containing names or contact information will be deleted after the study has concluded.

In the unfortunate event of a breach of privacy, the DNA sample(s) that are involved in the privacy breach will no longer be used in the study and the interview recording(s) will be deleted. All participants will also be notified that a breach of privacy has occurred with one or more samples and that, if they wish to be removed from the study because of such a breach, their sample and interview will be destroyed also, without penalty to the participants.

8. Data Retention

Plan for destruction of data

Any data containing genetic information, names or contact information of participants will be deleted or destroyed after the conclusion of this project.

Data Storage

Data will be stored on the researchers computer only and will be password-protected, should it contain any material that could be potentially linked to a participant.

Uses

The data and genetic material will only be used for this study only and will not be given out to other researchers or for other studies not mentioned in this proposal.

9. NIH Human Research Certification

Constance Ohlinger has completed the National Institute of Health Office of Extramural Research training course "Protecting Human Research Participants". Her certification number is 741986. Please see the attached Certificate of Completion.

DATE:_____

APPLICANTS SIGNATURE:_____

THESIS ADVISORS SIGNATURE:_____

The Wellesley College IRB approved the above proposal with the following letter:

WELLESLEY COLLEGE
INSTITUTIONAL REVIEW BOARD
(Committee on the Use of Humans as Experimental Subjects)

TO:	Constance Ohlinger, undergraduate student Wellesley College
FROM:	Nancy L. Marshall IRB Chair
SUBJECT:	Expedited Approval of "You are what you eat (and do): an epigenetic study on diet, exercise and obesity genes"
DATE:	October 19, 2011

Your proposed protocol "You are what you eat (and do): an epigenetic study on diet, exercise and obesity genes" has received human subjects approval through an expedited review of the Wellesley College IRB, under §46.110 (b) (1), "activities involving no more than minimal risk," and under category (3) Prospective collection of biological specimens for research purposes by noninvasive means, (i) mucosal and skin cells collected by buccal scraping or swab, skin swab, or mouth washings.

If there is any change to the actual risks and benefits of your research (i.e. any significant changes to recruitment plan, informed consent procedures, or unanticipated problems that contribute to the change), notify the IRB Chair promptly. Additional human subjects review or reporting may be required.

This approval expires on *October 19, 2012* by which date you should complete an annual progress report form, indicating whether the study is completed or ongoing, and whether there have been any significant changes to the risk for the study population, including the study design, the protocol, or the informed consent procedures.

III. Informed Consent Form

The following form was given to and signed by each participant involved in this study.

Human Informed Consent

Project: You Are What You Eat (And Do): An Epigenetic Study on Diet, Exercise and Obesity Genes

Student Researcher: Constance Ohlinger

Professor: Andrew Webb, Biological Sciences Department, Wellesley College

This form is used to inform you on this thesis project and ask for your voluntary participation. Should you wish to participate in this study after reading this form, please sign the appropriate box below. If necessary, please also have a parent or guardian sign as well.

Purpose of the Project:

Broadly, the purpose of this project is to connect lifestyle factors such as diet and exercise to the expression of genes that have been associated with obesity and to understand how those connections affect the way we see ourselves as individuals in society. This will involved a biological study paired with an anthropological study.

The biological portion of this study will need a sample of DNA from each of the participants. This will involve rubbing a swab stick on the inside of each cheek for approximately a minute. DNA from the swab sticks will then be amplified using a special form of PCR that looks at epigenetic markings on top of the DNA. These chemical markings control the expression of genes and can therefore affect our physiology and health. Importantly, these chemical markings can change throughout our lifetime in response to environmental factors such as diet and exercise. This study is therefore important in investigating the exact lifestyle factors that might affect the expression of obesity and diabetes-related genes and, therefore, the onset of those conditions.

The anthropological portion of the study will be in interview form and should last no longer than 20-25 minutes. The researcher and the participant will meet together and discuss the importance that epigenetic studies like this might have on the participants lifestyle choices and identity. A portion of the interview will also be spent on discerning nutrition and a health history of each participant. Should the participant feel uncomfortable about answering any question during the interview, they may skip that question without penalty.

If you participate, you will be asked to meet with the researcher at an arranged place and time. The researcher will then go over the project with you once more and ask you to sign this form stating that you understand the project and any

risks or benefits of participation. After this, you will be given a swab stick to rub on the inside of each cheek for approximately one minute. While the swab stick dries, the researcher will interview you on diet, nutrition and basic health history. A free-flowing discussion on epigenetic applications in society will then follow. For the ease of notetaking, the interview may be recorded. The researcher asks that you not mention your or others names during the interview for your privacy and the privacy of others. The recorded interview and the DNA sample will be numerically labeled, without any names, also to protect your privacy. Should you not wish to answer a question or should you not wish to have the interview recorded, you may skip or request so without penalty to you. Also, should you be quoted in the final thesis paper or any final production of the thesis results, pseudonyms will be used in place of your name.

Time required for participation: Approximately 20-30 minutes maximum

Potential Risks of Study include:
This study involves minimum risk to the participants, but any chance of risk should be mentioned here before you decide to participate.
There is a risk that you might swab too hard, creating temporary irritation on the inside of the cheek that might be uncomfortable or may become infected. This risk is extremely low, however, as there is no recorded incidence of harm from taking a buccal swab sample of DNA and the research will be using a sterile technique- using sterilized swabs and non-latex gloves- to avoid infection.
Because the sample will contain your complete DNA code and because we will be looking at genes connected with obesity and diabetes, there is a chance that outside agencies, such as employment or insurance companies, might get ahold of this information and use any information on health to unfairly discriminate participants from employment or coverage. This risk is also minimal, however, as no one but the researcher and her thesis advisor will have access to the samples. The samples will also be destroyed after use in this study so that they will not be used for purposes other than those outlined in this thesis study. Samples will also be coded so that they may not be traced back to the individual, with the key to the code held only by the researcher in a password-protected document.
Again, should you wish to withdraw participation at any time, you may do so without penalty by contacting the researcher at any time (see contact information below)

Benefits:
This study has the potential to make solid, biological connections between lifestyle and future health. This includes information on practices that could have lasting biological effects on our genetic code and, therefore, our health. The benefits to you, the participant will be in the satisfaction and fulfillment of having donated to the progression of scientific research into better treatment for obesity, a condition which affects 33.8% of the US according to the CDC. Should you also

wish to see the results of your own genetic sample and see if the genes we investigate are expressed or not, you may also contact the researcher.

How confidentiality will be maintained: Confidentiality of biological samples donated to this study will be maintained by a coding system known only to the researcher. The key to the code will be kept in a password-protected document, and only the researcher will know this password. Recorded interviews will be numbered and will not contain any names, addresses or information that could directly link the file to the donor. DNA samples will also be coded, with no attached name. No outside party will have access to the names, samples or interviews, this includes insurance companies or potential employers. To further assure that this is the case, DNA samples will be destroyed after use in this study.

Board Approval
This study has been approved by the Institutional Review Board at Wellesley College, an institution put in place by the college to make sure that all researchers conduct ethical practices that fall under federal regulations, that they explain clearly all aspects of the project to their participants and do everything possible to protect the privacy of the participant. If you have any questions about your rights, please contact the Committee for the Protection of Human Subjects at Wellesley College at 781-283-2508.

If you have any further questions or concerns about this study, please feel free to contact:
Constance Ohlinger at cohlinge@wellesley.edu
Phone: 831-521-6811

106 Central St
Unit 5031
Wellesley, MA, 02481

Or Professor Andrew Webb at dwebb@wellesley.edu

Voluntary Participation:

By signing this form, I understand that participation in this study is completely voluntary and there will be no negative consequences should I refuse to participate.

I understand that I may terminate participation and have my DNA sample and information removed from the study at any time by contacting the above persons.

By signing this form, I agree that I have read and understand the above information and give my consent to participate in this thesis study.

I understand the information given to me, and I have received answers to any questions I have about the research procedure.

To the best of my knowledge and belief, I have no health problems that would increase my risk of participation.

I am of 18 years or older.

Signature of Participant:_____
Name of Participant:_____ **Date:**_____

Signature of Parent/ Guardian:_____
Name of Parent/Guardian:_____ **Date:**_____

IV. Proposed Methods

Human Experiment Approval by IRB
Approval for human experimentation should be approved by institutional IRB boards. The approval should be under the conditions appropriate for observation of participants on a long-term basis as well as use of DNA under secure circumstances. Signature for informed consent of participants should be collected, DNA samples and corresponding observational data may also be numerically rather than nominally stored. For the safety of the participants, DNA should also be destroyed post-use.

Participant Observation
Participant observation and participation would be approved under IRB standards. Willing participants having given informed consent will be followed, observed and, optimally, lived-with for a length of time. Considering time availability and cost, this length of time will optimally be a week per participant. Observation will be both qualitative and quantitative, recording values for calorie intake and an observation of exercise and eating habits. Further observation and recording of results may be conducted as described by H. Russell Bernard[13].

Tissue Collection, DNA extraction, Quantitation
Adipose tissue would likely be collected through surgical removal by liposuction. Other tissue types may be collected through biopsy techniques. For buccal swabs, the BuccalAmp DNA Extraction Kit (Epicentre #BQ0901S) may be used. DNA should be extracted from the tissue samples with the DNeasy Blood and Tissue Kit (Qiagen #69581) as per manufacturer's instructions. Extracted DNA samples can be quantitated using a Nanodrop (ND-1000 Spectrometer) and stored at -20°C for long-term storage and at 4°C when in use.

Bisulfite Modification, Bisulfite Sequencing and MS-PCR
DNA samples may be modified using the EZ DNA Methylation Gold Kit (Zymo Research D5005S) according to manufacturer's instructions. MS-PCR should be run as described by James Herman[9], with 100ng DNA in 1X PCR Master Mix (Promega #M7505), DEPC treated H_2O and 300 ng of forward and reverse primer. Primer sequences for methylated and unmethylated DNA and PCR conditions should be appropriate for the gene promoter sequence and amplicon desired for investigation. PCR products with 1X loading buffer (Novagen #69180) may be run through a 3% Agarose Gel (Sigma-Aldrich #9012-36-6) in TAE Buffer with 1X SYBR safe DNA gel stain (Invitrogen #S33102) at 100V. DNA may then be visualized under UV trans-illumination. The PCR product may also be sequenced directly.

RT-PCR

RNA may be isolated from tissue samples using the SV Total RNA Isolation System (Promega #Z3100) as per manufacturer's instructions. RNA concentration may then be quantitated using the Nanodrop (ND-1000 Spectrometer). RT-PCR may then be conducted using the Superscript II Reverse Transcriptase (Invitrogen #18064-022) as per manufacturer's instructions and visualized through gel electrophoresis as described above in *Bisulfite Modification, Bisulfite Sequencing and MS-PCR*

Western Blot

Nitrocellulose membrane (Invitrogen #LC2000) may be placed in a Blocking Solution of 5% Blotting Grade Blocker (Bio-Rad #170-6404) in 1X TBS for an hour at 25°C. The blocking solution may then be incubated for 12 hours with primary antibody solution. The membrane is then washed repeatedly with 1X TBS. The membrane is then incubated at 25°C for 1 hour with the second antibody added before washed again with 1X TBS. Luminol (Denville Scientific Inc. #E2400) is added to the membrane for 1 minute. Protein presence may then be visualized with a ChemiDoc XRS Imager (Bio-Rad #170-8265).

V. Interview Details and Guiding Questions

The following are transcripts of the how the researcher explained epigenetic and epigenetic phenomena to participants. Following this transcript is a list of questions that were frequently asked to most participants. Some anthropological and social data was also collected from observation as well, without the prompting of interview-related questions but through the initiation of a diet and health-related discussion. It should be noted that many participants were also asked questions about their diet, exercise and lifestyle habits. These questions are not included here, as the answers were not ultimately used in this thesis.

Explaining Epigenetics:

> "Epigenetics is like, the things you eat, or your stress levels or any carcinogens you are exposed to during your lifetime, if you're exposed to a famine, that will put markings on your DNA that will change your gene expression in certain ways. And then the markings can get passed on. So, if there's a famine, children are going to be smaller. Those are epigenetic changes. And if those children grow up and have access to excess foods, then they can develop obesity and diabetes more readily. There are studies where a famine in one generation is related to obesity two generations later. Or in holocaust victims, their offspring have higher rates of post-traumatic stress disorder. Other examples are like- folic acid supplementation in women who are pregnant can cause different expression of obesity genes in offspring. A lot of studies are showing these kinds of trans-generational effects, but the markings are known to change in one lifetime as well."

Frequently asked questions posed to participants:

-What do you know about epigenetics? (If nothing, epigenetics was explained as above.)

-Knowing this about epigenetics, what does that make you think about?

-How often do you think about genetics? Does genetics affect any part of your life?

-Would knowing that your actions can change your gene expression change anything you do in day-to-day life?

-What do you think sets you apart as an individual?

-What do you think lifestyle choices say about a person?

-Do you think epigenetics would change those assumptions?

-Can you think of an instance where food is meaningful to you? How would it be meaningful?

-Have you ever gone on any kind of specific kind of diet- vegan, vegetarian, weight-loss, paleolithic…? What made you do so?

-If you had to change your diet for a 'healthier' epigenetic code, do you think that might make you feel different around your friends and family?

-So, after this interview and after learning a bit about epigenetics, do you think you'll change anything you do on a regular basis?

-Are there any last impressions you have about epigenetics?