Ned Tijdschr Klin Chem Labgeneesk 2006; 31: 187-193

Adaptation to the conditions of existence[1]

F.A.J. MUSKIET

'Typically Western' diseases derive from cultural trends that have changed our environment and by which we have pushed our conditions of existence beyond our evolutionary-established flexibility to adapt. Biochemical mechanisms by which our phenotype remains adapted to the environment include adjustment of DNA sequence and adjustments via epigenetics and endogenous sensors. Adjustment of DNA base sequence is a slow process by which a species remains adapted to its (changing) environment and from which eventually new species occur. Famine and microorganisms are among the principal environmental factors that have shaped and still shape our DNA base sequence. An example is the sickle cell gene that confers heterozygote survival advantage against malaria infection in countries like Kenya and Nigeria. There are several other mutations indicating that humans still evolve. Medium and short term adaptations occur by epigenetics, which is the study of changes in gene expression that are *not* accompanied by alterations of DNA sequence. The epigenome derives from DNA methylation and histone modification. Epigenetic marks are inherently labile and constitute the basis of cell differentiation and parent-of-origin specific effects via 'imprinting'. The epigenome also confers environmental instructions to the genome. These marks constitute a 'memory' by which information on the environment can potentially be transmitted to the next generation(s). Folate is one of many environmental factors that modulate phenotype by epigenetic mechanisms. Uniparental disomy and mutations in the epigenetic machinery are classical examples of epigenetic derangement. Some rare diseases derive from mutations, epigenetic derangements, or combination of these, affecting a single locus. A recent study linked epigenetic mechanisms to the hypothesis of the 'developmental origins of disease'. Mismatch between expected and actual environments may explain occurrence of typically Western diseases. Much progress has been made in the epigenetics of cancer. Tumorigenesis models with mutually-amplifying epigenetic disregulation and mutation have e.g. been postulated. Short term adaptation to the environment occurs by endogenous 'sensors'. For instance, peroxisome-proliferator activated receptors (PPARs) are nuclear transcription factors that function as lipid sensors. Upon activation by dietary fatty acids, they induce the coordinated expression and repression of proteins involved in intermediary metabolism, growth and differentiation, and inflammatory responses. Harmony between our millions of years old genome and environment may increase our numbers of years in health. For clinical chemistry, achievement of this state of 'optimal homeostasis' requires rethinking of the use of reference values and the use of algorithms for 'risk assessment' that enable early intervention. Also *Homo sapiens* needs to adapt to the conditions of existence on which its genome has been shaped during evolution.

Keywords: evolution, mutation, natural selection, epigenetics, developmental origin of disease, nuclear transcription factors, PPAR, reference values, risk assessment

'Adaptation to the conditions of existence' is the single most important outcome of evolutionary processes. It refers to morphological, physiological and behavioral characteristics that are genetic in nature and improve an organism's ability to survive and reproduce successfully under the prevailing environmental conditions. 'Natural selection' (Charles Darwin, 1859), also known as 'survival of the (evolutionary) fittest' (Herber Spencer, 1851), is the main driving force in evolution. Central to natural selection is 'environment', which refers to all factors that can influence an organism or a group of organisms. Environmental factors comprise external physical conditions (e.g. climate, diet, microorganisms) that affect and influence growth, development and survival, but also social and cultural conditions (e.g. stressful environment) that affect the nature of an individual or community. Biochemically, adaptation occurs by various mechanisms that differ in the rapidity by which they alter phenotype. Our genetic material serves as 'hardware' for translation to phenotype. It is however the environment that ultimately determines the base sequence of this hardware (i.e. by mutation and selection) and at least in part also how (by 'software') it

Pathology and Laboratory Medicine, University Medical Center Groningen

Correspondence: prof. dr. Frits A.J. Muskiet, CMC-V, Room Y 1.147, University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands
E-mail. f.a.j.muskiet@lc.umcg.nl

[1] Dit artikel en de drie volgende abstracts behoren bij het Wetenschappelijk symposium 'Epigenetica' gehouden op 12 april 2006 tijdens het NVKC Voorjaarscongres te Lunteren

becomes translated into our biochemical, physiological and morphological characteristics. After many years of interest in the 'hardware' (e.g. 'human genome project'), the medical community is now beginning to appreciate the importance of the 'software'. The rapidly increasing insights into the translation of genotype to phenotype, its plasticity and the influence of environment are exciting, take us right back to our origin as a species, and will revolutionize our thinking regarding the etiology, pathophysiology, treatment and (most importantly) prevention of disease.

Many, if not all, 'typically Western' diseases derive from cultural trends that have changed our environment and by which we have voluntarily pushed the conditions of existence beyond our evolutionary-established flexibility to adapt. Fortunately, the ensuing conflict between this new environment and our slowly adapting genome has not affected our evolutionary fitness. Witnessed by the rapid expansion of the world population, we have in the past centuries been able to eliminate many unfavorable 'conditions of existence' (mainly famine and infection), but have meanwhile introduced new, unfavorable, conditions that notably cause disease after reproductive age. Knowledge of the underlying mechanisms of the conflict might be the first step to its solution, with the ultimate goal to increase the number of years in health, rather than an increase of lifespan. This contribution addresses three (highly interconnected) biochemical mechanisms by which we adapt to our environment and speculates on their messages for clinical chemists.

Adaptation of base sequence

Adaptation by adjustment of DNA base sequence is probably the most widely known mechanism by which a species remains adapted to its (changing) environment and by which eventually new species occur. Ultimately, all adaptive mechanisms trace down to genetics. The evolution theory teaches us that the environment selects from the wide variety of existing genetic combinations and also from the new, spontaneously formed, mutations to confer evolutionary fitness to those that are adapted best. Famine and microorganisms, including viruses, bacteria and parasites, are among the most compelling environmental factors that have shaped and still shape our DNA base sequence. The resulting adjustments are, however, small. The molecular clock hypothesis postulates about 0.2% change in our nuclear DNA per million years ($1.7*10^{-9}$ substitutions/site, year) but may vary widely, dependent on (e.g. latitude-associated) mutation rates, gene flow, genetic drift and variable forces of selective pressure (i.e. natural selection, sexual partner selection, within species competition, competition with other species).

An example of parasite-orchestrated 'natural selection in action' might come from the sickle cell gene (or other hemoglobinopathy associated genes), which still spreads in malaria endemic countries. *In vitro* studies show that, at low oxygen tension, the malaria parasite grows poorly in HbS containing erythrocytes, while prospective studies reveal a heterozygote (HbAS) survival advantage during the first 5 years of postnatal life in countries like Kenya (1) (Figure 1) and Nigeria. Other examples showing that we still evolve are cystic fibrosis (resistance to tuberculosis, cholera?), familial hypercholesterolemia (Gram negative infection?), the 1,000 years old HbC gene (that is a 'private gene' of the Mossi in N-Ghana and confers resistance to malaria) and the 700 years old 32 base-pair deletion in the chemokine receptor CCR5 gene (small pocks?, HIV?) (2). The rapid unraveling of the evolutionary background of our genome stimulates rethinking of 'genetic disease' and appreciation of 'polymorphism' as an efficient manner to confer evolutionary advantage to certain heterozygous traits (a process also referred to as 'balancing selection'). Such conditions remind us that evolution is not necessarily elegant, since, if necessary, it takes the inevitable death of the homozygous mutant state for granted.

Adaptation by epigenetics

Medium (several generations) and short term adaptation may occur by epigenetic mechanisms. 'Epigenetics' (Dr. R. Feil) refers to the study of changes in gene expression that are *not* accompanied by alterations of DNA sequence. The major difference between genetics and epigenetics is the static nature of DNA as opposed to the dynamics of epigenetics. Mutation is basically irreversible while epigenetic modifications are inherently labile, although differences in stability do exist. Epigenetic mechanisms include reversible DNA modifications (notably CpG methylation), alterations in the protein constituents of chromatin (histones) and interaction of microRNAs with the genome. The resulting 'epigenome' ('epigenotype') constitutes not only the background of parent-of-origin specific effects (by imprinting during gametogenesis and embryogenesis) and the basis of cell differentiation (which is conserved during mitosis), but is also a reflection of the 'environmental instructions for the genome' aiming at phenotypic 'fine tuning'.

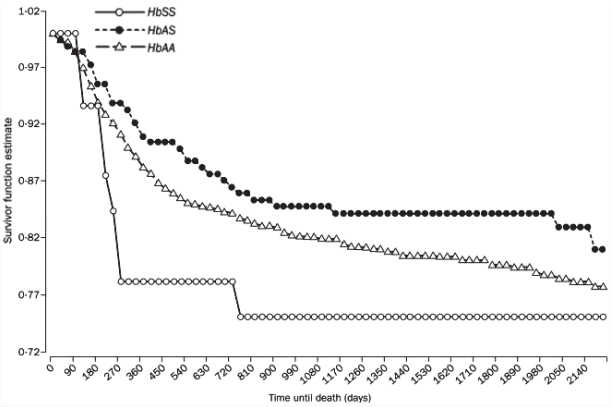

Figure 1. Natural selection acting on newborns with HbAA, HbAS and HbSS in Kenya (1). Widespread polymorphisms have proven to be evolutionary successes. In some cases, these 'adaptations to the conditions of existence' may turn into disadvantages by cultural trends that cause changes in environmental circumstances. Taking these circumstances for granted supports the qualification of these genes as 'disease susceptibility genes'. (Reprinted with permission from Elsevier.)

It has become clear that environmental changes may, via biological signaling and signal transduction systems, become translated into an altered epigenome and thereby into an adjusted phenotype. These pathways will greatly increase our understanding of environment-gene interactions and their outcomes in terms of health and disease. Although not as yet deciphered in epigenetic terms, it gains acceptance that, also in humans, maternally transmitted signals may cause the fetus to adapt to an expected postnatal environment. This environment may be either that of the mother, or constitute the mother herself (e.g. in maternal disease). Mismatches between this expected environment and the actual environment are likely to be at the basis of the 'fetal origins of disease hypothesis' (prof. dr. M.A. Hanson). This hypothesis, also known as the 'Barker hypothesis' and 'developmental origins of disease', was initially based on the observed relation between low birth weight and cardiovascular disease at adult age. Low birth weight is a proxy for intrauterine under- or malnourishment, but also short disruptions during the various sensitive periods of development may initiate a process of 'programming' that lead to phenotypic adjustments that are not necessary for immediate survival, but in expectation of a certain environment at postnatal life.

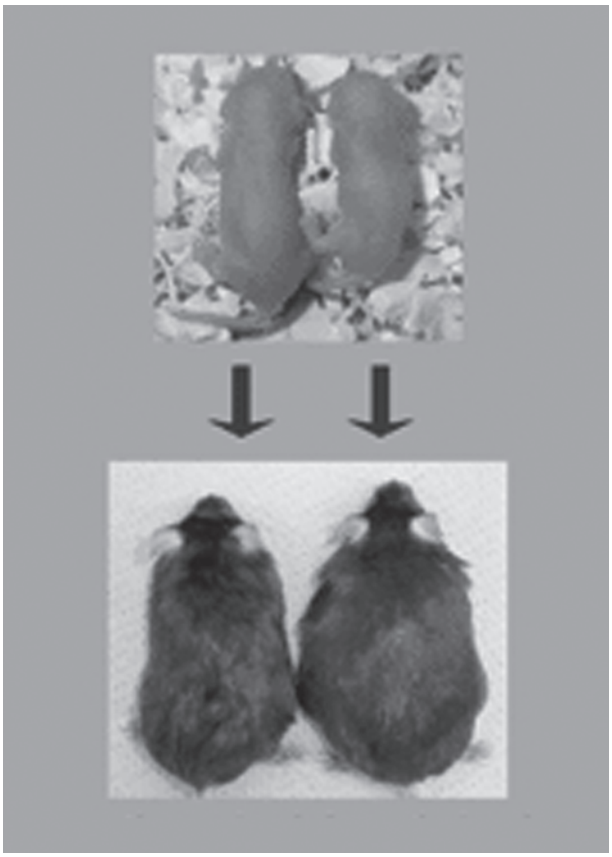

Figure 2. The low birth weight pup of the undernourished mother develops obesity on a postnatal high fat diet (5). Low birth weight is a proxy for the quality of intrauterine nutrition. Undernourished and malnourished fetuses are likely to be 'programmed'. Poor maternal macronutrient supply in Western pregnancy is rare. Suboptimal placental function, micronutrient deficiencies and disbalanced maternal diets may cause 'programming' that becomes not necessarily reflected by low birth weight. (Reprinted with permission from Elsevier.)

At present it is recognized that these 'programmed' babies raised in a mismatched (affluent) society have high risk of *many* conditions and diseases, such as the metabolic syndrome, coronary heart disease, diabetes mellitus type 2, schizophrenia and a variety of others at later life (3, 4). The hypothesis is supported by animal models that (e.g.) show that it is the pup of the undernourished mother during pregnancy and lactation that develops obesity at postnatal life when fed a high fat diet after weaning (Figure 2) (5).

Epigenetic marks (i.e. phenotypic characteristics) may be transmitted to future generations, since it is becoming clear that not all of these are necessarily erased during meiosis. This process is likely to confer 'transgenerational memory' regarding the prevailing environmental conditions (e.g. availability of nutrition, stressful environment) as experienced by previous generations with the evolutionary advantage to conserve reproductive ability, and not necessarily 'health' or longevity. A person's vulnerability to disease may consequently start as early as prior to conception and even as early as during oogenesis in the uterus of the maternal grandmother. It also implies that 'susceptibility to disease' does not necessarily originate from genetically demonstrable changes and that phenotype may be heritable, at least to some extent. Of importance is in this context to reemphasize that epigenetic marks are inherently unstable and that this type of phenotype inheritance should not be taken as support for Lamarck's theory of 'directed evolution through inheritance of acquired characteristics' (6).

As all mechanisms also those in epigenetics are sensitive to mistakes. The classical example comes from uniparental disomy (UPD), i.e. inheritance of 2 chromosomes from one parent and none of the other. UPD may, dependent on whether it derives from the mother or father, cause overexpression or underexpression of imprinted genes. Examples are the Angelman, Prader-Willi and Beckwith-Wiedemann syndromes (dr. R. Feil). Interestingly, each of these conditions may derive from classical mutation, epigenetic derangement or a mixture of mutation and epigenetic derangement affecting a single locus. Other classical examples are mutations in components of the epigenetic machinery, such as the Rett syndrome. This syndrome derives from mutations in a methyl-CpG-binding protein (MeCP2), which under normal conditions binds to single methylated CpG sites in the genome. Much progress has been made in the epigenetics of cancer. Silencing of tumor suppressor genes by methylation, activation of growth genes by demethylation and other epigenetic aberrations, such as genome-destabilizing global demethylation, are emerging as important causes of non-inheritable cancer that affects somatic cells (7). Both epigenetic disregulation and mutation, acting either as first or second 'hits', are envisioned to play mutually amplifying roles in tumorigenesis (dr. E. Ballestar).

Folate, and probably one-carbon metabolism in general, is intimately involved in epigenetic modulation of gene expression and thereby establishment of phenotype. For example, supplementation of female

mice with extra folic acid, vitamin B_{12}, choline and betaine from 2 weeks prior to conception until weaning induced augmented methylation of a retrotransposon within the so called 'agouti-gene', which is a gene that determines the color of their coat. The intervention (partially) silenced the agouti gene by methylation and thereby caused the coat color of the offspring to shift permanently from yellowish into the brownish (pseudo-agouti) phenotype, while there was also evidence of transgenerational transmission (8). A recent study shows that inheritance occurs via the maternal allele, but that it is not based on maintenance of DNA methylation (9). Hyperhomocysteinemic patients on hemodialysis showed global and locus-specific DNA hypomethylation. Subsequent folic acid supplementation augmented both global and locus-specific DNA-methylation, as witnesses from the switch of abnormal biallelic expression to normal monoallelic expression for a number of genes with known sensitivity to methylation (10). These studies illustrate that dietary components may have direct effects on the expression/repression of genes through epigenetic mechanisms, that the acquired metastable marks may produce life-long echoes (11) and that these marks may potentially become transmitted to the next generation(s).

Adaptation through 'endogenous sensors'

A third, rapidly acting, mechanism of adaptation is by interaction of the environment with proteins which in our body serve roles as intermediates between the environment and our genome with the aim to directly or indirectly alter gene expression. Examples are peroxisome proliferator activated receptors (PPARs) and sterol regulatory element binding proteins (SREBPs). These are nuclear transcription factors that can be considered as main switches in the coordinated expression and repression of a variety of (key) enzymes in intermediary metabolism, thermoregulation, energy partitioning, growth and differentiation, and also inflammatory responses (Figure 3) (12). Fatty acids, notably polyunsaturated fatty acids and their eicosanoid metabolites, are among the most powerful naturally occurring PPAR ligands. Like steroid hormone receptors, ligand-activated PPARs bind to specific sequences in the promoter regions of the target genes (named PPAR response elements) to activate transcription upon binding of their respective ligands. PPARs, but also the vitamin D receptor (VDR), bind to DNA as heterodimers with the retinoid X receptor (RXR) (13). This lays emphasis on the dependence of a healthy phenotype on certain dietary (i.e. environmental) constituents (fatty acids, vitamin A, vitamin D), the balance between dietary constituents (e.g. fatty acids/vitamin D, vitamin D/A) and on non-dietary environmental influences like sunlight (vitamin D).

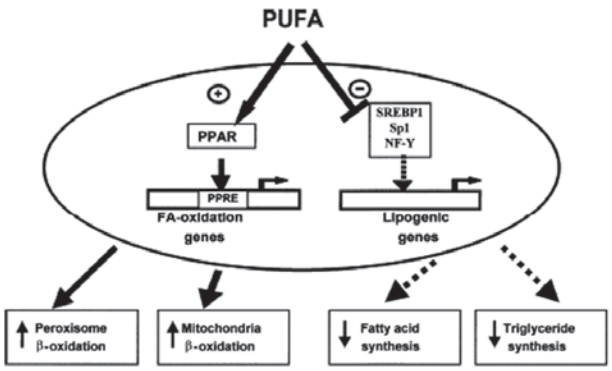

Figure 3. PPARs and SREBPs are examples of the many endogenous sensors by which we 'fine regulate' our phenotype to the prevailing environmental circumstances (12). Following their binding to PPARs, dietary constituents (i.e. notably fatty acids) may cause coordinated expression of many genes within a certain metabolic pathway (e.g. fatty acid beta-oxidation) and the expression of genes with widely differing function (e.g. intermediary metabolism, inflammation, growth) FA, fatty acids; NF-Y, nuclear factor Y; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator-activated receptor response element; PUFA, polyunsaturated fatty acids; Sp1, stimulatory protein 1; SREBP-1, sterol regulatory element binding protein-1; TG, triglycerides.

PPAR-alpha and the glucocorticoid receptor are among the fetal sensors that are under epigenetic control. They have recently been implicated in the 'developmental origins of disease' hypothesis (Prof. Dr. M.A. Hanson). Dietary protein restriction of pregnant rats reduces fetal growth and, in the fetal liver, causes diminished DNA methylation and concomitant activation of the genes for PPAR-alpha and the glucocorticoid receptor. The observed changes proved persistent up to at least 6 days after weaning and could be prevented by fortification of the protein-restricted diet with folic acid (14). These studies suggest the need of balance between dietary constituents such as certain fatty acids, vitamin A and those involved in one-carbon metabolism (e.g. folate). PPARs are targeted by drugs to lower serum triglycerides (fibrates: PPAR-alpha) and to improve glucose homeostasis (thiazolidinediones: PPAR-gamma). However, their natural ligands are fatty acids, notably fish oil fatty acids, and their eicosanoid metabolites, of which we have low intakes via our current, typically Western, diet. Fish oil reduces serum triglycerides, while studies with fish oil fatty acids (i.e. notably EPA and DHA) in rats revealed that in brain they modulate the expression and repression of a sizeable number of genes that are involved in structure, energy metabolism, neurotransmission, signal transduction and regulation (15). Clearly, the outcome of research on the pathophysiology of Western disease provides us with information on the environmental conditions at which our genome functions best. Ironically however, these conditions might have been predicted, since the evolution theory teaches us that these conditions are equal to those at which our genome has evolved.

Importance for clinical chemistry

Why is this important to clinical chemists? The first is to realize that purely genetic disease is rare, that many papers open with the statement that disease derives from interaction of genetic susceptibility (certain polymorphisms) and environment, but that there is nothing wrong with our genome and that the majority of common diseases are likely to derive from (changes of) environment *per se*. None of the

many mutations and polymorphisms that are implicated in cardiovascular disease (e.g. FH, apo-E, MTHFR) confer sufficient absolute risk to explain its epidemiology and this applies to the other intensely studied Western diseases as well. 'Genetic susceptibility to disease' is a forgery of history from an evolutionary point of view, and its acceptance as 'a biological fact' in notably public health might distract from the great influence of environment. 'Environmental susceptibility to disease' seems more appropriate since there was first an environment and then came a perfectly adapted, but largely misunderstood, polymorphic gene. Obviously, we do not blame our genes for the inability to cope with the ingestion of concentrated hydrochloric acid, but somehow fail to appreciate the similarity with the consumption of saturated fat, or with low vitamin D status, probably because the devastating effects of many environmental influences become obscured by the long period after which disease ensues. It is still the dose that makes the poison or causes deficiency, and any species will eventually get sick, or even become extinct, by voluntarily pushing the conditions of existence beyond its genetic flexibility to adapt.

The second is that we are in the business of classification into health and disease and in this capacity aware of the subclinical interface between these entities. Clearly, it might not be of high practical value to communicate to clinicians that the sickle cell gene is an evolutionary success, or that the LDL-receptor defects that cause familial hypercholesterolemia have become widespread because of heterozygote advantage. Another subject is however the issuing of reference values that are remote from our evolutionary determined homeostatic setpoint reflecting optimal interaction between environment and our genome. 'There is nothing wrong' since the clinical chemical and hematological profiles comply with Western references is not very reassuring if we observe the epidemiology of Western disease and take both primary and secondary prevention seriously.

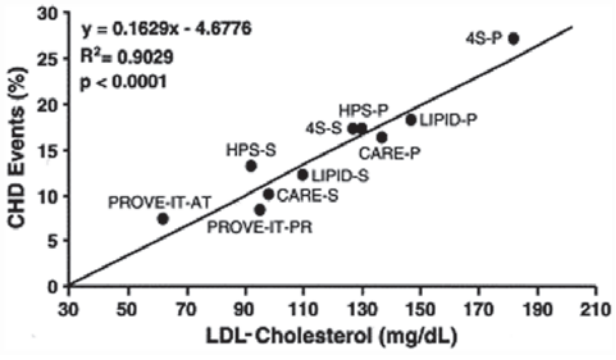

Figure 4. Secondary prevention trials with statins indicate lowest coronary heart disease risk at LDL-cholesterol levels of 50-70 mg/dL (1.3-1.8 mmol/L) (16). The current target values for lipid lowering therapy are identical to those encountered in hunter-gatherer populations with no deaths from cardiovascular disease. These data emphasize the importance of the environment (diet) on which our genes have evolved during the past 2.5 million years of evolution. (Reprinted with permission from Elsevier.)

Examples of the latter may derive from cholesterol, 25-hydroxyvitamin D and folate. Primary and secondary (Figure 4) (16) prevention trials with statins indicate lowest coronary heart disease risk at an LDL-cholesterol of 1.3-1.8 mmol/L (50-70 mg/dL), which is consistent with levels encountered in primates in the wild and hunter-gatherer populations with no deaths from cardiovascular disease. The issuing of a <4.7 mmol/L reference for LDL-cholesterol does not add to the prevention of a disease that in our country accounts for about 38% of annual deaths. Why would we advice to increase calcium intake when vitamin D status is within the reference range of apparently healthy subjects living in a country that ranks in the highest regions regarding both dietary calcium intake and osteoporosis prevalence? Recent trials showing no effect of vitamin D supplementation in osteoporosis (17-19) have been conducted with too low dosages (400 IU/day) (17, 19) or did not reach sufficiently high 25-hydroxyvitamin D levels by using a 800 IU/day dose (18). Dependent on baseline values, a 1,200-2,200 IU/day dose is needed (20, 21) to reach the estimated 75-80 nmol/L minimum serum 25-hydroxyvitamin D level that is required for maximum parathyroid hormone suppression, maximum calcium absorption, and fracture prevention (20-22). The vitamin D receptor is located in almost all tissues studied so far, and 75-80 nmol/L is still below the levels encountered in primates in the wild and traditionally living hunter-gatherers (23, 24). Finally, it does not seem justified to lean back now that the first randomized controlled trials (RCTs) do not show causal relationships between folate and cardiovascular disease in secondary prevention trials (25, 26). From an epidemiological point of view, a 4-30 nmol/L serum folate reference range does not seem appropriate in a country where 90% of the population does not reach the 300 μg/day Dutch folate recommended dietary allowance and 70% of the women do not reach the 200 μg average folate need (27). Moreover, the 300 μg/day Dutch folate recommendation is at least 100 μg short of the recommendations issued by most other countries. The mere derivation of evidence from RCTs (and preferably RCT meta-analyses) is not a synonym for compliance to the rules of Evidence Based Medicine (28), consequently also not to the rules of Evidence Based Laboratory Medicine, and by all means devoid of insight into the biological game of life that aims at adaptation to the conditions of existence.

Conclusions

Knowledge of the molecular bases that govern the interaction between environment and our genome, such as notably deriving from DNA base sequence, epigenetics and endogenous sensors, is important to the solution of many (if not all) typically Western diseases. The present conflict between environment and genome might be settled in healthcare, notably by massive treatment with drugs like statins, thiazolidinediones, bisphosphonates and the many for obesity that are currently on the shelves of pharmaceutical companies. Prevention seems, however, a

more attractive and certainly less costly option. Clinical chemists might contribute by supporting the correct interpretation of laboratory data. Central theme in this interpretation might be 'optimal homeostasis', which requires rethinking of the sense of 'reference values' and research on parameters and algorithms that support appropriate 'risk assessment'. *Homo sapiens* is part of nature and like all living organisms, also *homo sapiens* needs to adapt to the conditions of existence on which its genome has been shaped during the past 2.5 million years of evolution.

Literature

1. Aidoo M, Terlouw DJ, Kolczak MS, McElroy PD, Kuile FO ter, Kariuki S, Nahlen BL, Lal AA, Udhayakumar V. Protective effects of the sickle cell gene against malaria morbidity and mortality. Lancet 2002; 359: 1311-1312.
2. Balter M. Evolutionary genetics. Are humans still evolving? Science 2005; 309: 234-237.
3. Godfrey KM, Barker DJ. Fetal nutrition and adult disease. Am J Clin Nutr 2000; 71 (5 Suppl): 1344S-1352S.
4. Hanson MA, Gluckman PD. Developmental processes and the induction of cardiovascular function: conceptual aspects. J Physiol 2005; 565: 27-34.
5. Yura S, Itoh H, Sagawa N, Yamamoto H, Masuzaki H, Nakao K, Kawamura M, Takemura M, Kakui K, Ogawa Y, Fujii S. Role of premature leptin surge in obesity resulting from intrauterine undernutrition. Cell Metab 2005; 1: 371-378.
6. Balter M. Genetics. Was Lamarck just a little bit right? Science 2000; 288: 38.
7. Esteller M. Aberrant DNA methylation as a cancer-inducing mechanism. Annu Rev Pharmacol Toxicol 2005; 45: 629-656.
8. Waterland RA, Jirtle RL. Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. Nutrition 2004; 20: 63-68.
9. Blewitt ME, Vickaryous NK, Paldi A, Koseki H, Whitelaw E. Dynamic reprogramming of DNA methylation at an epigenetically sensitive allele in mice. PLoS Genet 2006; 2: e49.
10. Ingrosso D, Cimmino A, Perna AF, Masella L, De Santo NG, De Bonis ML, et al. Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia. Lancet 2003; 361: 1693-1699.
11. Gluckman PD, Hanson MA, Morton SM, Pinal CS. Life-long echoes - a critical analysis of the developmental origins of adult disease model. Biol Neonate 2005; 87: 127-139.
12. Clarke SD, Gasperikova D, Nelson C, Lapillonne A, Heird WC. Fatty acid regulation of gene expression: a genomic explanation for the benefits of the mediterranean diet. Ann N Y Acad Sci 2002; 967: 283-298.
13. Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. Nature 2000; 405: 421-424.
14. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. J Nutr 2005; 135: 1382-1386.
15. Kitajka K, Puskas LG, Zvara A, Hackler L Jr, Barcelo-Coblijn G, Yeo YK, Farkas T. The role of n-3 polyunsaturated fatty acids in brain: modulation of rat brain gene expression by dietary n-3 fatty acids. Proc Natl Acad Sci USA 2002; 99: 2619-2624.
16. O'Keefe JH Jr, Cordain L, Harris WH, Moe RM, Vogel R. Optimal low-density lipoprotein is 50 to 70 mg/dl: lower is better and physiologically normal. J Am Coll Cardiol 2004; 43: 2142-2146.
17. Grant AM, Avenell A, Campbell MK, McDonald AM, MacLennan GS, McPherson GC, et al.; RECORD Trial Group. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. Lancet 2005; 365: 1621-1628.
18. Porthouse J, Cockayne S, King C, Saxon L, Steele E, Aspray T, et al. Randomised controlled trial of calcium and supplementation with cholecalciferol (vitamin D3) for prevention of fractures in primary care. BMJ 2005; 330: 1003.
19. Jackson RD, LaCroix AZ, Gass M, Wallace RB, Robbins J, Lewis CE, et al; Women's Health Initiative Investigators. Calcium plus vitamin D supplementation and the risk of fractures. N Engl J Med 2006; 354: 669-683.
20. Heaney RP. The Vitamin D requirement in health and disease. J Steroid Biochem Mol Biol 2005; 97: 13-19.
21. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int 2005; 16: 713-716.
22. Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA 2005; 293: 2257-2264.
23. Vieth R. Why the optimal requirement for Vitamin D3 is probably much higher than what is officially recommended for adults. J Steroid Biochem Mol Biol 2004; 89-90: 575-579.
24. Muskiet FAJ. Evolutionaire geneeskunde. U bent wat u eet maar u moet weer worden wat u at. Ned Tijdschr Klin Chem Labgeneesk 2005; 30: 163-184.
25. Lonn E, Yusuf S, Arnold MJ, Sheridan P, Mc-Queen MJ, Pogue J, Probstfield J, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. N Eng J Med 2006; 354: 1-11.
26. Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, Wang H, Nordrehaug JE, Arnesen E, Rasmussen K. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med 2006; 354: 1578-1588.
27. RIVM report 350010001/2004. Methode voor schatting van de prevalentie van inadequate innemingen van micronutriënten. (http://www.rivm.nl/bibliotheek/rapporten/350010001.pdf).
28. Sackett DL, Straus SE, Scott Richardson W, Rosenberg W, Haynes RB. Evidence based medicine. How to practice and teach EBM. Churchill Livingstone, Edinburgh, 2000, 2nd ed, pp 261.

Samenvatting

Aanpassing aan de leefomstandigheden. Muskiet FAJ. Ned Tijdschr Klin Chem Labgeneesk 2006; 31: 187-193.

'Typisch Westerse' ziekten komen voort uit culturele trends die onze leefomgeving hebben veranderd en waarmee we onze leefomstandigheden voorbij onze evolutionair bepaalde flexibiliteit hebben getrokken. Biochemische mechanismen waarmee ons fenotype aangepast blijft aan de omgeving omvatten aanpassing van de DNA-volgorde en aanpassingen via de epigenetica en endogene sensoren. Aanpassing van onze DNA-basevolgorde is een langzaam proces waarmee een soort aangepast blijft aan de (veranderende) omgeving en waaruit uiteindelijk nieuwe soorten ontstaan. Hongersnoden en micro-organismen behoren tot de belangrijkste omgevingsfactoren die onze DNA-basevolgorde vorm hebben gegeven en nog steeds geven. Een voorbeeld is het sikkelcelgen dat in landen zoals Kenia en Nigeria een heterozygootvoordeel met zich meebrengt voor het overleven van malaria-infectie. Er zijn diverse andere mutaties die aangeven dat mensen nog steeds evolueren. Middellange en kortetermijnaanpassingen geschieden door middel van de epigenetica: veranderingen in genexpressie die *niet* gepaard gaan met veranderingen in DNA-volgorde. Het

epigenoom komt tot stand door middel van DNA-methylering en histonmodificatie. Epigenetische merktekens zijn inherent labiel en vormen de basis van celdifferentiatie en ouder-specifieke effecten door middel van 'imprinting'. Het epigenoom geeft ook instructies door vanuit de omgeving naar het genoom. Deze merktekens vormen een 'geheugen' waarmee gegevens van de omgeving potentieel kunnen worden overgedragen op de volgende generatie(s). Folaat is één van de vele omgevingsfactoren die het fenotype moduleren door middel van epigenetische mechanismen. Het erven van twee chromosomen van één ouder en geen van de ander, en mutaties in de epigenetische machinerie zijn klassieke voorbeelden van epigenetische ontsporing. Enkele zeldzame ziektes ontstaan door mutaties, epigenetische ontsporingen of combinatie hiervan, die een enkele locus aantasten. Een recente studie legde een verband tussen epigenetische mechanismen en de hypothese van de 'ontwikkelingsoorsprong van ziekte'. Een disbalans tussen de verwachte en werkelijke omgeving kan het ontstaan van typisch Westerse ziekten verklaren. Veel vooruitgang is geboekt in de epigenetica van kanker. Tumorgenesemodellen met elkaar wederzijds versterkende epigenetische disregulatie en mutatie zijn o.a. gepostuleerd. De kortetermijnaanpassing aan de omgeving geschiedt door middel van 'sensoren'. Zo zijn peroxisoom-proliferator geactiveerde receptoren (PPARs) bijvoorbeeld nucleaire transcriptiefactoren die functioneren als sensoren voor vetten. Indien geactiveerd door vetzuren uit de voeding, induceren ze de gecoördineerde expressie en repressie van eiwitten die betrokken zijn in het intermediair metabolisme, groei en differentiatie, en ontstekingsreacties. Harmonie tussen ons miljoenen jaren oude genoom en de omgeving kan het aantal jaren in gezondheid doen toenemen. Voor de klinische chemie vereist het bereiken van deze toestand van 'optimale homeostase' een heroverweging van het gebruik van referentiewaarden en het gebruik van algoritmen die een vroege interventie mogelijk maken. Ook *Homo sapiens* dient zich aan te passen aan de leefomstandigheden gedurende welke haar genoom tijdens de evolutie vorm heeft gekregen.

Ned Tijdschr Klin Chem Labgeneesk 2006; 31: 193-194

Fundamentals of epigenetics and its relevance to human disease

R. FEIL

The term 'epigenetic' is used to refer to heritable patterns of gene expression that occur without changes in the DNA sequence. Epigenetic regulation plays an important role in animal and plant development, and is required in particular to achieve stable repression of genes in specific cell types and at defined developmental stages (1). In mammals, there are many examples of epigenetic inactivation of one of the two alleles of genes. These include X-chromosome inactivation in female somatic cells (2), and imprinted genes, a subset of about a group of essential genes whose expression depends on whether they are inherited from the mother or the father (3).

The allelic repression of imprinted genes depends on epigenetic marks, the imprints, which are placed onto the gene upon passage through either the female or the male germ line (3). Some eighty imprinted genes have been identified in humans and mice to date. These rather unusual genes are clustered in the genome. They are organized in evolutionarily conserved chromosomal domains, some of which are more than a thousand kilobases in size. Mouse studies have shown for several imprinted genes, including the insulin-like growth factor 2 (*IGF2*) gene, that they play key roles in the regulation of growth and cellular proliferation, both in the embryo and in the extra-embryonic lineages. Deregulation of the epigenetic marks that regulate imprinted genes is causally involved in different disease syndromes of aberrant growth, such as the Silver-Russell Syndrome (SRS), Beckwith-Wiedemann Syndrome (BWS), and Transient Neonatal Diabetes Mellitus (TNDM) (4). The somatic maintenance of imprinting is frequently perturbed in cancer as well, and is thought to be an early event in tumourigenesis.

The somatic maintenance of imprinting is a highly complex process that involves DNA methylation, covalent histone modifications, and recruitment of non-histone proteins to the chromatin (3). Different kinds of environmental stress, including embryo culture and assisted reproduction technologies, may perturb the maintenance of these epigenetic marks (5, 6). Also dietary changes may disrupt the maintenance of epigenetic marks, and can thus heritably affect gene expression (6). Recent studies in the mouse suggest that genomic imprinting is particularly susceptible to perturbation in the early embryonic cells that give rise to the extra-embryonic tissues. Possibly, this could be explained by the finding that, in contrast to the embryo and the adult animal, imprinting of certain genes in the placenta does not require DNA methylation for its somatic maintenance (7, 8). Our current studies address to which extent this kind of imprinting is evolutionarily conserved in humans, and could contributes to imprinting disorders such as the Silver-Russell and Beckwith-Wiedemann Syndromes (9).

Institute of Molecular Genetics, Montpellier, France

Correspondence: dr. Robert Feil, Institute of Molecular Genetics, CNRS, UMR-5535, 1919 route de Mende, 34293 Montpellier cedex 5, France
E-mail: robert.feil@igmm.cnrs.fr