Prospective Foods To Prevent Early-Onset Obesity: An Evolutionary Hypothesis About Human Ancestral Diet And Fetal Programming

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I. Abstract

This thesis proposes a novel diet for parents in the prevention of early-onset obesity in offspring. This diet includes green tea, grapes, soy, curries, plants from the broccoli and onion family, and a balanced ratio of fatty acids, especially polyunsaturated fatty acids (PUFAs), and represents a departure from the modern western diet towards one closer to an ancestral diet. Incorporation of these foods has the potential to protect offspring against the effects of prior exposure to a diet high in saturated fat consumed by mothers and fathers throughout their lives, especially near the time of offspring conception. While the causes of the sudden surge in childhood obesity rates are still not clear, it has been proposed that maternal diets may predispose offspring to weight-gain. During critical windows in fetal development, including egg and sperm formation, modifications to DNA can cause "programming" changes that are meant to increase early infant survival, but persist into adulthood. This phenomenon is termed epigenetics (meaning "on top of genetics") because it is not the actual genetic code (nucleotide sequence) that is altered, but instead the expression of genes that is modified by non-genetic factors such as the gestational environment. Epigenetics allows an organism to 'customize' its gene activity when exposed to 'predictable' environmental stimuli, and thereby offers adaptation over multiple generations to environmental circumstances. This thesis synthesizes information scattered across the primary literature on how energy/nutrient abundance during fetal development may shape epigenetic programming by providing critical signals about the environmental energy abundance during early development that ultimately influences adult expression of those genes that regulate fat metabolism. Specifically, parents consuming a modern diet high in saturated fat may be contributing to the current childhood obesity epidemic through epigenetic programming of their offspring to store fat. In contrast, it is proposed that plant-based substances typical of the diet of human's

evolutionary ancestors may prevent such fetal epigenetic programming for fat storage. This thesis explores the possibility that modest additions of plant-based substances, such as the incorporation of omega-3 polyunsaturated fats or drinking tea during pre- and post-natal nutrition, may prove beneficial in preventing the transmission of obesity from one generation to the next.

II. Introduction

A surge of obesity and related deaths in the last hundred years is reaching epidemic proportions, with a third of Americans categorized as overweight and another third as obese (World Health Organization, 2000). Furthermore, the prevalence of obesity-associated metabolic 'dysfunctions', such as diabetes, cardiovascular disease and hypertension, is also increasing (Ford, Giles, & Dietz, 2002). Childhood obesity rates have also increased over the last forty years, from 5% in 1971 to 17% in 2008 (Ogden & Carroll, 2010). While the causes of childhood obesity are still not clear (Moraru et al., 2006), it has been proposed that maternal diets may predispose children to early-onset obesity. Such a predisposition for early-onset obesity has been documented from maternal diets low in protein (Bol, Delattre, Reusens, Raes, & Remacle, 2009) or high in saturated fats (Table 1). These latter findings suggest that an important link between diet and genetics may contribute to the early onset of childhood obesity, although as explained in this thesis, the search for dietary components involved in trans-generational regulation of fat metabolism is ongoing.

Obesity can have several contributing factors, including activity level, diet, and genetics. There is mounting evidence that diet may have important interactions with an

individual's genetics, thus creating significant diet-gene interactions (Simopoulos, 2010). It has recently been established that parental diet influences diet-gene interactions, producing offspring with increased susceptibility to early-onset obesity persisting well into adulthood (Elahi, Cagampang, Mukhtar, Anthony, Ohri, & Hanson, 2009). Understanding how parental diet influences these diet-gene interactions is critical in developing strategies to prevent the enduring effects of early-onset obesity.

Consumption of specific dietary factors may have an even greater influence on the regulation of metabolism in an individual than others. For example, saturated and unsaturated fatty acids (Vallim & Salter, 2010; Jump, 2002), simple sugars (Jeffcoat, 2007), or secondary plant compounds including polyphenols (small carbon-based molecules consisting of several OH-group-containing rings) (Fang et al., 2003) have been shown to either turn on, or inhibit, genes controlling the storage of fat following their consumption. Additionally, a maternal high-fat diet (HFD), i.e. a diet comprised of over thirty percent of caloric intake as saturated fats during pre-natal development, produces a significant trans-generational effect in offspring fat metabolism. Such lasting effects increase the risk for obesity persisting into adult-hood in these individuals through long-term activation or silencing of entire gene networks controlling fat accumulation (McCurdy, et al., 2009). Correlation studies have documented an increase in obesity risk from the diet of both mothers and fathers lasting at least two generations in multiple human cohorts (Kaati, Bygren, & Edvinsson, 2002). Interestingly, diet can also produce protective effects against obesity. For example, protective effects against metabolic fetal programming were found when total maternal fat intake was reduced during pregnancy (McCurdy et al., 2009). This suggests that diet can bi-directionally influence the expression of genes associated with fat metabolism, rendering offspring vulnerable or resistant to developing obesity.

The propensity to transmit a susceptibility to obesity to offspring relies, in part, on the faithful passing on of parental genes and their respective proteins, including marks on this DNA that program certain genes for either sustained activation or inactivation (i.e. 'epigenetics'). DNA contains the genes that, in turn, contain instructions on how to construct every protein of a cell, with proteins serving in a multitude of functions including as enzymes that perform all metabolism. These instructions are read from each respective gene via the process of gene expression. How frequently and how much the information of these genes is copied is strongly influenced by changes in DNA structure that put it into an "open" (readable) or "closed" (not-readable) state (Annunziato, 2008). The open state, or active gene expression, results in protein formation, while the closed state inactivates, or 'silences,' the expression of that gene and prevents the protein formation. During critical developmental windows, such as fetal development (gestation), lasting changes to the state of the DNA can cause "programming" changes that persist into adulthood. These epigenetic changes in programming represent changes in the genetic expression that arise from non-genetic factors (other than changes in the genetic code or nucleotide sequence), such as the gestational environment.

Epigenetics allows an organism to customize its gene activity in a lasting manner when exposed to environmental stimuli, that presumably occurred with 'predictable' regularity throughout human evolution, and provides a relatively fast-acting (on an evolutionary scale) adaptation to the environment over the course of one to two generations. In response to fetal exposure to particular dietary nutrients, or lack there of, offspring may be able to increase their chances of survival. This can occur through the opposing mechanisms of either increasing fat storage capabilities or increasing baseline fat

oxidation. The latter is proposed to occur following fetal exposure to dietary compounds in food consumed by humans and produced by the food plants and animals in response to environmental stress. These stress 'signals' presumably suggest a near-approaching famine or winter months. As a result, offspring would be energy efficient (fat utilization produces more energy per gram in weight), which may serve to prolong life during an imminent famine. Consequently, by preferentially using fat for fuel, these offspring would additionally be obese-resistant. Conversely, increased fat storage in rodent models is seen in response to fetal exposure to a diet high in saturated fats (Elahi et al., 2009) or post-natal over-nutrition of excess calories during lactation (Chen et al., 2008). These epigenetic modifications that increase fat storage capabilities presumably increase offspring survival by maximizing energy storage during times of abundance. Such a response may possibly have conferred survival advantages in an ancestral environment with uncertain energy availability (and perhaps characterized by long energy shortages, punctuated by shorter opportunities with high energy availability). However, in light of today's constantly energy-abundant diet, the same response is harmful.

Overcoming epigenetic programming of metabolism for lasting activation of fat storage is critical in preventing, or reversing, such maladaptive fat metabolism. Little research has thus far been conducted on factors that may reverse or prevent aberrant programming effects. However, one study shows that maternal supplementation with omega-3 polyunsaturated fatty acids (PUFAs) during lactation reversed fetal programming caused by the steroid Dexamethasone and produced offspring that are obesity-resistant throughout adulthood (Wyrwoll, Mark, Mori, Puddey, & Waddell, 2006). Other dietary constituents in the parental diet that may contribute to the prevention of early-onset obesity should be explored. For example, plant-based polyphenols found in tea, soy, and

wine decrease the accumulation of fat following administration in human cell cultures and rat models via immediate, but not necessarily lasting, gene regulation (Sakurai, Mochizuki, & Kemeji, 2009; Rayalam, Della-Fera, Yang, Park, Ambati, & Baile, 2007; Lagouge, et al., 2006). These immediate effects last only minutes, but recruit proteins that can cause permanent gene silencing. However, compared with immediate effects of polyphenols on fat metabolism, their role in preventing or reversing aberrant, lasting epigenetic fetal programming on metabolic regulation has not been extensively studied. Due to their immediate diet-gene interactions, phytochemicals (plant-produced compounds including polyphenols) consumed by parents in soy, tea, grapes, turmeric, broccoli, onions, and related plants presumably counter harmful fetal programming of metabolism. Parents consuming excessive saturated fat could supplement their diet with plant-based fats and polyphenols to produce obesity-resistant offspring who preferentially burn fat rather than storing it.

This thesis presents a novel and comprehensive summary of the effects of parental intake of dietary factors that oppose fat accumulation and potentially promote epigenetic reprogramming towards metabolism active in fat burning. A specific goal of the thesis is to identify dietary factors that presumably prevent and/or reverse epigenetic effects in offspring caused by a parental diet high in saturated fats. An increased intake of such fat-storage-discouraging dietary factors, e.g. PUFAs and select phytochemicals, from plants would represent a return to the diet of human's evolutionary ancestors when obesity was a rarity. A return to such a diet may ameliorate the increasing prevalence of early-onset obesity in our society. Here, different dietary compounds (i.e. sub-classes of fats, sugars, polyphenols, etc.) prevalent in ancestral versus modern diets are compared with respect to their potential to influence epigenetic reprogramming that bi-directionally alters the risk

for obesity later in life. This information should help formulate a diet that may prevent deleterious effects of fetal programming on obesity.

III: An evolutionary perspective

This thesis proposes that a return to our "ancestral diet" may prevent or reverse harmful epigenetic programming that occurs following fetal exposure to a "modern diet." Human's ancestral diet had been suggested to be abundant in unsaturated fats and phytochemicals found in plant-rich diet (Naughton, O'Dea, & Sinclair, 1986; Simopoulous, 2001). The "modern diet" is largely animal-based and higher in saturated fats (U.S. Department of Agriculture, 2008). An ancestral diet may have promoted efficient use of fat as fuel and conservation of sugar, which is important during frequent periods of near-starvation (Owen, Morgan, & Kemp, 1967). In contrast, the modern western diet stimulates the utilization of sugar as a predominant energy source and promotes the storage of fat as well as the conversion of excess sugar into fat (Vallim & Salter, 2010). Seasonal variety has presumably always been present in the ancestral diet, and a rarity of saturated fats and sugars, combined with annual winter fasting may reconcile the opposing actions between the two diets in regulating whether fat should be burned or stored. Sugars and saturated fat abundant in the summer and fall may thus have promoted fat storage in preparation for the winter. Seasonal variation in the modern diet is virtually absent and is typically represented by continual abundance of sugars and saturated fat.

On the other hand, phytochemicals and PUFAs are produced as defense compounds by plants and are most abundant during environmentally adverse (stressful) periods such as winter months (Dixon & Paiva, 1995; Savchenko et al., 2010). Additionally, omega-3 PUFAs in some algae are the most abundant fat present in December through February

(Honya et al., 1993) and fat from cold-water fish and other seafood would have significant omega-3 fat ('fish oil') stores. Hence, phytochemicals and omega-3 PUFAs may have signaled the presence of winter and the presence of energy shortages. These signals, in turn, presumably promote fat utilization in human consumers to prevent starvation.

Interestingly, the ancestral and modern diets would theoretically also produce opposing effects on the programming of gene regulation in offspring, suggesting that nutrient abundance during fetal development may shape epigenetic programming and cause lasting influences on fat accumulation and the burning of fat for fuel in offspring (Jump, 2008; Vallim & Salter, 2010). This diet-gene regulation is likely accomplished through nutrients physically interacting with DNA controlling entire gene networks related to fat metabolism. Hence, saturated fats, omega-3 PUFAs, and stress-induced phytochemicals presumably provide a signal about environmental energy abundance and accordingly influence the expression of the key genes controlling metabolism. Diet-induced epigenetic programming may thus predispose a fetus to be better at storing fat or burning fat, depending on what it is exposed to during early development. The modern diet could be increasing the risk in offspring to have increased fat stores in order to make use of the abundant energy available and enhance survival. Conversely, an ancestral diet high in phytochemicals and unsaturated fats presumably predisposes offspring to being starvation-resistant by utilizing efficient metabolism of fat. This thesis therefore proposes that a return to a plant-rich diet may curb childhood obesity rates and possibly produce obese-resistant offspring.

IV. Parental Saturated Fat Intake Increases Offspring Weight

It is emphasized in this thesis that parental consumption of a modern western diet increases susceptibility to obesity in children via epigenetic mechanisms, and is contributing to the current surge in early-onset obesity. Using a variety of animal models, a parental diet high in saturated fat has repeatedly been demonstrated to trigger epigenetic programming that increases offspring susceptibility to weight gain early in life as illustrated in Table 1 and 2. Early weight gain was seen in offspring as a result of either mothers or fathers consuming a diet high in saturated fat (Ng et al., 2010; Elahi et al., 2007). Long-term epigenetic programming in offspring occurred in response to exposure to a high-fat diet occurring during either fetal development or early post-natal stages, e.g. lactation (Chen et al., 2008). Increased susceptibility to obesity, apparently caused by saturated fat-induced fetal programming, persisted into adulthood, irrespective of whether or not the offspring themselves also consumed a diet high in saturated fats (White et al., 2009; Ng et al., 2010).

The consequences of epigenetic programming in offspring induced by a parental diet high in saturated fat are not limited to fat accumulation. A parental diet high in saturated fat also increases blood glucose levels in offspring, thus contributing to an increased diabetes risk (Chen et al., 2008). Parental diets can also impair secretion of hormones regulating offspring's satiety ultimately leading to overeating (Gallou-Kabani, et al., 2007). Finally, parental high-fat diets have been linked to inflammation in the offspring, which exacerbates obesity-related health problems (Elahi et al., 2009; Monteiro & Azevedo, 2010). Prevention of these harmful long-term metabolic programming into adulthood is pivotal to reducing obesity risk.

A recent study showed that supplementation of maternal diets with long-chain omega-3 PUFAs prevented aberrant metabolic effects induced by early steroid exposure that produced early-onset obesity in control-diet rats (Wyrwoll et al., 2006). PUFAs (e.g. omega-3 and omega 6 fatty acids) were abundant in the ancestral diet, and omega-3 PUFAs are commonly found in foods such as fish, nuts, and certain seeds (Simopoulos, 2001). Both classes of PUFAs have been shown to override the short-term activation of fat and sugar metabolism genes by glucose and saturated fat (Clarke, Gasperikova, Nelson, & Lapillonne, 2002; Limatta, Towle, & Clarke, 1994). Hence, PUFA exposure *in utero* may prevent harmful fetal programming induced by the modern diet high in saturated fat.

Government recommendations suggest a daily intake of saturated fat of no more than 10% of total calories, yet the average American intake levels exceeds this amount (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010). Early-onset obesity could be induced in rodents via mothers or fathers consuming a diet with saturated fat comprising 34% of total caloric intake. Additionally, consumption of PUFAs increases immediate fat utilization and reduces fat storage depots, thereby contributing to their role in regulating fat metabolism (Sampath & Ntambi, 2004). Modest dietary supplementation of PUFAs with balanced ratios of omega-3 and -6 fatty acids comprising 10% of total caloric intake is recommended in the prevention of cardiovascular disease (Calder et al., 2010). This value is similar to PUFA intake from the Mediterranean diet (Razquin, Martinez, Martinez-Gonzalez, Mitjavilia, & Marti, 2009). Thus maternal intake of PUFAS at least 10% total calories may also prove powerful in the prevention of metabolic epigenetic programming in offspring induced by a diet high in saturated fats.

PUFAs can apparently control genes involved in fat metabolism by turning on the machinery involved in promoting or inhibiting gene expression. For example, PUFAs increases the activity of enzymes that promote the open state of DNA thus allowing for increased gene expression. Through control over these classes of enzymes in variety of mechanisms, PUFAs regulate immediate, short-term epigenetic regulation at this may extend to long-term fetal programming involved in epigenetic regulation of fat metabolism (Mochizuki, Suruga, & Fukami, 2006; Fan, Spencer, Wang, Moyer, & Chapkin, 2003). Through these varied mechanisms, PUFAs may ameliorate the harmful programming caused by saturated fats that contribute to the development of obesity in offspring especially when the different classes of fats are consumed together. To date, no study has directly assessed trans-generational effects of regular parental PUFA consumption on the reversal and/or prevention of saturated fat-induced metabolic programming in offspring. In order to elucidate the different epigenetic fetal programming mechanisms induced by parental saturated fat versus unsaturated fat consumption, more research utilizing physiologically relevant fat intake levels of specific sub-classes of fatty acids is needed. For instance, a rodent study using a maternal diet high in saturated fat (greater than 30% of total calories), where a portion of the mothers consume omega-3 and -6 fats in a balanced fashion and/or individually, may be able to address this fundamental question pertaining to PUFA's protective effects against fetal exposure to saturated. Early post-natal supplementation of PUFAs, e.g. during lactation, may also reverse metabolic programming as seen in Wyroll et al. (2006), but replication of this finding using parental saturated fat intake is necessary for clarification.

Year-round abundance of high levels of saturated fat has only appeared recently in the modern diet. The "high-fat diet" described in Table 1 and 2, potently signals to DNA to

increase fat storage capabilities via epigenetic regulation that persists into adulthood. By including PUFAs common in the ancestral diet, but rare in the modern diet (Simopoulos, 2001), these harmful fetal fat-storage adaptations may be reduced or eliminated. Hence, parental consumption of PUFAs may help reduce early-onset obesity susceptibility induced by the modern diet high in saturated fats.

V. Phytochemicals Regulate Epigenetic Machinery Increasing Fat Burning

Phytochemicals is an umbrella term used to describe plant-based compounds presumably abundant in the ancestral diet (Eaton & Konner, 1985), but lacking in the modern diet consumed in Western countries today, which is low in plant-based foods relative to animal-based products. Sub-classes of phytochemicals (e.g. polyphenols) are produced in plants for protection against environmental stresses including cold weather, UV damage, pathogen attack, and soil nutrient deficiencies (Dixon & Paiva, 1995). This thesis discusses polyphenols from green tea, red grapes, soy, turmeric, and sulfides from members of the broccoli and onion families (Tables 3 and 4). Ultimately, environmental stresses could reduce plant biomass and thus limit energy abundance in the environment and possibly result in famine. Consumption of plants containing these phytochemicals may provide a signal to the consumer of a future food-shortage. These signals may consequently increase the utilization of fat for energy to prevent starvation. Increased fat oxidation has been demonstrated after consumption of the above plant-based foods through phytochemical-gene interactions (Table 4). As the ancestral diet was presumably rich in fruits, vegetables, and leafy greens, phytochemicals may affect epigenetic regulation during fetal development to predispose offspring to be starvation-resistant and programmed for the utilization of fat for fuel.

There is much evidence from within-generation studies that phytochemicals induce expression of enzymes that mark DNA for immediate inactivation (gene silencing) or make DNA inaccessible through epigenetic programming potentially controlling fat metabolism (Table 3). These pre-natal diet-epigenetic interactions provide a mechanism for plant compounds to potentially influence genetic programming and produce obesity-resistant offspring. It has not been assessed if the epigenetic effects listed in Table 3 cause the fat utilization effects described in Table 4. Effectively if the epigenetic effects are related to the metabolic ones, phytochemical abundance *in utero* may predispose offspring to increase fat utilization throughout adult-hood, thus presumably enhancing survival should similar stress-inducing energy constraints persist or return. Alternatively, phytochemicals that inhibit epigenetic silencing of genes may reverse harmful fetal epigenetic programming caused by saturated fats if exposure of the former occurs early in development.

The same phytochemical apparently can operate through different epigenetic mechanisms to achieve the same outcome, obesity-resistance where fat is preferentially utilizes for fuel. Often, the gene-silencing and reactivation is site-specific. For example, polyphenols in green tea inhibit the expression of genes that turn on the immune response (Ito et al, 2002), while reactivating genes involved in cellular protection from damage (Pandey, Shukla, & Gupta, 2009). Both mechanisms promote cell health despite what appears to be opposing control over epigenetic regulation. More research is required to fully understand these complicated nutrient-epigenetic interactions.

Some polyphenols prevalent in green tea and soy have the ability to reverse epigenetic programming and reactivate “silenced” genes (Pandey, Shukla, & Gupta, 2009;

Fang, Chen, & Sun, 2005). Compounds in soy have already proved useful in reversing programming in an obesity-susceptible breed of mice (Dolinoy, Weidman, & Waterland, 2006), but these trans-generational investigations do not address parental dietary influences induced by saturated fats. These same polyphenols potently increase the utilization of fat for fuel in fat cells *in vitro* (Sakurai, Mochizuki, & Kemeji, 2009), and, when provided as supplements in the diet, increase weight loss in rats (Ronis, Chen, & Badeaux, 2009). Hence, these polyphenols may potently reverse harmful fetal programming by saturated fatty acids if provided through the mother or in the early post-natal diet, e.g. during lactation.

Other plant compounds found in turmeric, green tea, broccoli-related plants, and garlic/onions inhibit or enhance the immediate behavior of enzymes involved in epigenetic regulation described in Table 3 (Balasubramanyam, Varier, & Altaf, 2004; Ito et al., 2002; Myzak, Tong, Dashwood, Dashwood, & Ho, 2007; Druesne, Pagniez, & Mayeur, 2004). These immediate effects last only minutes, but recruit other epigenetic enzymes that transfer methylgroups onto the DNA like a road block and regulate gene expression over the long-term. If these latter proteins are recruited through continued consumption of polyphenols, the potential to silence genes inlolved in fat storage remains a possibility. These phytochemicals may thus play a larger role during fetal development than in adults. Generational studies are needed to investigate the magnitude of these actions. In addition to their ability to recruit enzymes involved in gene silencing, the above-mentioned plants increases fat utilization when consumed chronically (i.e. over the long-term) (Table 4); hence, they all remain potential epigenetic regulators of metabolism when combined with parental consumption of the modern diet rich in saturated fat.

Lastly, resveratol is a polyphenol found in grape skins (and thus wine) that is proposed to mimic fasting conditions in the body, i.e. increased fat oxidation (Howitz et al., 2003). Additionally, resveratrol increased resting metabolism and decreased food intake in non-human primates through the same mechanism (Dal-Pan, Blanc, & Aujard, 2010). *In vivo s*upplementation with resveratrol increased mitochondrial density in rats, which suggests an increase in resting fat oxidation for fuel. However, these mechanisms are not classified as "epigenetic" *per se* and no trans-generational studies have been conducted. Because of its nearly-identical gene expression profile to caloric restriction (Baur, et al., 2006), resveratrol (consumption of grape juice or supplementation) administration during pregnancy may produce obesity-resistant offspring. Individuals interested in consuming red wine for its health benefits, but wanting to largely avoid the alcohol may not need to drink much if they combine wine with a vegetable-rich meal, which may have additive effects.

Synergystic regulation of enzymes involved in long-term epigenetic programming and immediate, short-term metabolic regulatory effects are seen when particular classes of phytochemicals are consumed together. For instance, compounds in red wine and soy are thought to regulate epigenetic enzymes through separate mechanisms, but potentiate each other's effects on fat utilization when consumed together at lower doses (Rayalam, Della-Fera, Yang, Park, Ambati, & Baile, 2007). These findings suggest that a return to a varied ancestral, plant-based diet may prove more effective than individual phytochemical supplements in protecting against deleterious fetal programming. Further examination of these nutrient-nutrient interactions will be important to fully explore these synergistic effects. Nutrient-nutrient interactions may not be limited to phytochemicals, and may extend to polyphenol interactions with PUFAs. The combination of varied nutrients

(polyphenols and fatty acids) remains unexplored in this regard, but would be supported by the hypothesis that an ancestral-based diet is preventive to obesity.

Limited generational studies have investigated whether chronic parental consumption of particular plants may potentially reverse and/or prevent saturated-fat-induced epigenetic programming of metabolism in offspring. Within epigenetic studies examining trans-generational epignetic programming, only soy has been investigated (Dolinoy, Weidman, & Waterland, 2006). However, this latter study uses an obesity-inducing animal model not comparable to a maternal diet high in fat. Further research must thus be completed to confirm the role of phytochemicals in trans-generational programming. As these compounds are generally safe and non-toxic, human studies using a combination of vegetables, spices, and plant-based beverages to formulate an 'ancestral' diet during pre-natal development may prove useful in elucidating their preventative role in the epigenetic programming of metabolism induced by the modern diet.

VI. Conclusions

Plant-based dietary nutrients in combination with unsaturated fats may be able to prevent, or even reverse, fetal epigenetic programming by high levels of saturated fats that results in early-onset obesity. These former nutrients include polyunsaturated omega-3 fatty acids and phytochemicals, both powerful inhibitors of fat accumulation and regulators of epigenetic programming of offspring for obesity resistance. Parents consuming a modern diet high in saturated fat may be contributing to the current childhood obesity epidemic by triggering epigenetic programming, but modest additions of certain components of an ancestral diet during pre- and post-natal nutrition may counteract these effects.

Mechanisms behind epigenetic programming by saturated fats have been extensively investigated (Table 1 & 2), and the logical next step is to expand trans-generational epigenetic research to unsaturated fats and phytochemicals. Very little is known about whether the latter nutrients are able to effect an *in vivo* re-programming. Critical windows of exposure, e.g. pre-natal versus lactation, also remain to be determined to most effectively counteract harmful epigenetic programming. It does seem likely, however, that several of the above-mentioned phytochemicals as well as PUFAs (all as constituents abundant in the ancestral diet) would play a role in fetal (and also life-long offspring) health and energy metabolism.

This thesis proposes that continuous pre-natal consumption of green tea, grapes, soy, curries (rich in turmeric), broccoli-related plants (e.g. kale, cauliflower, brussels sprouts, etc.), onion-related plants (e.g. garlic, leeks, and chives), and polyunsaturated fatty acids will effectively eliminate harmful epigenetic metabolic programming by parental diets high in saturated fats. As effects of the former phytochemicals and PUFAs can be synergistic, consuming combinations of these foods will likely produce strong protective effects at lower doses. Furthermore, it is possible that these epigenetic regulations affecting fat metabolism can persist through several generations. Hence, a varied and colorful diet, with green tea, red wine, yellow curry, throughout one's lifetime is likely the best defense against early-onset obesity in offspring caused by the modern diet consumed today.

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Parental Diet	Offspring Diet	Tissue Harvested	Effects	Reference
Mothers fed HFD (34%) prior to mating	Reduced litter sizes to mimic post-natal over-nutrition	Post-natal d20	Maternal obesity alone or combined with over nutrition produces fatter pups with poor blood sugar control & altered appetite hormone levels	Chen et al. 2008
Mothers fed HFD their entire life or mating through lactation	Offspring fed HFD from weaning	Post-natal d120	Any maternal HFD influences offspring obesity	Howie et al. 2009
Maternal HFD (60%); Pair-fed mothers consumed fewer calories from the HFD	Offspring fed HFD (60%)	Post-natal week 18	Maternal obesity increases offspring obesity and may be required for fetal programming	White et al. 2009
Paternal HFD (34%)	N/A	Post-natal week 13	Paternal HFD impairs offspring insulin secretion and increases body fat	Ng et al. 2010
Maternal omega-3 intake (34% of total fat)	Offspring were cross-fostered with omega-3 diet during lactation and post-weaning	Post-natal month 6	Post-natal n-3 PUFA supplementation reverses steroid-induced fetal programming of metabolism in offspring	Wyroll et al. 2006
Maternal LFD (10%) followed by HFD-induced obesity (60%)	N/A	Post-natal month 6	A maternal LFD increases number of offspring that are obese- and diabetes-resistant	Gallou-Kabani et al. 2007
Mothers fed a HFD (60%) during early offspring gestation	N/A	Gestation d15	Placentas undergo sex- and diet-specific fetal programming; Maternal HFD reduces genetic imprinting in female offspring	Gallou-Kabani et al. 2010
Maternal HFD	Offspring HFD	Post-natal week 36	Maternal HFD causes hypertension, inflammation, high cholesterol, more body fat, and fatty livers in offspring	Elahi et al. 2009

Table 1. A chart of recent epigenetic studies investigating the influences of a parental fat intake on fetal programming concerning obesity, diabetes, and other symptoms of metabolic dysfunction in rodents. Abbreviations: HFD- High-fat diet

Parental Diet	Offspring Diet	Tissue Harvested	Effects	Reference
Maternal HFD (35%)	N/A	Gestation d130	Maternal HFD causes a fetal fatty liver and greater amounts of gene-specific activation	Aagaard-Tillery et al. 2008
Maternal HFD (32%) followed by a switch to a LFD	Offspring HFD (32%)	3rd trimester and post-natal d180	A LFD during gestation reduces fat-induced oxidative damage in fetal livers caused by maternal HFD	McCurdy et al. 2009
Parental dietary questionnaire	N/A	Forms filled out at 32 week into gestation and at offspring age 10	Offspring post-natal dietary intake correlates with pre-natal maternal diet over post-natal maternal diet or paternal diet	Brion et al. 2010
Two-generational cohorts	N/A	Cohorts from 1890, 1905, and 1920	Exposure to food abundance during critical post-natal developmental windows correlates with grandchild CVD mortality and diabetes	Kaati, Bygren, & Edvinsson, 2002

Table 2. A chart of recent epigenetic studies investigating the influences of a parental fat intake on fetal programming concerning obesity, diabetes, and other symptoms of metabolic dysfunction in primates. Abbreviations: CVD- Cardiovascular Disease; HFD- High-fat diet; LFD- Low-fat diet

Plant	Design	Results	Reference
Green Tea	In vitro	Treatment of EGCG directly re-expressed specific genes and increases their protein abundance in multiple cancer cell lines	Fang et al. 2003
	In vitro	Several tea polyphenols indirectly and directly reverse epigenetic gene silencing	Lee, Shim, & Zhu 2005
	In vitro	EGCG extensively re-expressed antioxidant genes in cancer cell lines	Pandey, Shukla, & Gupta 2009
	In vivo	Theophylline suppresses expression of inflammatory genes	Ito et al. 2002
Turmeric	In vitro	Curcumin prevents inflammatory gene activation	Li et al. 2008
	In vivo	Curcumin supplementation contributes to gene repression	Balasubramanyam et al. 2004
Soy	In vitro	Genistein indirectly reactivates silenced genes	Fang et al. 2005
	In vivo	Genistein supplementation in mice contributes to epigenetic gene silencing	Day et al. 2002
	In vivo	Maternal genistein supplementation in Agouti mice increases the prevalence of obese-resisant offspring	Dolinoy et al. 2006
	In vivo	Maternal genistein supplementation in Agouti mice reverses environmentally-induced epigenetic programming	Dolinoy et al. 2007
Broccoli Family	In vitro	Administration of sulforaphane increases gene expression and may reverse gene silencing	Myzac et al. 2004
	In vivo	Acute supplementation of sulforaphane demonstrates epigenetic effects in human subjects for several hours following consumption	Myzac et al. 2007
Onion Family	In vitro	Administration of disulfides in vivo metabolites increased gene activity	Druesne, et al. 2004

Table 3. A chart of known epigenetic regulatory effects in phytochemicals from various plants. Abbreviations: EGCG- (-)-epigallocatechin-3-O-gallate

Plant	Design	Results	Reference
Green Tea	In vitro	EGCG reduces fat accumulation in fat cells increasing insulin sensitivity, although older, larger fat cells typical of obesity have impaired uptake of the polyphenol	Sakurai et al. 2009
Turmeric	In vivo	Curcumin supplementation in rats reduces fat accumulation despite a high-fat diet	Ejaz et al. 2009
Soy	In vivo	Adolescent rats fed genistein upregulate gene activity involved in fat oxidation and supplementation prevents symptoms of metabolic dysfunction in adult rats consuming a high-fat diet	Ronis et al. 2009
	In vitro	Adminstration of genistein onto rat adipose cells decreases the conversion of excess sugar into fat and increases the break down of fat for energy	Kandulska et al. 1999
Soy + Red Wine	In vitro	Combining resveratrol adminstration with genistein decreases fat accumulation in a potentiated fashion in adipose cells	Rayalam et al. 2007
Red Wine	In vitro	Resveratrol increases longevity in yeast through mimicking caloric restriction	Howitz et al. 2003
	In vivo	Resveratrol increases fat oxidation and mitochondrial density in rats	Lagouge et al. 2006
	In vivo	Resveratrol reduces seasonal weight-gain in non-human primates by reducing food intake and increasing resting metabolic rate	Dal-Pan, Blanc, & Aujard, 2010
	In vivo	Resveratrol increases survival in mice fed a high-fat diet by reversing gene activation caused by the latter	Baur et al. 2006
Broccoli Family	In vivo	Sulforaphane supplementation over 7 weeks downregulates genes involved in fat and cholesterol synthesis	Rodríguez-Cantu et al. 2011
Onion Family	In vitro	Allyl Isothiocyanate administration increased fat oxidation in rat fat cells	Okulicz, 2010

Table 3. Phytochemicals from several plants regulate fat metabolism decreasing the accumulation of fat within cells. Abbreviations: EGCG- (-)-epigallocatechin-3-O-gallate

VIII. References

Aagaard-Tillery, K., Grove, K., Bishop, J., Ke, X., Fu, Q., McKnight, R., et al. (2008). Developmental Origins of Disease and Determinants of Chromatin Structure: Maternal Diet Modifies the Primate Fetal Epigenome. *Journal of Molecular Endocrinology*, 91-102.

Annunziato, A. (2008). DNA Packaging: Nucleosomes and Chromatin. *Nature Education*, 1-4.

Balasubramanyam, K., Varier, R., & Altaf, M. (2004). Curcumin, a Novel p300/CREB-binding Protein-specific Inhibitor of Acetytransferase, Repress the Acetylation of Histone/Nonhistone Proteins and Histone Acetyltransferase-dependent Chromatin Transcription. *Journal of Biological Chemistry, 279* (49), 51163-71.

Baur, J., Pearson, K., Price, N., Jamieson, H., Lerin, C., Kalra, A., et al. (2006). Resveratrol Improves Health and Survival of Mice on a High-calorie Diet. *Nature, 444* (16), 337-42.

Bol, V., Delattre, A., Reusens, B., Raes, M., Remacle, C. (2009). Forced Catch-up Growth After Fetal Protein Restriction Alters the Adipose Tissue Gene Expression Program Leading to Obesity in Adult Mice. *American Journal of Physiology – Regulatory, Integrative, and Comparative Physiology, 297*, R291-9.

Brion, M., Ness, A., Rogers, I., Emmett, P., Cribb, V., Smith, G., et al. (2010). Maternal Macronutrient and Energy Intakes in Pregnancy and Offspring Intake at 10 y: Exploring Parental Comparisons and Prenatal Effects. *The American Journal of Clinical Nutrition, 91*, 748-56.

Brooks, G., Fahey, T., & Baldwin, K. (2005). *Exercise Physiology: Human Bioenergetics and Its Applications. 4th ed.* New York: McGraw Hill.

Canto, C., & Auwerx, J. (2009). Caloric Restriction, SIRT1 and Longevity. *Trends in Endocrinology and Metabolism, 20* (7), 325-331.

Carone, B., Fauquier, L., Habib, N., Shea, J., Hart, C., & Li, R. (2010). Paternally Induced Transgenerational Environmental Reprogramming of Metabolic Gene Expression in Mammals. *Cell, 143*, 1084-96.

Chen, H., Simar, D., Lambert, K., Mercier, J., & Morris, M. (2008). Maternal and Postnatal Overnutrition Differentially Impact Appetite Regulation and Fuel Metabolism. *Endocrinology, 149*, 5348-56.

Choi, S.-W., & Friso, S. (2009). *Nutrients and Epigenetics. 1st edition.* Boca Raton: CRC Press.

Christodoulides, C., & Vidal-Puig, A. (2010). PPARs and Adipocyte Function. *Molecular and Cellular Endocrinology, 318*, 61-8.

Clarke, S., Gasperikova, D., Nelson, C., & Lapillonne, A. (2002). Fatty Acid Regulation of Gene Expression: A Genomic Explanation for the Benefits of the Mediterranean. *Annals New York Academy of Sciences*, 283-98.

Cropley, J., Suter, C., Beckman, K., & Martin, D. (2006). Germ-line Epigenetic Modifications of the Murine Avy Allele by Nutritional Supplementation. *Proceedings of the National Academy of Science, 103,* 17308-12.

Dal-Pan, A., Blanc, S., & Aujard, F. (2010). Resveratrol Suppresses Body Mass Gain in a Seasonal Non-human Primate Model of Obesity. *BMC Physiology*, *10* (11), 1-10.

Dashwood, R., & Ho, E. (2007). Dietary Histone Deacetylase Inhibitors: From Cells To Mice To Man. *Seminars in Cancer Biology, 17*, 363-9.

Day, K., Bauer, A., desBordes, C., Zhuang, Y., Kim, B.-E., Newton, L., et al. (2002). Genistein Alters Methylation Patterns in Mice. *American Society for Nutritional Sciences, 132*, 2419S-2423S.

Dixon, R., & Paiva, N. (1995). Stress-induced Phenylpropanoid Metabolism. *The Plant Cell, 7,* 1085-97.

Dolinoy, D., Huang, D., & Jirtle, R. (2007). Maternal Nutrient Supplementation Counteracts Bisphenol A-induced DNA Hypomethylation in Early Development. *Proceedings of the National Academy of Science, 104* (32), 13056-61.

Dolinoy, D., Weidman, J., & Waterland, R. (2006). Maternal Genistein Alters Coat Color and Protects Avy Mouse Offspring from Obesity by Modifying the Fetal Epigenome. *Environmental Health Perspectives, 114* (4), 567-72.

Druesne, N., Pagniez, A., & Mayeur, C. (2004). Diallyl Disulfide (DADS) Increases Histone Acetylation and p21 Expression in Human Colon Tumor cell Lines. *Carcinogenesis, 25* (7), 1227-36.

Eaton, S., Konner, M. (1985). Paleolithic Nutrition – A Consideration of Its Nature and Current Implications. *New England Journal of Medicine*, 312, 283-9.

Ejaz, A., Wu, D., Kwan, P., & Meydani, M. (2009). Curcumin Inhibits Adipogenesis in 3T3-L1 Adipocytes and Angiogenesis and Obesity in C57/BL Mice. *The Journal of Nutrition*, *139* (5), 919-25.

Elahi, M., Cagampang, F., Mukhtar, D., Anthony, F., Ohri, S., & Hanson, M. (2009). Long-term Maternal High-fat Feeding from Weaning Through Pregnancy and Lactation Predisposes Offspring to Hypertension, Raised Plasma Lipid and Fatty Liver in Mice. *British Journal of Nutrition, 102*, 514-9.

Fan, Y., Spencer, T., Wang, N., Moyer, M., & Chapkin, R. (2003). Chemopreventive N-3 Fatty Acids Activate RXR-α in Colonocytes. *Carcinogenesis*, *24* (9), 1541-8.

Fang, M. Z., Chen, D., & Sun, Y. (2005). Reversal of Hypermethylation and Reactivation of p16, RARB, and MGMT Genes by Genistein and Other Isoflavones from Soy. *Cancer Prevention, 11* (19), 7033-41.

Fang, M., Wang, Y., & Ai, N. (2003). Tea Polyphenol (-)-Epigallocatechin-3-Gallate Inhibits DNA Methyltransferase and Reactivates Methylation-silenced Genes in Cancer Cell Lines. *Journal of Cancer Research, 63*, 7563-70.

Ford, E., Giles, W., & Dietz, W. (2002). Prevalence of the Metabolic Syndrome Among US Adults. *Journal of American Medical Association*, *287* (3), 356-9.

Gallou-Kabani, C., Gabory, A., Tost, J., Karimi, M., & Mayeur, S. (2010). Sex- and Diet- Specific Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placenta Under a High-Fat Diet. *PLoS ONE, 5* (12), 1-13.

Gallou-Kabani, C., Vige, A., & Gross, M.-S. (2007). Resistance to High-fat Diet in the Female Progeny of Obese Mice Fed a Control Diet During the Periconceptual, Gestation, and Lactation Periods. *Endocrinology and Metabolism, 292*, E1095-E1100.

German, J., & Dillard, C. (2010). Saturated Fats: A Perspective from Lactation and Milk Composition. *Lipids, 45*, 915-23.

Godfrey, K., Lillycrop, K., Burdge, G., Gluckman, P., & Hanson, M. (2007). Epigenetic Mechanisms and the Mismatch Concept of the Developmental Origins of Health and Disease. *Pediatric Research, 61*, 5R-10R.

Haggarty, P. (2010). Fatty Acid Supply to the Human Fetus. *Annual Review of Nutrition, 30*, 237-55.

Holness, M., Caton, P., & Sugden, M. (2010). Acute and Long-term Nutrient-led Modifications of Gene Expression: Potential Role of SIRT1 as a Central Co-ordinator of Short and Longer-term Programming of Tissue Function. *Journal of Nutrition, 26* (5), 491-501.

Honya, M., Kinoshita, T., Iskikawa, M., Mori, H., Nisizawa, K. (1994). Seasonal Variation in the Lipid Content of Cultured *Laminaria Japonica* Fatty Acids, Sterols, β-carotene, and Tocopherol. Journal of Applied Phycology, *6*, 25-9.

Howie, G., Sloboda, D., Kamal, T., & Vickers, M. (2009). Maternal Nutritional History Predicts Obesity in Adult Offspring Independent of Postnatal Diet. *The Journal of Physiology, 587* (4), 905-15.

Howitz, K., Bitterman, K., Cohen, H., Lamming, D., Lavu, S., Wood, J., et al. (2003). Small Molecule Activators of Sirtuins Extend Saccharomyces Cerevisiae Lifespan. *Nature, 425* (11), 191-6.

Hudgins, L., Hellerstein, M., Seidman, C., Neese, R., Diakun, J., & Hirsch, J. (1996). Human Fatty Acid Synthesis Is Stimulated by a Eucaloric Low Fat, High Carbohydrate Diet. *The Journal of Clinical Investigation, 97* (9), 2081-91.

Ito, K., Lim, S., & Caramori, G. (2002). A Molecular Mechanism of Action of Theophylline: Induction of Histone Deacetylase Activity to Decrease Inflammatory Gene Expression. *Proceedings of the National Acadamy of Science, 99* (13), 8921-6.

Jeffcoat, R. (2007). Obesity - A Perspective Based on the Biochemical Interrelationship of Lipids and Carbohydrates. *Medical Hypotheses, 68*, 1159-71.

Jump, D. (2002). Dietary Polyunsaturated Fatty Acids and Regulation of Gene Transcription. *Current Opinion in Lipidology*, 155-164.

Jump, D. (2008). N-3 Polyunsaturated Fatty Acid Regulation of Hepatic Gene Transcription. *Current Opinion in Lipidology, 19*, 242-7.

Junien, C., & Nathanielsz, P. (2007). Report on the IASO Stock Conference 2006: Early and Lifelong Environmental Epigenomic Programming of Metabolic Syndrome, Obesity, and Type II Diabetes. *Complications of Obesity* (pp. 487-502). Bangkok: Obesity.

Kaati, G., Bygren, L., & Edvinsson. (2002). Cardiovascular and Diabetes Mortalitity Determined by Nutrition During Parents' and Grandparents' Slow Growth Period. *European Journal of Human Genetics, 10*, 682-8.

Kandulska, K., Nogowski, L., & Szkudelski, T. (1999). Effects of Some Phytoestrogens on Metabolism of Rat Adipocytes. *Reproductive Nutrition Development, 39*, 497-501.

Karastwegiou, K., & Mohamed-Ali, V. (2010). The Autocrine and Paracrine Roles of Adipokines. *Molecular and Cellular Endocrinology, 318*, 69-78.

Kucharski, R., Meleszka, J., & Foret, S. (2008). Nutritional Control of Reproductive Status in Honeybees Via DNA Methylation. *Science, 319* (5871), 1827-30.

Lagouge, M., Argmann, C., Gerhart-Hines, Z., Maziane, H., Lerin, C., Daussin, F., et al. (2006). Resveratrol Improves Mitochondrial Function and Protects Against Metabolic Disease by Activating SIRT1 and PGC1α. *Cell, 127* (6), 1109-22.

Lancet. (2006). Curbing the Obesity Epidemic. *Lancet*, 1549.

Lee, W., Shim, J.-Y., & Zhu, B. T. (2005). Mechanisms for the Inhibition of DNA Methyltransferases by Tea Catechins and Bioflavonoids. *Molecular Pharmacology, 68* (4), 1018-1030.

Li, H.-L., Liu, C., & Couto, G. (2008). Curcumin Prevents and Reverses Murine Cardiac Hypertrophy. *Journal of Clinical Investigation, 118* (3), 879-93.

Limatta, M., Towle, H., & Clarke, S. (1994). Dietary Polyunsaturated Fatty Acids Interfere with the Insulin/Glucose Activation of L-type Pyruvate Kinase Gene Transcription. *Molecular Endocrinology, 8*, 1147-53.

Lucock, M. (2007). *Molecular Nutrition and Genomics: Nutrition and the Ascent of Humankind.* Hoboken: Wiley.

McCurdy, C., Bishop, J., Williams, S., Grayson, B., Smith, S., Freidman, J., et al. (2009). Maternal High-fat Diet Triggers Lipotoxicity in the Fetal Livers of Nonhuman Primates. *The Journal of Clinical Investigation, 119*, 323-5.

Mochizuki, K., Suruga, K., & Fukami, H. (2006). Selectivity of Fatty Acid Ligands for PPARα which Correlates Both with Binding to Cis-element and DNA Binding-independent Transactivity in Caco-2 Cells. *Life Sciences, 80*, 140-5.

Monteiro, R., & Azevedo, I. (2010). Chronic Inflammation in Obesity and the Metabolic Syndrome. *Mediators of Inflammation*, 1-10.

Moraru, E., Luchian, A., Bozomitu, L., Rusu, T., Sacaci, P., Antonesei, L., et al. (2006). Obesity in Children: New Controversies and Present Prevention Measures. *Revista Medico-Chirurgicala a Societatii de Medici Naturalisti Din Lasi*, *114* (3), 921-7.

Myzak, M., Karplus, A., Chung, F.-L., & Dashwood, R. (2004). A Novel Mechanism of Chemoprotection by Sulforaphane: Inhibition of Histone Deacetylase. *Cancer Research, 64*, 5767-74.

Myzak, M., Tong, P., Dashwood, W.-M., Dashwood, R., & Ho, E. (2007). Sulforaphane Retards the Growth of Human PC-3 Xenographs and Inhibits HDAC Activity in Human Subjects. *Experimental Biology and Medicine, 232* (2), 227-34.

Naughton, J., O'Dea, K., & Sinclair, A. (1986). Animal Foods in Traditional Australian Aboriginal Diets: Polyunsatured and Low in Fat. *Lipids , 21*, 684-90.

Ng, S.-F., Lin, R., Laybutt, R., Barres, R., & Owens, J. (2010). Chronic High-Fat Diet in Fathers Programs β-cell Dysfunction in Female Rat Offspring. *Nature, 467*, 963-7.

Ogden, C., Carroll, M., Curtin, L., Lamb, M., & Flegal, K. (2010). Prevalence of High Body Mass Index in US Children and Adolescents, 2007-2008. *The Journal of the American Medical Association*, *303* (3), 242-9.

Okulicz, M. (2010). Multidirectional Time-Dependent Effect of Sinigrin and Allyl Isothiocyanate on Metabolic Parameters in Rats. *Plant Foods and Human Nutrition, 65*, 217-24.

Owen, O., Morgan, A., & Kemp, H. (1967). Brain Metabolism During Fasting. *Journal of Clinical Investigation, 46* (10), 1589-95.

Pandey, M., Shukla, S., & Gupta, S. (2009). Promoter Demethylation and Chromatin Remodeling by Green Tea Polyphenols Leads to Re-expression of GSTP1 in Human Prostate Cancer Cells. *International Journal of Cancer, 126*, 2520-33.

Ravelli, G.-P., Stein, Z., & Susser, M. (1976). Obesity in Young Men after Famine Exposure in Utero and Early Infancy. *New England Journal of Medicine, 295*, 349-53.

Rayalam, S., Della-Fera, M. A., Yang, J.-Y., Park, H. J., Ambati, S., & Baile, C. (2007). Resveratrol Potentiates Genistein's Antiadipogenic and Proapoptotic Effects in 3T3-L1 Adipocytes. *Journal of Nutrition, 137*, 2668-73.

Razquin, C., Martinez, J., Martinez-Gonzalez, M., Mitjavilia, M., & Marti, A. (2009). A 3 Years Follow-up of a Mediterranean Diet Rich in Virgin Olive Oil is Associated with High Plasma Antioxidant Capacity and Reduced Body Weight Gain. *European Journal of Clinical Nutrition, 63* (12), 1387-93.

Rodriguez-Cantu, L., Gutierrez-Uribe, J., Arriola, J., Diaz-De La Garza, R., Faheys, J., & Serna-Saldivar, S. (2011). Broccoli Sprouts and Extracts Rich in Glucosinolates and Isothiocyanates Affect Cholesterol Metabolism and Genes Involved in Lipid Homeostasis in Hamsters. *Journal of Agricultural and Food Chemistry, 59* (4), 1095-1103.

Ronis, M., Chen, Y., Badeaux, & Badger, T. (2009). Dietary Soy Protein Isolate Attenuates Metabolic Syndrome in Rats via Effects on PPAR, LXR, and SREBP Signaling. *Journal of Nutrition, 139* (8), 1431-8.

Sakurai, N., Mochizuki, K., & Kemeji, H. (2009). (-)-Epigallocatechin Gallate Enhances the Expression of Genes Related to Insulin Sensitivity and Adipocyte Differentiation in 3T3-L1 Adipocytes at an Early Stage in Differentiation. *Nutrition, 25*, 1047-56.

Sampath, H., & Ntambi, J. (2004). Polyunsaturated Fatty Acid Regulation of Gene Expression. *Nutrition Reviews, 62* (9), 333-9.

Savchenko, T., Walley, J., Chehab, E., Xiao, Y., Kaspi, R., Pye, M. et al. (2010). Arachidonic Acid: An Evolutionary Conserved Signaling Molecule Modulates Plant Stress Signaling Networks. *The Plant Cell, 22, 3193-205.*

Sharma, A., & Staels, B. (2007). Review: Peroxisome Proliferator-Activated Receptor Y and Adipose Tissue -- Understanding Obesity-Related Changes in Regulation of Lipid and Glucose Metabolism. *The Journal of Clinical Endocrinology & Metabolism , 92* (2), 386-95.

Simopoulos, A. (2001). Evolutionary aspects of diet, essential fatty acids and cardiovascular disease. *European Heart Journal Supplements. 3. Supplement D*, D8-21.

Simopoulos, A. (2010). Genetic Variants in the Metabolism of Omega-6 and Omega-3 Fatty Acids: Their Role in the Determination of Nutritional Requirements and Chronic Disease Risk. *Experimental Biology and Medicine, 235*, 785-95.

Stoger, R. (2008). The Thrifty Epigenotype: an Acquired and Heritable Predisposition for Obesity and Diabetes? *BioEssays, 30* (2), 156-66.

Towle, H., & Kaytor, E. (1997). Regulation of the Expression of Lipogenic Enzyme Genes By Carbohydrate. *Annual Review of Nutrition, 17*, 405-33.

U.S. Department of Agriculture, Agricultural Research Service. (2008). Nutrient Intakes from Food: Mean Amounts Consumed per Individual, One Day, 2005-2006.

U.S. Department of Agriculture and U.S. Department of Health and Human Services. *Dietary Guidelines for Americans, 2010.* 7th Edition, Washington, DC: U.S. Government Printing Office, December 2010.

Vallim, T., & Salter, A. (2010). Regulation of Hepatic Gene Expression by Saturated Fatty Acids. *Prostaglandins, Leukotrienes, and Essential Fatty Acids, 82* (4-6), 211-8.

Veneman, A. (2003). *Agriculture Fact Book 2001-2002.* Washington, DC: U.S. Government Printing Office.

White, C., Purpera, M., & Morrison, C. (2009). Maternal Obesity is Necessary for Programming Effect of High-fat Diet on Offspring. *American Journal of Physiology - Regulatory, Integrative, and Comparative Physiology, 296*, R1464-72.

World Health Organization. (2000). *Obesity: preventing and managing the global epidemic. Report of a WHO consultation.* Geneva: World Health Organization.

Wyrwoll, C., Mark, P., Mori, T., Puddey, I., & Waddell, B. (2006). Prevention of Programmed Hyperleptinemia and Hypertension by Postnatal Dietary W-3 Fatty Acids. *Endocrinology, 147* (1), 599-606.

Yudkin, J. (1967). Evolutionary and Historical Changes in Dietary Carbohydrates. *The American Journal of Clinical Nutrition, 20* (2), 108-15.