# The thrifty epigenotype: an acquired and heritable predisposition for obesity and diabetes?

**Reinhard Stöger**

**Summary**

**Obesity and type 2 diabetes arise from a set of complex gene–environment interactions. Explanations for the heritability of these syndromes and the environmental contribution to disease susceptibility are addressed by the ''thrifty genotype'' and the ''thrifty phenotype'' hypotheses. Here, the merits of both models are discussed and elements of them are used to synthesize a ''thrifty epigenotype'' hypothesis. I propose that: (1) metabolic thrift, the capacity for efficient acquisition, storage and use of energy, is an ancient, complex trait, (2) the environmentally responsive gene network encoding this trait is subject to genetic canalization and thereby has become robust against mutational perturbations, (3) DNA sequence polymorphisms play a minor role in the aetiology of obesity and type 2 diabetes—instead, disease susceptibility is predominantly determined by epigenetic variations, (4) corresponding epigenotypes have the potential to be inherited across generations, and (5) *Leptin* is a candidate gene for the acquisition of a thrifty epigenotype. *BioEssays* 30:156–166, 2008.**


*''. . ..we do not always bear in mind, that though food may be now superabundant, it is not so at all seasons of each recurring year''*

Charles Darwin (The Origin of Species)

## Heritability: the thrifty genotype hypothesis

At the beginning of the 21st century, obesity and type 2 diabetes reached pandemic proportions.[1–4] An increase in the frequency of diabetes mellitus began to be noticed about 50 years ago. Why has a disease with an apparently heritable component become more prevalent in our species?

Neel put forward the idea that many diabetics carry allelic variations in a small number of genes that would make them: ''. . .*exceptionally efficient in the intake and/or utilization of food*''. To describe this hypothetical set of alleles, he coined the term ''thrifty'' genotype.[5] Physiologists did not distinguish between type 1 diabetes (formerly termed juvenile or insulin-dependent diabetes) and type 2 diabetes (formerly termed adult diabetes) at the time Neel's paper was published. The thrifty genotype hypothesis is predominantly focused on type 2 diabetes and obesity. Obesity, in particular abdominal obesity, often precedes type 2 diabetes and is strongly associated with, and predictive of this metabolic disease.[6]

Neel speculated that rapid environmental changes caused the rising incidence of diabetes. Dietary and cultural conditions of the developed, ''western world'' are the factors putting individuals with a thrifty genotype at a higher diabetes risk. The abundant and cheap supply of energy in the developed world has created an environment where calorie-dense foods are constantly available, and the requirement for physical activity has been greatly reduced.

Food and energy, however, have been and continue to be limited commodities for much of the world's population. Keeping our evolutionary history in mind, Neel noted that: ''. . . *it must be remembered that during the first 99 percent or more of man's life on earth, while he existed as a hunter and gatherer, it was often feast or famine*''.[5] Under these environmental conditions, thrifty alleles could certainly confer selective advantage. Carriers of a thrifty genotype would amass energy stores more efficiently during periods of abundant food supply and have: ''. . .*an extra pound of adipose reserve*. . .'' to increase the odds of survival during a period of starvation.[5] While beneficial in times of recurrent famine, a thrifty genotype would be a liability in an environment typical of westernized societies.[5]

The thrifty genotype hypothesis has generated continued attention over the years, and is attractive from a medical perspective.[7–12] Identification of thrifty allele variants could provide new targets for therapeutic intervention of type 2

Funding agency: This work was supported by NIH grant GM077464-01A1.
Reinhard Stöger, Department of Biology, University of Washington, Department of Biology, Box 351800, Seattle WA 98195-0002.
E-mail: stoeger@u.washington.edu
DOI 10.1002/bies.20700
Published online in Wiley InterScience (www.interscience.wiley.com).

diabetes and obesity, because they are considered likely culprits of this metabolic syndrome.[12]

## Uncertainties of the thrifty genotype hypothesis

Neel re-evaluated his evolutionary model 36 years after the original 1962 publication.[13] Acknowledging the complexity of non-genetic and genetic factors contributing to the metabolic disorder, he suggested a broadening of the original concept, but concluded: "*... that the concept of a thrifty genotype remains as viable as when first advanced...*".[13]

Yet, thrifty alleles remain: "*...inherently speculative and difficult to prove*"[12] and are widely perceived as: "*...little more than a nebulous concept*".[14] Convincing examples of thrifty allelic variants have not been reported. In other words, and to paraphrase Thomas Huxley:[15] is this beautiful hypothesis slain by an ugly fact?

The validity and general applicability of Neel's hypothesis has been disputed. Reasonable doubt has been cast on the fundamental assumption that food security was commonly lower in prehistoric and ancient societies that practiced a hunter-gatherer lifestyle.[16,17] The hypothesis does not provide strong arguments to explain sudden changes in disease incidence that occur over short periods of time and affect large population fractions. Moreover, it has been argued that, during the short history of *Homo sapiens,* famines would not have provided sufficient selective advantage for the penetration of a thrifty genotype in modern populations, culminating with the suggestion that it may be time to stop the search for thrifty alleles.[18]

Why has the search for variations in genes conferring metabolic thrift so stubbornly refused to yield any results supporting Neel's hypothesis? The answer may be surprisingly simple: we all bear thrifty alleles.

## The old trait "metabolic thrift"—only thrifty genotypes exist

Limited food supply is a fact of life. Most animal species populating the Earth both past and present, experience fluctuations in food supply and at times famine. Thus, there has been ample time and selection pressure during evolution to mold genomes robust against environmental heterogeneity. The struggle for food and existence is much older than humankind. Starvation and famine are probably two of the oldest and strongest forces driving natural selection.[19] Encoding into genomes the trait of "metabolic thrift"—the capacity to efficiently acquire, store and expend energy—almost certainly began at the root of the tree of life. Because energy efficiency is so vital for survival and fitness, it is possible that a window of opportunity never opened for the establishment of "unthrifty" alleles during life's history.

It is likely that all human genotypes are thrifty and encode only small differences in energy efficiency—rare, monogenic disorders and uncommon Mendelian forms of type 2 diabetes are probably the exception, not the rule. As a species, we carry nothing but thrifty genotypes and are maladapted for the environment created by modern industrialized societies. The prototype for this environment is found in North America, whose population is composed of a broad diversity of genotypes.

According to the National Center for Health Statistics, 65.2% of adults in the USA were overweight or obese in the year 2002.[20] For women, aged 20–74, the survey found that race and ethnicity influences the occurrence of obesity. We do not know why African American women (50%) have a higher prevalence of obesity than Mexican women (39%) and white women (31%).[20] A simple genotype–phenotype correlation would make these ethnic differences evident also among adult males. But it does not. In the USA, male obesity rates (28–29%) are the same, independent of African American, Mexican or European genetic backgrounds.[20] This suggests, for males at least, that a wide range of different genotypes encode a very similar potential to store and burn energy. The trait "metabolic thrift" is deeply rooted in our genomes.

## A robust trait: genetic canalization

Within our 3-billion-base genome, single base differences are found, on average, every 100–300 nucleotides.[21] We expect that certain combinations of these single nucleotide polymorphisms (SNPs) would render some genotypes thriftier than others. We would also expect that the human genome be selected for the presence of safeguards that protect genetic networks encoding important traits.

Extreme sequence conservation would indicate strong purifying selection acting in parallel on diverged lineages. On the other hand, mechanisms described as "canalization" may stabilize polygenic traits. The classic concept of canalization was conceived to explain the constancy of a wild-type phenotype in natural populations of a single species, regardless of abundant environmental and genetic heterogeneity.[22,23] Canalization is a helpful metaphor. It illustrates how a specific cell type or trait is formed and guided during ontogeny; a deep and narrow canal would produce a very stable and robust phenotype that displays minimal variability.

The process whereby a trait becomes buffered against allelic heterogeneity is termed "genetic canalization" (see review in Ref. 27).[24–26] This reduced sensitivity to allelic variations evolves under diverse conditions, as mathematical models predict. For example, Kawecki demonstrated the emergence of genetic canalization for quantitative traits in response to a fluctuating environment, where the direction of selection frequently alternates and affects the entire population.[26] Such changes in the direction of selection could be caused by fluctuations in food supply.

Evolution of genetic canalization may occur even in the absence of selection for optimal phenotypes. It has been

argued that canalization is tightly linked with the stability of the developmental process of an organism.[28] That is, without robust gene networks operating during development, selection for an optimal phenotype later in life could not proceed.[28] Moreover, through their models of conditions leading to genetic canalization, Siegal and Bergman showed that increasingly complex gene networks also evolve a greater insensitivity to mutations.[28] Metabolic networks fulfill the criteria of complexity.[29,30]

I propose that metabolic thrift is subject to genetic canalization and, as a result, this complex trait is relatively insensitive to new allelic variants that appear in human populations by chance. The complexity of the metabolic system, together with historical selection, could account for the evolution of a canalized genetic network (Fig. 1A). This predicts that most polymorphisms identified in candidate genes assumed to play a role in obesity and type 2 diabetes will have a neutral or mild effect on the phenotype. Practically, this means that genomic screens designed to detect genetic variations are blunt tools in the search for the molecular basis of these complex health conditions. After years of intense work, allelic variants associated with type 2 diabetes have been confirmed for only ten loci in the human genome.[31] Genetic canalization of metabolic thrift may explain the difficulty and limited success in identifying disease-predisposing allelic variants, and why Neel described type 2 diabetes to be: "*. . .a geneticist's nightmare*".[32]

## Pima and Nauruans

My proposition that all human genomes are almost equally thrifty is incompatible with Neel's original thrifty genotype hypothesis. For this reason, it is worthwhile examining examples in support of his theory. Pima Indians in Arizona and Nauru people from the Micronesian South Pacific islands appear to be carriers of thrifty genotypes (eg Refs 33,34). Both populations are thought to have endured repeated bouts of food shortage and starvation. These conditions, paired with relative isolation, likely created strong selective pressures, thereby providing a classic situation for natural selection in favor of thrifty alleles. Upon transitioning to a Western lifestyle obesity and type 2 diabetes increased dramatically in these people. Pima and Nauruans have one of the highest recorded age–sex-adjusted incidence rates of type 2 diabetes (~25 cases/1000 per year) among world population groupings.[35–38] These examples do seem consistent with Neel's idea of a thrifty genotype.

## Rapid adjustment of Nauruans

The exceptionally high prevalence of type 2 diabetes in Nauruans dropped somewhat in recent years, although their Western lifestyle has not changed.[39] The decline of disease incidence can be interpreted as the most rapid natural selection-event documented in a human population.[9] However, the time frame appears suspiciously short for the reduction or removal of extreme metabolic thrift from the Nauruan gene pool by natural selection. Alternative explanations for the rapid adjustment of Nauruans are "epigenetics" or "programming" in early life as proposed by the "thrifty phenotype" hypothesis.[40]

## Epigenetics

A current definition of epigenetics is "*. . .the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence*".[41] These changes determine a gene's transcription potential, which can range from high expression to complete gene inactivity. The expression states of the two X chromosomes in somatic cells of females exemplify mitotic epigenetic inheritance in

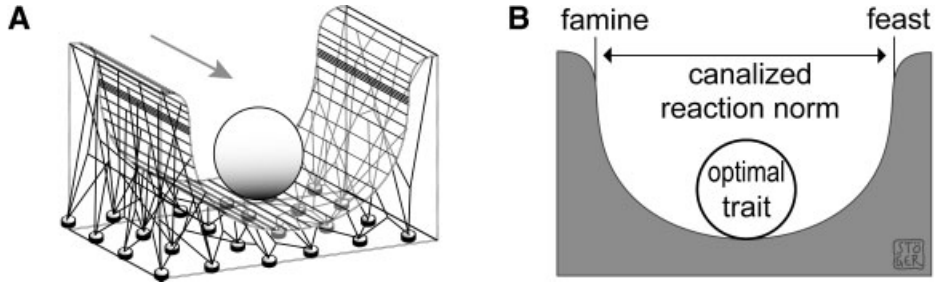


**Figure 1. A:** Developmental programs that establish the complex trait "metabolic thrift" (efficient acquisition, storage and use of energy), have become resistant to genetic perturbations over evolutionary time and "canalized". In analogy to Waddington's illustrations of epigenetic landscapes, the segment of a virtual canal is shown. The trait (ball) is established as it rolls down the canal during development. Large gene networks underlie these virtual "canals": bolts represent genes and the extending strings their interactions that form the canal. Gene variations would have a trivial affect on the overall canal. **B:** The cross section of a canal is shown. Without environmental input, the optimal phenotype of the trait would be fixed at the bottom of the canal. However, if the trait has potential for phenotypic plasticity, it will be able to respond to environmental conditions within the range of a "canalized reaction norm". Famine and feast are two such environmental extremes that have occurred repeatedly during the history of life.

mammals. Genes stay transcriptionally active on one of the X chromosomes, while the same genes on the second X chromosome are silenced during embryonic development and then remain transcriptionally inactive throughout a female's lifetime.

Known molecular components and processes underlying the inheritance of epigenetic information include DNA methylation, histone modifications, remodeling of chromatin and a variety of non-coding RNAs.[42] Cytosine methylation is the best-understood epigenetic inheritance system (reviewed in Ref. 43). In mammalian DNA, this epigenetic modification is typically found on cytosines of $^{5'}$-CG-$^{3'}$ dinucleotides, but also occurs at a low frequency on non-CG cytosines.[44] Dense methylation of CG dinucleotides around the promoter region is a very good indicator of long-term gene silencing (reviewed in Ref. 43), and generally correlates with histone and chromatin modifications of transcriptionally inactive loci (reviewed in Ref. 45).

During critical stages of development, epigenetic information can be established in response to both intrinsic and environmental cues, (reviewed in Refs 46–48) which may affect genomic loci in somatic cells, but also specific loci in germline cells.[49] The view emerges that epigenetic mechanisms are mediators between the environment and the genome.

In the mouse, genomic DNA methylation patterns are largely erased and reestablished during preimplantation development; loci carrying parental-specific imprints are the exception. Such reprogramming of the genome also takes place during the course of germ-cell maturation, where ''old'' epigenetic marks are cleared, and replaced by gamete-specific epigenetic marks.[50] The efficiency of these two epigenetic reprogramming events lacks accurate estimates for non-imprinted, autosomal loci, which comprise most of the mammalian genome. That is, established histone modifications, DNA methylation patterns and other epigenetic marks may not always be completely erased from the genome in every cell during gametogenesis and embryogenesis. As a result, tissue-specific cells of an individual can be epigenetically and functionally mosaic, even though their primary DNA sequence is identical.

Essential for the ''thrifty epigenotype'' hypothesis advanced in this essay is the distinct possibility of meiotic inheritance: epigenetic variations that establish how genetic information is used—or disused—can be transmitted through the germline to subsequent generations (reviewed in Ref. 55).[51–54]

## Epigenetics in obesity and type 2 diabetes?

A possible role for epigenetics in the etiology of obesity and type 2 diabetes has been foreshadowed and documented in a vast number of studies both in humans and animal models. For good reasons, most of the attention was, and still is, directed towards somatic tissues in the fetus and early infant and how environmental conditions influence their growth and function. The intrauterine environment appears to be a considerable determinant of the body fat mass of an individual later in life. A classic example is the ''Dutch Hunger Winter'' study.[56] Ravelli and colleagues examined the occurrence of obesity in about 300,000 19-year-old males that were born before, during or after a severe eight-month famine in the Netherlands in 1944–1945. A significantly higher incidence of obesity was observed in the cohort of young adults whose mothers had been exposed to famine during the first two trimesters of pregnancy.[56] This finding was interpreted in the context of Dörner's idea, whereby the future function of hypothalamic centers regulating food intake is influenced by the amount of calories available at crucial times of development.[57,58]

## Environmental contribution: the thrifty phenotype hypothesis

In 1992 Hales and Barker proposed a new hypothesis concerning the causes and origins of type 2 diabetes, emphasizing the nutritional conditions in early life.[40] Their ''thrifty phenotype'' hypothesis suggests that during gestation and early postnatal life an individual becomes programmed for nutritional thrift in order to adapt to and survive in an environment of limited resources and poor nutrition. Once established, this acquired metabolic phenotype is maintained throughout the lifetime of the individual, and does not change.

Under the thrifty phenotype hypothesis, type 2 diabetes and related symptoms arise if the metabolic program is set to ''thrift'' and does not match the westernized environment that an individual may encounter later in life. Environmental programming of metabolic pathways during early life is thought to induce lasting changes in the structure and function of an organism in response to certain stimuli.[40,59] The limited period during which metabolic programming occurs suggests that the evolution of this process was likely driven by selection for optimal completion of prenatal and early postnatal development, rather than by selection for adaptive capability during adult life.

Epigenetics was not explicitly mentioned in the original paper by Hales and Barker,[40] but it is now considered an integral component of the thrifty phenotype hypothesis. A considerable number of recent reviews suggest epigenetic mechanisms to be the basis of fetal programming.[60–67]

## Plasticity of epigenetic information

Rapid adjustment and optimization, at times necessary for survival, require a type of plasticity that genomes encoding highly complex traits can neither achieve nor afford.[68] Epigenetic mechanisms may have evolved in part because they provide solutions to this conundrum. Without harming the integrity of the genome, epigenetic information enables the interpretation of genetic information in response to a given environment. Under extreme conditions such as famine, the

epigenetic information superimposed on the genetic network would enhance metabolic thrift encoded in the genes themselves. When environmental conditions return to ''normal'' in the following generation, optimal fitness for the population is maintained because genetic information was not permanently altered. Under this prevailing view, the evolutionarily valuable trait metabolic thrift is not compromised and future generations get their own chance to re-interpret the genotype in accordance with the environmental conditions encountered during early life.

The decline of type 2 diabetes documented in Nauruans could be explained by the thrifty phenotype hypothesis and, by extension, epigenetic reprogramming of the genome. Ample nutrition during gestation and early postnatal life, as experienced by the younger generations of islanders, may have reduced the very high prevalence for diabetes, as Hales and Barker proposed.[40]

## Canalized reaction norms

The trait metabolic thrift is proposed to remain sensitive to environmental changes, while it continues to be relatively insensitive to genetic changes. That is, genetic canalization of a trait does not exclude phenotypic variability. A ''canalized reaction norm'' defines the pattern of all possible phenotypes expressed from a canalized gene network in response to a range of different environments (Fig. 1B).[25,27] Famine and abundant food supply are environmental conditions setting the limits of the canalized reaction norm. Anywhere within the boundaries of the canal, however, there is potential for phenotypic development (Fig. 1B).

Without environmental input, the optimal phenotype of a genetically determined and canalized trait would develop at the bottom of the canal (Figs. 1B and 2). With environmental input—during the metabolic programming phase(s)—epigenetic modifications could promote phenotypic adjustment, causing a shift in the position of the trait within the canal. Extreme conditions would lead to the establishment of the trait furthest away from the genetically determined, phenotypic optimum (bottom of the canal). Nevertheless, expression of the trait at the established position within the canal would provide the best-available response to these extreme conditions during development (Fig. 2).

Under this model, the risk of developing metabolic disorders later in life increases with the distance between the phenotype optimal under a given set of environmental conditions and the phenotype established early in life. Thus, either famine or feast experienced during development may cause an individual's predisposition for type 2 diabetes and obesity. The disease risk would be markedly heightened by a mismatch between the environments encountered during the metabolic programming phase and adult life, as the thrifty phenotype hypothesis implies.[40]

## Phenotype versus genotype

The thrifty phenotype hypothesis provides a compelling alternative explanation for the aetiology of obesity and type 2 diabetes. The phenotype acquired during early life seems to confer a much stronger susceptibility to these syndromes than the genotype.

Twentieth-century biology and medicine emphasized the distinction between genotype and phenotype. Johannsen introduced both terms in 1909 to describe two sources of trait variation that he had observed while breeding genetically ''pure'' and mixed lines of bean plants: variation due to environmental factors and variation due to heterogeneous, genetic backgrounds.[69] He concluded that environmentally induced trait variation is not heritable and thereby influenced how we evaluate ideas about heredity.

The advent of molecular biology introduced a mechanistic understanding of the nature and heritability of the genotype. The enthusiasm inspired by this revolution in biology continues to resonate today and, as a result, genetic variation in our nuclear and mitochondrial genomes are widely assumed to be the only cause of heritable diseases. By contrast, the environment is thought to be relevant only insofar as it may modulate mutation rates and influence the penetrance of disease-promoting genotypes. Hence, the outcome of environmentally induced phenotypic variation is always perceived to be somatic and thus not heritable. Ever since the genotype has been equated with the primary DNA sequence of an organism, heritability of an acquired phenotype has been deemed an implausible proposition.

Slowly, this notion is changing. Jablonka and Lamb, for instance, have discussed how the heritable component of a trait is not always confined to the base composition of the genome (eg Refs 70,71). Heritable effects induced by environmental factors have been documented in humans and rodents, some of which have been attributed to epigenetic changes and span at least two generations (reviewed in Ref. 47).[65,72–78] Dietary variations change the epigenetic state at a specific genomic locus in the germline and affect the phenotype in offspring.[49]

Solid evidence for transgenerational epigenetic inheritance in mammals is still slim. This idea has been lingering at the periphery of biology, in part because it is difficult for geneticists to detect this phenomenon in genetically heterogeneous, outbred populations. Nevertheless, epigenetics has been suggested to form part of an alternative, ''soft inheritance'' system.[79]

## Transgenerational inheritance of a ''thrifty epigenotype''

I have argued above that genetic variants play a modest role in the aetiology of type 2 diabetes and obesity, as a result of genetic canalization. Therefore, heritability of these metabolic syndromes must have a strong, non-genetic component.

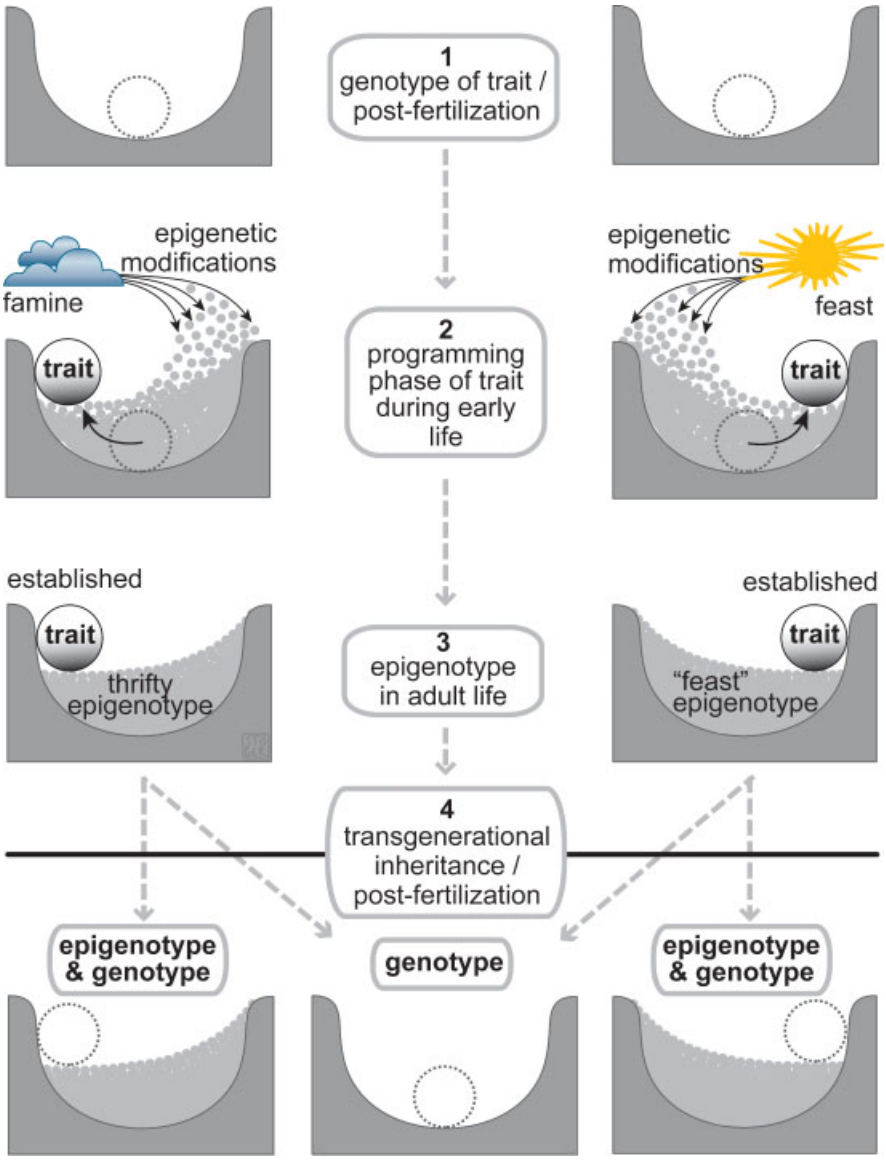


**Figure 2.** Hypothetical effects of either famine or feast during various stages of human life. (1) The genotype encoding the trait "metabolic thrift" is shown prior to environmental input (shape of canal). The dotted ball indicates the developmental potential of the trait (position within the canal), if all epigenetic information has been erased. (2) The metabolic gene network is responsive to environmental conditions during certain periods of gestation and early postnatal life (fetal programming); epigenetic modifications promote programming and adjustment of the trait to the existing conditions (shift of ball from the bottom of the canal)—this is to ensure progression of development. However, the risk to develop metabolic disorders in adult life increases with the distance between the optimal phenotype (bottom of the canal) and the established phenotype. An individual may have acquired a "thrifty epigenotype" if his or her metabolic phenotype was established during a time of famine or malnutrition. (3) During adult life, the phenotype and the underlying epigenetic settings are firmly established. The established phenotype of the trait cannot adjust to new conditions; disease risk is further increased, if environmental conditions that existed during the programming phase have changed from famine to food abundance, or vice versa. (4) A particular epigenotype could occasionally be transmitted to offspring if epigenetic information is not erased in germ cells, or if environmental conditions would either reinforce or newly impose a particular epigenotype in the parental germ line. Transgenerational inheritance of an acquired trait may, therefore, occur and predispose to obesity and type 2 diabetes.

An acquired phenotype and the underlying epigenetic modifications of the genome—the epigenotype—could be passed down to subsequent generations. For example, a ''thrifty epigenotype'' is acquired under conditions of malnutrition or famine at a critical time of development. Theoretically, gametes could carry the blueprint for this particular epigenotype and thereby transmit it through multiple generations (Fig. 2). Repeated bouts of hunger during the reproductive lifetime of an individual, or in successive generations could (a) fail to erase, (b) reinforce, or (c) newly impose, epigenetic marks in germline cells. This inherited thrifty epigenotype would augment epigenetic marks established during the metabolic programming phase in early life of an individual in an unchanged, impoverished environment. In contrast, an inherited thrifty epigenotype could diminish the impact of epigenetic marks that would establish a metabolic trait in response to abundant food supply, if these more agreeable conditions were to prevail during the programming phase of an individual.

DNA methylation may be one of the epigenetic modifications involved in response to environmental cues. Alterations of cytosine methylation patterns have been detected in germline cells two generations after male mice were exposed to a fungicide.[80] Dietary methyl-supplementation during pregnancy resulted in a change of the phenotype, which is linked to the DNA methylation state at the $A^{vy}$ and $Axin^{Fu}$ loci in the mouse genome.[81,82]

Consider the possibility that a thrifty epigenotype involves promoter methylation of a gene or a gene network, thereby increasing an individual's odds of survival during famine. Severe or prolonged energy constraints could cause a complete transcriptional shutdown and hypermethylation of an environmentally responsive gene(s). DNA methylation could initiate additional epigenetic changes such as histone modifications and chromatin remodeling—or vice versa—and, in this way, promote and establish long-term gene silencing (reviewed in Ref. 45).

Conceptually, inheritance of a thrifty epigenotype differs from inheritance of a new allelic variation of a gene. The entire gene network, or a large proportion of it, can be modulated and fine-tuned simultaneously by epigenetic processes. To achieve similar adjustment by classical mutations, many random genetic changes would have to occur by chance within the genome of a germ cell—an unlikely scenario. Thus, in the case of inheritance of a thrifty epigenotype, we may expect multiple genomic loci to be epigenetically modified.

Epigenetic silencing of a normally active gene can phenocopy a genetic mutation and may be described as ''epimutation''.[83,84] Methylation and silencing of autosomal gene promoters is thought to result from stochastic and random events and is often deemed abnormal. The hypothetical process described above implies that promoter methylation, seemingly aberrant in nature, could represent an environmental imprint that is maladapted for conditions of abundant food supply; the term ''epimutation'' may at times be misleading and not reflect the true basis of the epigenetic modification.

## Heritable epigenotypes and disease

Transgenerational inheritance of epigenetic states that cause disease is not a new concept[83,85] and was first proposed to result from incomplete erasure of epigenetic marks in germ cells.[85] Support of this model comes from studies of human imprinting disorders, where alleles of certain genes are not expressed correctly because they carry a faulty parental-specific epigenetic mark.[86,87] Incomplete epigenetic resetting and silencing of retrotransposons during early development could also lead to variations in disease risk and ''nonMendelian'' inheritance.[88] Aberrant silencing of the human DNA mismatch repair gene *MLH1* is the first documented example of a heritable epimutation associated with cancer susceptibility.[54,84] An intriguing study showed how alterations of DNA methylation induced by the endocrine disruptor vinclozolin can promote transgenerational disease.[75] These insights led to suggestions that heritability of type 2 diabetes and obesity may also have an epigenetic component.[38,89–92]

Genetic studies of type 2 diabetes and obesity are often based on family histories. Because humans reproduce relatively slowly, only two to three generations are typically included in these studies. It is at least plausible that inheritance of epigenetic marks affecting metabolic genes could persist over this time span.

## Implications and predictions

- The thrifty epigenotype is anticipated to be present at significantly higher frequencies in populations experiencing recurrent food shortages. Individuals exposed to these conditions will have a characteristic epigenetic profile, which could differ markedly from individuals native to rich, developed countries. Whole-genome association studies on populations with a heritable predisposition for diabetes and obesity will benefit by scanning for epigenetic variations. The thrifty epigenotype may possibly leave an evolutionary footprint on promoters of metabolic genes, if cytosine methylation plays a role in fetal programming. Spontaneous deamination of methylated cytosines in germline cells is thought to be the main cause for the higher-than-expected C to T base transition frequency observed in mammalian genomes.[93,94] Intermediate levels of CG dinucleotides could indicate occasional promoter methylation in the germline and possible transgenerational inheritance of a

variable epigenotype at a particular locus. ''Sinking'' CpG islands may be a hallmark of environmentally responsive genes, which become methylated only under certain conditions, such as energy depletion and are passed in this state through the germ line.

- The question then arises which genes are most likely to hold epigenetic memories of the environmental past. Primary candidates are genes directly associated with energy acquisition, storage and utilization.
- *Leptin* (*LEP*), is thought to be one of the best thrifty gene candidates,[9,12] since it encodes a hormone regulating appetite and energy homeostasis (reviewed in Ref. 95). However, *LEP* mutations and allelic variants associated with obesity are very rare in humans.[96,97] In Pima Indians, for example, plasma leptin concentrations are reportedly lower in individuals with a tendency to gain weight; yet allelic *LEP* variants have not been identified.[97,98] Thrifty epigenetic variants of *LEP* could possibly explain low plasma concentrations of this fat hormone. The promoter region of *LEP* is methylated in somatic tissues of human and mouse and displays epigenetic variation.[99,100] It is tempting to speculate that *LEP* is responsive to environmental cues and can acquire a thrifty epigenotype.
- Epigenetic programming of the metabolic gene network might also affect traits, which are indirectly associated with energy homeostasis. An increased risk of schizophrenia has been found in the cohort of individuals that were conceived during the aforementioned Dutch Hunger Winter (reviewed in Ref. 103).[101,102] Certain behavioral characteristics could impart survival advantage during famine, as Prentice and colleagues suggested in a recent and insightful discussion of the thrifty genotype hypothesis.[14] The tendency for physical activity or inactivity is possibly one such trait.[10,14]
- The number of sequence polymorphisms identified to be associated with type 2 diabetes and obesity is likely to stay small; those few causative genetic variants, which influence disease risk, are expected to modulate the expression and epigenetic modification of the metabolic gene network.

## Conclusions

Unraveling the relationship between genotype and phenotype continues to be a fundamental problem in Biology. It gains a certain amount of urgency as we try to understand the causes of obesity and type 2 diabetes—metabolic disorders that have grown into a global health challenge. In this context, I have considered three theoretical concepts: (i) the thrifty genotype hypothesis, (ii) genetic canalization, and (iii) the thrifty phenotype hypothesis.

- With the thrifty genotype hypothesis, Neel contributed the pivotal insight that the history of the human genome might stand in the way of some individuals to live a healthy life in an environment created by contemporary, technologically advanced societies. In this essay, I propose that most human genotypes are equally maladjusted for this westernized lifestyle. The complex trait metabolic thrift, encoded by a highly connected gene network is optimized to meet fluctuating conditions of famine and abundance.
- The trait has become refractory to allelic variations through genetic canalization. Although not absolute, this proposed insensitivity to mutations suggests that allelic variations play only a small part in the aetiology of type 2 diabetes and obesity. The precise molecular mechanisms underlying genetic canalization are not known. Hence, the challenge will be to provide empirical evidence that this type of process indeed buffers the trait metabolic thrift.
- Phenotypic variation—the differences among individuals in their tendency for type 2 diabetes and obesity—likely arises from variations in programming events during critical stages of development, according to the thrifty phenotype hypothesis. Epigenetic mechanisms have the qualities needed for this kind of programming.

Technology for the genome-wide detection of epigenetic signals has been developed[42,104] and we can expect a wealth of data to come from metabolic studies that make use of these research tools. It will be a formidable task to determine all members of the epigenetically regulated, metabolic gene network. Multiple cell types and tissues will have to be tested and compared. A good proportion of these epigenetic studies will make use of animal models; in such systems, conditions for feast and famine can be controlled and metabolic changes, as well as temporal epigenetic changes, can be closely monitored.

Identification of epigenetic signatures typical for the metabolic gene network will provide a molecular entry point to explore critical aspects of the thesis. For example, at which developmental time point(s) is a thrifty epigenotype established? What are the signaling pathways that relay information on environmental conditions to the metabolic gene network? Is the epigenotype of an individual detected in DNA of peripheral blood leukocytes suitable for diagnostic testing, or will the epigenotype present in fat and muscle cells provide clearer information on the environmental past? Is a particular epigenotype at all times erased in the germline, or do children occasionally inherit their epigenotype from their parents and grandparents?

Future generations may find our current way of life to be reflected ''on'' their genes—as inherited epigenotypes, predisposing them to obesity and type 2 diabetes.

## Acknowledgments

It is a pleasure to thank David I.K. Martin, Diane Genereux, Alice Burden, Charles Laird and Lisa Chakrabarti, as well as

anonymous reviewers for critical, but helpful comments on this manuscript. A great body of relevant work was not cited here due to text size limitations.

## References


1. Manson JE, Bassuk SS. 2003. Obesity in the United States: a fresh look at its high toll. Jama 289:229–230.
2. Zimmet P, Magliano D, Matsuzawa Y, Alberti G, Shaw J. 2005. The metabolic syndrome: a global public health problem and a new definition. J Atheroscler Thromb 12:295–300.
3. Prentice AM. 2006. The emerging epidemic of obesity in developing countries. Int J Epidemiol 35:93–99.
4. Smyth S, Heron A. 2006. Diabetes and obesity: the twin epidemics. Nat Med 12:75–80.
5. Neel JV. 1962. Diabetes mellitus: a ''thrifty'' genotype rendered detrimental by ''progress''? Am J Hum Genet 14:353–362.
6. Ford ES, Williamson DF, Liu S. 1997. Weight change and diabetes incidence: findings from a national cohort of US adults. Am J Epidemiol 146:214–222.
7. Eaton SB, Konner M, Shostak M. 1988. Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. Am J Med 84:739–749.
8. Ravussin E. 2002. Cellular sensors of feast and famine. J Clin Invest 109:1537–1540.
9. Diamond J. 2003. The double puzzle of diabetes. Nature 423:599–602.
10. Chakravarthy MV, Booth FW. 2004. Eating, exercise, and ''thrifty'' genotypes: connecting the dots toward an evolutionary understanding of modern chronic diseases. J Appl Physiol 96:3–10.
11. Prentice AM, Moore SE. 2005. Early programming of adult diseases in resource poor countries. Arch Dis Child 90:429–432.
12. Lazar MA. 2005. How obesity causes diabetes: not a tall tale. Science 307:373–375.
13. Neel JV. 1999. The ''thrifty genotype'' in 1998. Nutr Rev 57:S2–9.
14. Prentice AM, Rayco-Solon P, Moore SE. 2005. Insights from the developing world: thrifty genotypes and thrifty phenotypes. Proc Nutr Soc 64:153–161.
15. Huxley TH. 1870. Biogenesis and Abiogenesis. The Presidential Address to the Meeting of the British Association for the Advancement of Science at Liverpool. In: Discourses Biological and Geological: Essays by Huxley TH. 1897. New York: D. Appleton and company, p 229–271
16. Cordain L, Miller J, Mann N. 1999. Scant evidence of periodic starvation among hunter-gatherers. Diabetologia 42:383–384.
17. Benyshek DC, Watson JT. 2006. Exploring the thrifty genotype's food-shortage assumptions: A cross-cultural comparison of ethnographic accounts of food security among foraging and agricultural societies. Am J Phys Anthropol 131:120–126.
18. Speakman JR. 2006. Thrifty genes for obesity and the metabolic syndrome--time to call off the search? Diab Vasc Dis Res 3:7–11.
19. Darwin C. 1859. The origin of species by means of natural selection or the preservation of favoured races in the struggle of life. First published 1857, by John Murray. Burrow JW, editor. Published by Penguin Books 1968. Penguin Classics 1985, London, New York, Victoria, Toronto, Auckland: Penguin Group. ISBN 0-14-043205-1.
20. Leavitt MO. 2005. Chartbook on Trends in the Health of Americans. chapter 18/Table 73. Bernstein AB, Makuc DM, Freid VM, editors: National Center for Health Statistics (NCHS) Centers for Disease Control and Prevention (CDC).
21. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, et al. 2001. The sequence of the human genome. Science 291:1304–1351.
22. Waddington CH. 1942. Canalization of development and the inheritance of acquired characters. Nature 150:563–565.
23. Schmalhausen II. 1949. Factors of evolution: the theory of stabilizing selection. Edited by T. Dobzhansky; translated by I. Dordick/ Philadelphia: Blakiston; Chicago/London: University of Chicago Press, reprinted 1986.
24. Wagner GP. 1988. The influence of variation and development constraints on the rate of multivariate phenotypic evolution. J Evol Biol 1:45–66.
25. Stearns SC, Kaiser M, Kawecki TJ. 1995. The differential genetic and environmental canalization of fitness components in *Drosophila melanogaster*. J Evol Biol 8:539–557.
26. Kawecki TJ. 2000. The evolution of genetic canalization under fluctuating selection. Evolution Int J Org Evolution 54:1–12.
27. Debat V, David P. 2001. Mapping phenotypes: canalization, plasticity and developmental stability. Trends in Ecology & Evolution 16:555–561.
28. Siegal ML, Bergman A. 2002. Waddington's canalization revisited: developmental stability and evolution. Proc Natl Acad Sci USA 99:10528–10532.
29. Jeong H, Tombor B, Albert R, Oltvai ZN, Barabasi AL. 2000. The large-scale organization of metabolic networks. Nature 407:651–654.
30. Wagner A. 2002. The large-scale structure of metabolic networks: a glimpse at life's origin. Complexity 8:15–19.
31. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, et al. 2007. A Genome-Wide Association Study of Type 2 Diabetes in Finns Detects Multiple Susceptibility Variants. Science 316:1341–1345.
32. Neel JV. 1976. Diabetes mellitus—a geneticist's nightmare. In: The Genetics of Diabetes Mellitus. Creutzfeldt W, Köbberling J, Neel JV, editors. Berlin, New York: Springer-Verlag. p 1–11.
33. Zimmet P, Dowse G, Finch C, Serjeantson S, King H. 1990. The epidemiology and natural history of NIDDM--lessons from the South Pacific. Diabetes Metab Rev 6:91–124.
34. Dowse GK, Zimmet PZ, King H. 1991. Relationship between prevalence of impaired glucose tolerance and NIDDM in a population. Diabetes Care 14:968–974.
35. West KM. 1974. Diabetes in American Indians and other native populations of the New World. Diabetes 23:841–855.
36. Zimmet P, Pinkstone G, Whitehouse S, Thoma K. 1982. The high incidence of diabetes mellitus in the micronesian population of Nauru. Acta Diabetol Lat 19:75–79.
37. Sugarman JR, Hickey H, Hall T, Gohdes D. 1990. The Changing Epidemiology of Diabetes Mellitus Among Navajo Indians. W J Med 153:140–145.
38. Schulz LO, Bennett PH, Ravussin E, Kidd JR, Kidd KK, et al. 2006. Effects of traditional and Western environments on prevalence of type 2 diabetes in pima indians in Mexico and the US. Diabetes Care 29:1866–1871.
39. Dowse GK, Zimmet PZ, Finch CF, Collins VR. 1991. Decline in incidence of epidemic glucose intolerance in Nauruans: implications for the ''thrifty genotype''. Am J Epidemiol 133:1093–1104.
40. Hales CN, Barker DJ. 1992. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35:595–601.
41. Riggs AD, Martienssen RA, Russo VEA. 1996. Historical overview of epigenetic mechanisms 1–4. In: Epigenetic mechanisms of gene regulation. Russo VEA, Martienssen RA, Riggs, editors. New York: Cold Spring Harbor Laboratory Press. p 1–4.
42. Bernstein BE, Meissner A, Lander ES. 2007. The mammalian epigenome. Cell 128:669–681.
43. Bird A. 2002. DNA methylation patterns and epigenetic memory. Genes Dev 16:6–21.
44. Ramsahoye BH, Biniszkiewicz D, Lyko F, Clark V, Bird AP, Jaenisch R. 2000. Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. Proc Natl Acad Sci USA 97:5237–5242.
45. Lande-Diner L, Cedar H. 2005. Silence of the genes—mechanisms of long-term repression. Nat Rev Genet 6:648–654.
46. Jaenisch R, Bird A. 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 33:245–254.
47. Jirtle RL, Skinner MK. 2007. Environmental epigenomics and disease susceptibility. Nat Rev Genet 8:253–262.
48. Rando OJ, Verstrepen KJ. 2007. Timescales of genetic and epigenetic inheritance. Cell 128:655–668.
49. Cropley JE, Suter CM, Beckman KB, Martin DI. 2006. Germ-line epigenetic modification of the murine A vy allele by nutritional supplementation. Proc Natl Acad Sci USA 103:17308–17312.

50. Reik W, Dean W, Walter J. 2001. Epigenetic reprogramming in mammalian development. Science 293:1089–1093.
51. Morgan HD, Sutherland HG, Martin DI, Whitelaw E. 1999. Epigenetic inheritance at the agouti locus in the mouse. Nat Genet 23:314–318.
52. Rakyan VK, Chong S, Champ ME, Cuthbert PC, Morgan HD, et al. 2003. Transgenerational inheritance of epigenetic states at the murine Axin(Fu) allele occurs after maternal and paternal transmission. Proc Natl Acad Sci USA 100:2538–2543.
53. Rassoulzadegan M, Grandjean V, Gounon P, Vincent S, Gillot I, Cuzin F. 2006. RNA-mediated non-mendelian inheritance of an epigenetic change in the mouse. Nature 441:469–474.
54. Hitchins MP, Wong JJ, Suthers G, Suter CM, Martin DI, et al. 2007. Inheritance of a cancer-associated MLH1 germ-line epimutation. N Engl J Med 356:697–705.
55. Whitelaw NC, Whitelaw E. 2006. How lifetimes shape epigenotype within and across generations. Hum Mol Genet 15(2):R131–137.
56. Ravelli GP, Stein ZA, Susser MW. 1976. Obesity in young men after famine exposure in utero and early infancy. N Engl J Med 295:349–353.
57. Dörner G, Staudt J. 1972. Comparative morphologic studies of hypothalamic differentiation in the rat and man. Endokrinologie 59:152–155.
58. Dörner G. 1974. Environment dependent brain organization and neuroendocrine, neurovegetative and neuronal behavioral functions. Prog Brain Res 41:221–237.
59. Lucas A. 1991. Programming by early nutrition in man. In: The childhood environment and adult disease. Bock GR, Whelan J, editors. Ciba Foundation: Chichester: Wiley. p 38–50.
60. McKay JA, Williams EA, Mathers JC. 2004. Folate and DNA methylation during in utero development and aging. Biochem Soc Trans 32:1006–1007.
61. Holness MJ, Sugden MC. 2006. Epigenetic regulation of metabolism in children born small for gestational age. Curr Opin Clin Nutr Metab Care 9:482–488.
62. Wu G, Bazer FW, Cudd TA, Meininger CJ, Spencer TE. 2004. Maternal nutrition and fetal development. J Nutr 134:2169–2172.
63. Burdge GC, Hanson MA, Slater-Jefferies JL, Lillycrop KA. 2007. Epigenetic regulation of transcription: a mechanism for inducing variations in phenotype (fetal programming) by differences in nutrition during early life? Br J Nutr 97:1036–1046.
64. Wells JC. 2007. The thrifty phenotype as an adaptive maternal effect. Biol Rev Camb Philos Soc 82:143–172.
65. Burdge GC, Slater-Jefferies J, Torrens C, Phillips ES, Hanson MA, Lillycrop KA. 2007. Dietary protein restriction of pregnant rats in the F0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F1 and F2 generations. Br J Nutr 97:435–439.
66. Simmons RA. 2007. Developmental origins of diabetes: the role of epigenetic mechanisms. Curr Opin Endocrinol Diabetes Obes 14:13–16.
67. Meaney MJ, Szyf M, Seckl JR. 2007. Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. Trends Mol Med 13:269–277.
68. Kuzawa CW. 2005. Fetal origins of developmental plasticity: are fetal cues reliable predictors of future nutritional environments? Am J Hum Biol 17:5–21.
69. Johannsen W. 1909. Elemente der Exakten Erblichkeitslehre. Jena: Gustav Fischer.
70. Jablonka E, Lamb ML. 1995. Epigenetic Inheritance and Evolution:. The Lamarckian Dimension: Oxford University Press.
71. Jablonka E, Lamb ML. 2005. Evolution in Four Dimensions: Genetic, Epigenetic, Behavioral, and Symbolic Variation in the History of Life. Cambridge, Massachusetts: MIT Press. 462 p.
72. Dubrova YE, Plumb M, Gutierrez B, Boulton E, Jeffreys AJ. 2000. Transgenerational mutation by radiation. Nature 405:37.
73. Morgan WF. 2003. Non-targeted and delayed effects of exposure to ionizing radiation: I. Radiation-induced genomic instability and bystander effects in vitro. Radiat Res 159:567–580.
74. Pogribny I, Raiche J, Slovack M, Kovalchuk O. 2004. Dose-dependence, sex- and tissue-specificity, and persistence of radiation-induced genomic DNA methylation changes. Biochem Biophys Res Commun 320:1253–1261.
75. Anway MD, Cupp AS, Uzumcu M, Skinner MK. 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308:1466–1469.
76. Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, et al. 2006. Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet 14:159–166.
77. Whitelaw E. 2006. Epigenetics: sins of the fathers, and their fathers. Eur J Hum Genet 14:131–132.
78. Crews D, Gore AC, Hsu TS, Dangleben NL, Spinetta M, et al. 2007. Transgenerational epigenetic imprints on mate preference. Proc Natl Acad Sci USA 104:5942–5946.
79. Richards EJ. 2006. Inherited epigenetic variation—revisiting soft inheritance. Nat Rev Genet 7:395–401.
80. Chang HS, Anway MD, Rekow SS, Skinner MK. 2006. Transgenerational epigenetic imprinting of the male germline by endocrine disruptor exposure during gonadal sex determination. Endocrinology 147:5524–5541.
81. Waterland RA, Jirtle RL. 2003. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 23:5293–5300.
82. Waterland RA, Dolinoy DC, Lin JR, Smith CA, Shi X, Tahiliani KG. 2006. Maternal methyl supplements increase offspring DNA methylation at Axin Fused. Genesis 44:401–406.
83. Holliday R. 1987. The inheritance of epigenetic defects. Science 238: 163–170.
84. Suter CM, Martin DI, Ward RL. 2004. Germline epimutation of MLH1 in individuals with multiple cancers. Nat Genet 36:497–501.
85. Laird CD. 1987. Proposed mechanism of inheritance and expression of the human fragile-X syndrome of mental retardation. Genetics 117:587–599.
86. Reik W. 1989. Genomic imprinting and genetic disorders in man. Trends Genet 5:331–336.
87. Nicholls RD, Knoll JH, Butler MG, Karam S, Lalande M. 1989. Genetic imprinting suggested by maternal heterodisomy in nondeletion Prader-Willi syndrome. Nature 342:281–285.
88. Whitelaw E, Martin DI. 2001. Retrotransposons as epigenetic mediators of phenotypic variation in mammals. Nat Genet 27:361–365.
89. Pembrey M. 1996. Imprinting and transgenerational modulation of gene expression; human growth as a model. Acta Genet Med Gemellol (Roma) 45:111–125.
90. Gallou-Kabani C, Junien C. 2005. Nutritional epigenomics of metabolic syndrome: new perspective against the epidemic. Diabetes 54:1899–1906.
91. Gluckman PD, Hanson MA, Beedle AS. 2007. Non-genomic transgenerational inheritance of disease risk. Bioessays 29:145–154.
92. Godfrey KM, Lillycrop KA, Burdge GC, Gluckman PD, Hanson MA. 2007. Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. Pediatr Res 61:5R–10R.
93. Sved J, Bird A. 1990. The expected equilibrium of the CpG dinucleotide in vertebrate genomes under a mutation model. Proc Natl Acad Sci USA 87:4692–4696.
94. Weber M, Hellmann I, Stadler MB, Ramos L, Paabo S, et al. 2007. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat Genet 39:457–466.
95. Friedman JM, Halaas JL. 1998. Leptin and the regulation of body weight in mammals. Nature 395:763–770.
96. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, et al. 1997. Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387:903–908.
97. Farooqi IS, Keogh JM, Kamath S, Jones S, Gibson WT, et al. 2001. Partial leptin deficiency and human adiposity. Nature 414:34–35.
98. Ravussin E, Pratley RE, Maffei M, Wang H, Friedman JM, Bennett PH, Bogardus C. 1997. Relatively low plasma leptin concentrations precede weight gain in Pima Indians. Nat Med 3:238–240.
99. Noer A, Sorensen AL, Boquest AC, Collas P. 2006. Stable CpG hypomethylation of adipogenic promoters in freshly isolated, cultured, and differentiated mesenchymal stem cells from adipose tissue. Mol Biol Cell 17:3543–3556.
100. Stöger R. 2006. In vivo methylation patterns of the Leptin promoter in human and mouse. Epigenetics 1:155–162.

101. Susser E, Neugebauer R, Hoek HW, Brown AS, Lin S, et al. 1996. Schizophrenia after prenatal famine. Further evidence. Arch Gen Psychiatry 53:25–31.
102. Susser ES, Lin SP. 1992. Schizophrenia after prenatal exposure to the Dutch Hunger Winter of 1944-1945. Arch Gen Psychiatry 49:983–988.
103. Lumey L, Stein AD, Kahn HS, van der Pal-de Bruin KM, Blauw G, et al. 2007. Cohort Profile: the Dutch Hunger Winter Families Study. Int J Epidemiol 36:1196–1204.
104. Khulan B, Thompson RF, Ye K, Fazzari MJ, Suzuki M, et al. 2006. Comparative isoschizomer profiling of cytosine methylation: the HELP assay. Genome Res 16:1046–1055.