

INAUGURAL LECTURE

of

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Energy: a prime biological driver of life and death



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1. Introduction

The energy used by living systems is essentially derived from solar energy. At any moment, $\sim 2 \times 10^{17}$ W of solar energy strikes the earth. Of this, a third is reflected and, along with a relatively small contribution of energy derived from oceanic convection and conduction, is assimilated in the biosphere by photosynthetic organisms to produce the essential organic molecules utilized by final consumers (animals and humans). As shall be argued, the production of biological (chemical) energy and the way production can be affected are fundamental to human health, disease and ageing. This review will focus on research into the involvement of energy metabolism in non-communicable human diseases. Inherited mitochondrial disorders will provide a model to understanding the cellular processes and genetics.

2. Bioenergetics: concepts and turnover

The term “bioenergetics” originally refers to the study of the energy-conversion processes across the mitochondrial inner membrane, cytoplasmic membranes of bacteria and the photosynthetic thylakoid membranes found in the chloroplasts of plants¹. From this, it is already apparent that the pathways of energy transduction in living organisms rely on energy-conserving *membranes*, which use ion gradients across them to generate proton motive force (Δp). Proton motive force in turn can be used in several functional ways, as summarized in Figure 1. Proton motive force is mainly applied to generate adenosine triphosphate (ATP), which is the dominant currency of energy in cells. This chemiosmotic process, coupled to phosphorylation of ADP to form ATP, is called oxidative phosphorylation (OXPHOS). OXPHOS was uncovered by Peter Mitchell in 1961, who subsequently received the Nobel Prize in Chemistry (1978) for this discovery².

The numbers reflecting the daily production and consumption (turnover) of ATP in humans are astounding. The average human ATP turnover is 100 to 150 moles per day, which is equivalent to a mass of 50 to 75 kg of ATP ($\sim 4\,700$ kJ). As the total amount of ATP available in the average human body is only 0.1 moles, this pool has to be recycled 1 000 to 1 500 times per day. Furthermore, from

a daily nutritional intake of 8 700 kJ (~2 000 nutritional calories), a power equivalent of 100 W is the result. This is comparable to an incandescent household light bulb burning constantly. From these numbers, it should be evident that chemical energy production is a vital anabolic activity, used to drive all catabolic processes. As a result, any divergence of these important processes should indeed have severe consequences. Notwithstanding this, and the fact that humans are genetically adapted to a specific energetic milieu as shall be discussed later, cells can adapt and protect remarkably well during an energy crisis, mostly preventing cell death.

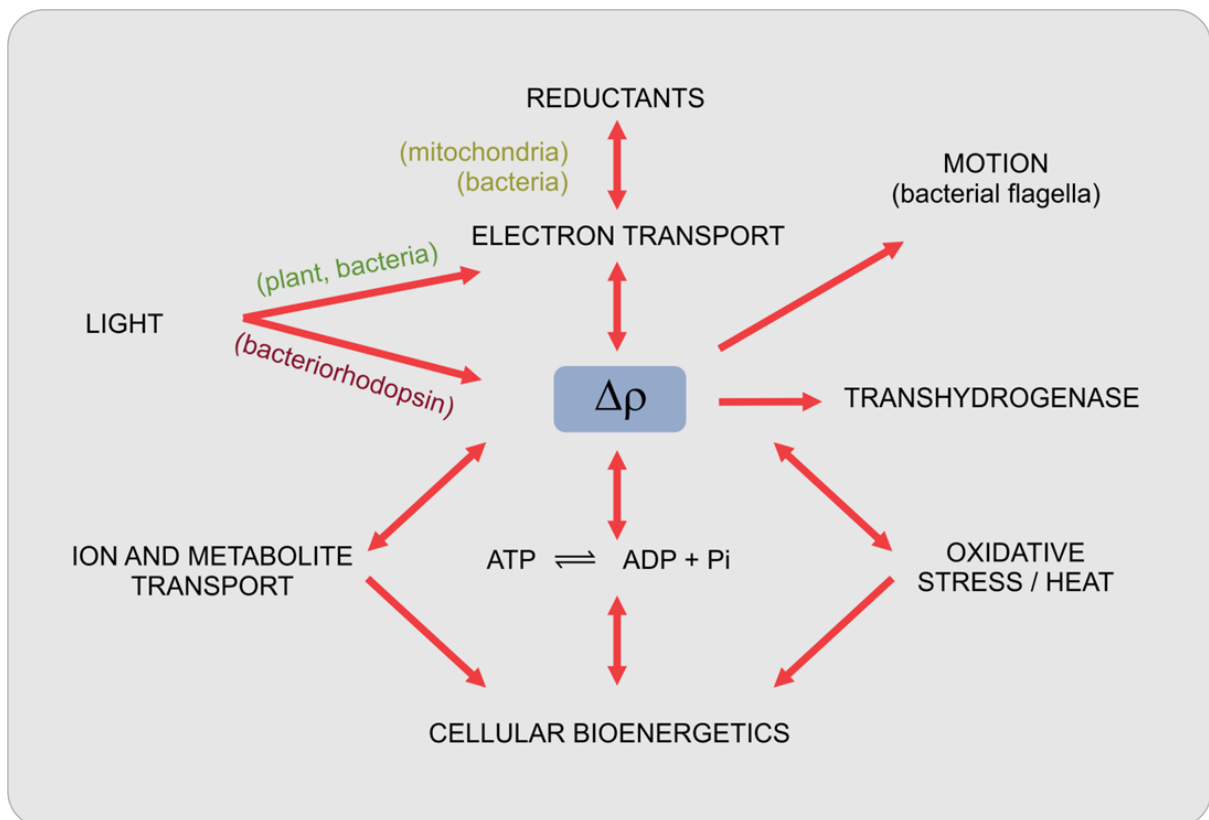


Figure 1. Proton motive forces (Δp) are central in bioenergetics pathways.

The pathways leading to the formation and the usages of proton motive force are indicated by red arrows (adapted from Nicholls and Ferguson¹).

3. The central role of mitochondrial bioenergetics in health, disease and ageing

Mitochondria are the organelles in eukaryotic cells which are primarily involved in energy metabolism. Although mitochondria are highly diverse in its role in the cell, some key features can be highlighted. Firstly, as mentioned before, the inner mitochondrial membrane functions as a capacitor, responsible for generating proton motive force required to generate ATP via the OXPHOS

system. The OXPHOS system consists of five enzyme complexes (complexes I to V) and is the entry point for reducing equivalents from carbohydrates, fats and proteins. By using these substrates and oxygen, it produces approximately 90% of the bodies' energy. Proton motive force not only results in ATP generation but, as shown in Figure 1, it is also used to transport ions, generate heat via uncoupling of the membrane, and produce reactive oxygen species (ROS) as a consequence of ineffective electron transfer through the membrane (electron leaking). Secondly, mitochondria contain their own small circular and independent genome in multiple copies, which is strictly maternally inherited³. From this genome (illustrated in Figure 2), only 13 of the estimated 1 500 mitochondrial proteins are produced⁴. Thirdly, mitochondria contain the elements for many other metabolic functions, such as cell signalling, calcium housekeeping, haeme and steroid synthesis, redox regulation and, importantly, programmed cell death (apoptosis).

The discovery in recent decades of the involvement of mitochondria in diseases results from a better *understanding of the combined roles* of mitochondria, in particular those of energy transduction, redox regulation, signalling and cell death. Mitochondrial involvement in neurodegenerative diseases⁵, cardiac diseases⁶, cancer⁷, diabetes⁸, HIV/AIDS and its treatments⁹, mitochondrial diseases¹⁰ and ageing¹¹ has now been well documented in scientific literature. In fact, mitochondrial dysfunction has been implicated, either in a primary or secondary way, in four of the ten most common causes of death world-wide¹².

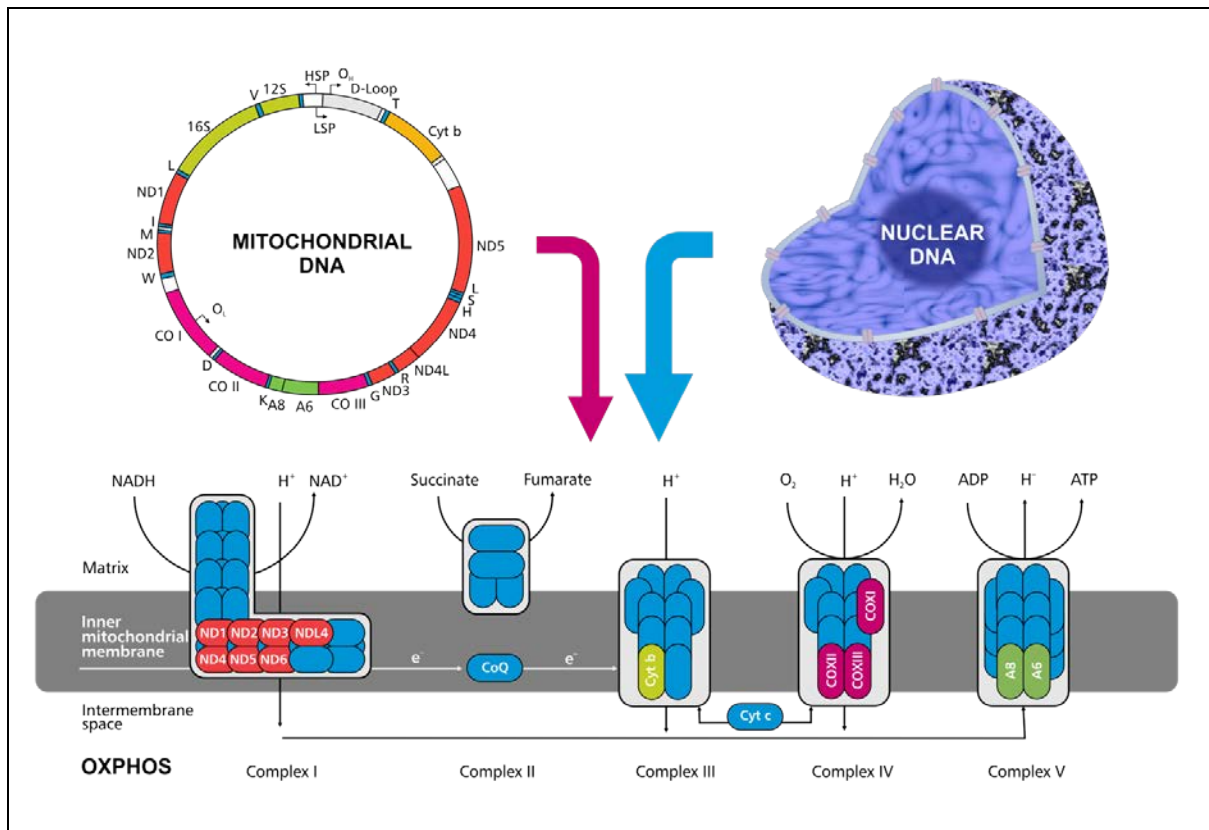


Figure 2. Bi-genomic expression of the mitochondrial OXPHOS system.

The OXPHOS system with the main reactions involved to finally produce ATP is indicated at the bottom. The mitochondrial genome and the 13 OXPHOS subunits encoded by this genome (colour-coded) are indicated at the top left. The remaining 76 genes of the OXPHOS system, as well as all other mitochondrial proteins are encoded by the nuclear genome (blue).

4. Cellular responses to energy crises: death, differentiation or adaptation

The complex system of mitochondrial energy production and the associated pathways which can accompany proton motive force (i.e. ROS production and uncoupling), are dependent on the energy sources that drive this process. An under-supply, or over-supply, or unbalanced source all can impact significantly on the products of this system, leading to a wide range of possible signalling pathways. A good example is the cellular consequences of perpetual over-supply of energy sources, which initiates several metabolic and signalling pathways leading to insulin resistance and diabetes mellitus type 2⁸. From studying the cellular consequences in diseases associated with energy dysfunction, it is clear that cells, depending on the tissues affected, can in fact respond differently to an energy crisis. Mitochondria can initiate *cell death* through a cascade of signals, which is dependent on energy status, often as a result of insufficient ATP production and increased and uncontrollable ROS production (oxidative stress). Alternatively, as in cancers, malignant transformation of cells (*differentiation*) is associated with an increased aerobic glycolysis and the conversion of glucose into

lactate (called the Warburg effect), which is in contrast to normal differentiated cells which predominantly rely on OXPHOS to generate ATP. However, an energy crisis can also result in *adaptive responses* which are foremost an effort to recover. As an example of this, our research group and others have shown that primary fibroblasts with a pathogenic mutation in complex I, the first and most crucial enzyme of the OXPHOS system, initiate several adaptive responses, many of which can be considered defensive^{13, 14}. The nature of these responses is intricate involving activation of signalling networks which enhance bioenergetic adaptation via mitochondrial biogenesis (recovery) or alternatively autophagy (self-digestion), and global nuclear transcription changes which implicates epigenetic changes to the genome¹⁴⁻¹⁶.

Among the defensive adaptive responses, the induction of metallothionein (MT) gene expression as a putative protective response to an energy deficiency has been identified¹³. Similar to glutathione, metallothioneins (MTs) are ubiquitously expressed, cysteine-rich peptides involved in modulating the redox balance and metal homeostasis in cells. MTs have been strongly associated with the prevention of oxidative stress and cell death in diseases where mitochondrial energy metabolism has been compromised¹⁷. We have shown in *in vitro* studies that MT over-expression protects against oxidative stress-induced cell death¹⁸. In addition, metabolomics investigations using MT knockout mice (mice where the genes for MTs have been made dysfunctional) revealed that mitochondrial metabolism is significantly affected by the absence of MTs. These effects were compounded when the mice were challenged by physical stress (exercise) and diet interventions¹⁹.

Investigations of the full extent of cellular responses (metabolic, signalling, transcription and epigenetic) to an energy crisis are imperative to understand what the nature of the consequences (cell death, differentiation, adaptation) will be. As indicated before, these responses can result in the pathology that is seen with so many of the major death-causing diseases of our time. The current shift towards considering biological questions on a global perspective (“omics”), as well as the matching advances in analytical tools, makes these challenging investigations possible.

5. Inherited mitochondrial disorders: a 15-year study on South African paediatric patients

Inherited mitochondrial disorders (MDs) are present in at least 1 in 5 000 live births²⁰ and are the most prevalent group of inherited metabolic disorders. They occur as a result of a deficiency of any one of the five OXPHOS enzymes. The key properties of these disorders are that: the clinical presentations are highly varied and can occur at almost any stage of life; they affect multiple and often unusual combination of organs; involvement in adults often occurs in classical mitochondrial

syndromes; they arise from a great number of nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) mutations and, as a result, have multiple forms of inheritance; they can be monogenic or found in more common multigenic pathological conditions (e.g. Parkinson's disease, cancer, diabetes), or induced by toxins and drugs (secondary defects); disease progression and outcome varies significantly.

MDs have been clinically recognised in patients of African origin in the past. However, the first major effort to better investigate African paediatric patients with MDs was initiated in 1997 by the paediatric neurologist, Prof Izelle Smuts, at the Department of Paediatrics, Steve Biko Academic Hospital at the University of Pretoria. Since then, the author and at a later stage other national and international collaborators joined this investigation with the aim of implementing a multidisciplinary research and diagnostic approach. The diagnostic strategy includes, among others, biochemical investigations in muscle biopsies, which were established in 2001 with refinements implemented on a regular basis. The first seminal publication of clinical phenotypes in an African patient cohort was published in 2010, which revealed that patients of Black African origin have a predominantly muscular phenotype compared to Caucasian patients, who predominantly presented with a neurological phenotype²¹. More extensive research investigations on an expanding cohort of patients followed, including metabolomics (to improve diagnostics) and molecular genetics investigations (to establish the cause of disease).

From metabolomics investigations in urine, the main findings were that organic acids are much more informative than currently recognized in the common diagnostic approach to these disorders²². Furthermore, a putative metabolic biosignature in urine for MDs was proposed²³. Following further validation, the outcome of these metabolomic investigations could be a significant improvement in pre-biopsy selection of patients, using a low-invasive selection approach.

To better understand the aetiology of MDs in South African patients, molecular genetic investigations on the above-mentioned cohort resulted in the first extensive case reports^{24, 25}, as well as a report on mtDNA involvement on the patient cohort using a next-generation DNA sequencing approach²⁶. The latter revealed a very low prevalence (~1% compared to the published norm of 10%) of known mtDNA mutations in African patients, with several possible novel mutations found. In addition to these results, the outcomes of these molecular genetic investigations were 1) a significant contribution to genetic data on African mtDNA sequences and 2) confirmation that full-length mtDNA sequencing should be the first choice over the single mutation screening strategy, based on Western mutation data, which is currently used in clinical practice when MDs are suspected.

A major challenge that lies ahead is to further resolve the aetiology of these complex disorders in the African population. The findings of our molecular genetic investigations are in line with the world-wide recognition that finding and proving disease-causing variants (mutations) in populations requires population-specific investigations, supported by knowledge of the genomics and life-style (diet, environment, etc.) of that population. With African population data thinly spread in most genomic and mutation databases, efforts such as the Southern African Human Genome Programme (SAHGP) are essential to providing the crucial genomic data required to better identify and understand the role of genetic variation in diseases in the diverse African population.

6. Human genetics is set up around an energetic environment and cannot adapt to rapid energetic changes: the rise of “Western” diseases and its implications in Africa

The increasing amount of scientific evidence and general awareness of the involvement of bioenergetics in rapidly increasing death-causing human diseases, such as cardiovascular and neurodegenerative diseases, cancer as well as ageing, have provided an impetus for geneticists, biochemists and evolutionary biologists to re-evaluate the underlying relation between genetics and energy. In academic forums on this topic, a perspective currently discussed (although not the topic of this manuscript but certainly relevant) is that life harnesses chemical energy and that evolution and the mechanics of natural selection only make sense in the light of energetics²⁷. The key to comprehending the genetics of energy is to recognize the close relationship between mitochondria and the rest of the cell (its host) as well as the relationship and differences between nDNA and mtDNA. Selected properties between these two genomes, which resemble a genetic David and Goliath, are compared in Table 1 and illustrated in Figure 2. Notable from this comparison are the differences in variability between the two genomes, with mtDNA much more variable. Consequently, the 13 structural proteins encoded by mtDNA, which are core subunits in the hydrophobic area where electrons flow through the OXPHOS system (Figure 2), are highly variable in energetic efficiency²⁸. Random variability of these proteins between different mtDNA lineages could thus erode the efficiency of the OXPHOS system, that is, the effectiveness of coupling to produce ATP instead of ROS or heat (uncoupling). Douglas C Wallace recently reviewed the role of bioenergetics on genetic variation and the implications for biological complexity and common diseases²⁹. He concluded that “the missing genetic variation for common human diseases is primarily mtDNA variation plus regional nDNA variants, both of which have been missed by large, inter-population association studies”. Considering these views and scientific knowledge of the process, there is an active interaction between our genes and the *energetic environment* in which we live, which

includes the amount and type of dietary energy sources, physical work, environmental challenges, such as pathogens and toxins, and climate (oxygen tension, temperature).

Table 1. Comparison of human nuclear and mitochondrial genome properties.

Nuclear Genome	Mitochondrial Genome
Size: ~3 000 000 000 base pairs (x2 for two alleles)	Size: ~16 500 base pairs
One set per nucleated cell, two alleles, arranged in 23 chromosome pairs	Thousands of circular copies per cell
Bi-parental inheritance	Uni-parental (maternal) inheritance
Extensive protection and repair, low mutation rate, but recombination and epigenetics occur	Little protection with no repair, high mutation rate (up to 40 × higher), no recombination, no epigenetics
Encodes ~20 000 genes, of which ~2 000 are involved in energy metabolism	Encodes 37 genes (for 13 structural proteins) exclusively involved in energy metabolism (OXPHOS function)
Very little sequence variation between populations, allele frequency changes occurs	More sequence variation between populations with clear geographical location (haplogroups)
Variants mostly neutral, less often deleterious	Variants more often deleterious, which are mostly removed by ovarian selection, or functional (adaptive)
Bioenergetics: sequence variation relatively slow but undergoes epigenetic changes (“plasticity”) in response to changing energy environment	Bioenergetics: sequence variation relatively fast and allows adaptation to changes in energy environment

Notwithstanding the potential of our genes to change and an apparent increase in the tempo by which this change has occurred over recent millennia³⁰, the human genome has in fact remained relatively unchanged. This is at least true since long before the end of the Paleolithic era ~10 000 years ago, at which time human diet started to change more rapidly³¹. It is well known that, over the last century, human lifestyle has changed significantly in ways that involve our energetic environment. This includes lower physical activity, increased exposure to exogenous chemicals, changes in micronutrient and macronutrient composition, as well as an increase in total daily energy intake and energy density³². From a bioenergetics point of view, in a very short time humans have thus introduced an energetic environment to their bodies, which still uses an ancient genetic blueprint that did not have the time to adapt genetically to these rapid changes.

As mentioned before, many diseases are linked to changes in our energetic environment and, in most cases, mtDNA or nDNA mutations or polymorphisms implicating cellular energy metabolism have been identified in these diseases²⁹. Our research group and others have also identified low-level mtDNA mutations in other common diseases, such as chronic fatigue syndrome (unpublished data) and hypertension³³. The disproportionate increase of so-called “Western” diseases such as hypertension, with a prevalence of ~70% in the Black African population³⁴, is particularly disconcerting. These diseases are associated with rapid urbanization and consequent changes in lifestyle and diet as well as stress³⁴⁻³⁶. A genetic predisposition predominantly involving genes of energy metabolism is likely to be found. Studies investigating these associations are progressing well and are indeed timely as, if the current hypothesis of energy involvement in these diseases remains to be valid, much can be done to prevent or alleviate the progression of these diseases.

7. Conclusions

When considering the term “energy consumption”, the industrial production and use of energy from fossil fuels, nuclear energy or sustainable sources for day-to-day household or economic reasons comes to mind first because of the social awareness of this issue and its immediate impact. There are, however, many parallels between *industrial energy* production and consumption with that of *biological energy* production and consumption in living organisms. As reviewed here, bioenergetics provides a fundamental basis to life and death which manifests itself in a much less acute way. Considering the current scourge of diseases associated with modern lifestyle and relatively rapid changes that humans bring on their energetic environment, it can be argued that energy consumption should be viewed currently as not only a global socio-economic crisis, but also a global biological crisis that should be addressed with equal, if not greater, urgency.

References

1. Nicholls DG, and Ferguson SJ. 2013. *Bioenergetics (4th Ed)*. Academic Press, London.
2. Mitchell P. 1961. *Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism*. Nature 191, 144-148.
3. Anderson S, Bankier AT, Barrell BG. 1981. *Sequence and organization of the human mitochondrial genome*. Nature 290, 457-465.
4. Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong S-E, Walford GA, Sugiana C, Boneh A, Chen WK, Hill DE, Vidal M, Evans JG, Thorburn DR, Carr SA, Mootha VK. 2008. *A mitochondrial protein compendium elucidates complex I disease biology*. Cell 134, 112-123.
5. Zsurka G, and Kunz WS. 2013. *Mitochondrial involvement in neurodegenerative diseases*. IUBMB Life 65, 263-272.

6. Madamanchi NR, and Runge MS. 2007. *Mitochondrial Dysfunction in Atherosclerosis*. Circulation Research 100, 460-473.
7. Gogvadze V, Orrenius S, Zhivotovsky B. 2008. *Mitochondria in cancer cells: what is so special about them?* Trends in Cell Biology 8, 165-173.
8. Fisher-Wellman KH, and Neufer PD. 2012. *Linking mitochondrial bioenergetics to insulin resistance via redox biology*. Trends in Endocrinology and Metabolism 23, 142-153.
9. Gerschenson M, and Brinkman K. 2004. *Mitochondrial dysfunction in AIDS and its treatment*. Mitochondrion 4, 763-777.
10. Smeitink J, van den Heuvel L, DiMauro S. 2001. *The genetics and pathology of oxidative phosphorylation*. Nature Reviews in Genetics 2, 342-352.
11. Finkel T, and Holbrook NJ. 2000. *Oxidants, oxidative stress and the biology of ageing*. Nature 408, 239-247.
12. <http://www.who.int/mediacentre/factsheets/fs310/en/>.
13. van der Westhuizen FH, van den Heuvel LP, Smeets R, Veltman JA, Pfundt R, Geurts van Kessel A, Ursing BM, Smeitink JAM. 2003. *Human Mitochondrial Complex I deficiency: investigating transcriptional responses by microarray*. Neuropediatrics 34, 14-22.
14. Elstner M, and Turnbull DM. 2012. *Transcriptome analysis in mitochondrial disorders*. Brain Research Bulletin 88, 285-293.
15. Reinecke F, Smeitink J, van der Westhuizen FH. 2009. *OXPHOS genes expression and control in mitochondrial disorders*. Biochimica et Biophysica Acta - Molecular Basis of Disease 1792, 1113-1121.
16. Wallace DC, Fan W. 2010. *Energetics, epigenetics, mitochondrial genetics*. Mitochondrion 10, 12-31.
17. Lindeque JZ, Levanets O, Louw R, van der Westhuizen FH. 2010. *The involvement of metallothioneins in mitochondrial function and disease*. Current Protein & Peptide Science 11, 292-309.
18. Reinecke F, Levanets O, Olivier Y, Louw R, Semete B, Grobler A, Hidalgo J, Smeitink JAM, Olckers A, van der Westhuizen FH. 2006. *Metallothionein-2A expression is inducible and protects against ROS-mediated cell death in rotenone treated HeLa cells*. Biochemical Journal 395, 405-415.
19. Lindeque JZ, Hidalgo J, Louw R, van der Westhuizen FH. 2013. *Systemic and organ-specific metabolic variation in metallothionein knockout mice challenged with swimming exercise*. Metabolomics 9, 418-432.
20. Darin N, and Tulinius M. 2000. *Neuromuscular disorders in childhood: a descriptive epidemiological study from western Sweden*. Neuromuscular Disorders 10, 1-9.
21. Smuts I, Louw R, du Toit H, Klopper B, Mienie LJ, van der Westhuizen FH. 2010. *An overview of a South African cohort of patients with mitochondrial disorders*. Journal of Inherited Metabolic Disease 33, S95-104.
22. Reinecke CJ, Koekemoer G, van der Westhuizen FH, Louw R, Lindeque JZ, Mienie LJ, Smuts I. 2012. *Metabolomics of urinary organic acids in respiratory chain deficiencies*. Metabolomics 8, 264-283.
23. Smuts I, van der Westhuizen FH, Louw R, Mienie LJ, Mason S, Engelke U, Koekemoer G, Reinecke CR. 2013. *Disclosure of a putative biosignature for respiratory chain disorders through a metabolomics approach*. Metabolomics 9, 379-391.
24. van der Westhuizen FH, Smet J, Levanets O, Messner-Roloff M, Louw R, van Coster R, Smuts I. 2010. *Aberrant ATP synthase synthesis resulting from a novel deletion in mitochondrial DNA in an African patient with progressive external ophthalmoplegia*. Journal of Inherited Metabolic Disease 33, S55-62.
25. Alston CL, Davison JE, Goffrini P, van der Westhuizen FH, He L, Peet AC, Gissen P, Meloni F, Ferrero I, Wassmer E, McFarland R, Taylor RW. 2012. *Recessive germline SDHA and SDHB mutations causing leukodystrophy and isolated mitochondrial complex II deficiency*. Journal of Medical Genetics 49, 569-577.

26. van der Walt EM, Smuts I, Taylor RW, Elson JL, Turnbull DM, Louw R, van der Westhuizen FH. 2012. *Characterization of mtDNA variation in a cohort of South African paediatric patients with mitochondrial disease*. European Journal of Human Genetics 20, 650-656.
27. Lane N, Martin WF, Raven JA, Allen JF. 2013. *Energy, genes and evolution: introduction to an evolutionary synthesis*. Philosophical Transactions of the Royal Society of London Series B 368, 20120253.
28. Ji F, Sharpley MS, Derbeneva O, Alves LS, Qian P, Wang Y, Chalkia D, Lvova M, Xu J, Yao W, Simon M, Platt J, Xu S, Angelin A, Davila A, Huang T, Wang PH, Chuang LM, Moore LG, Qian G, Wallace DC. 2012. *Mitochondrial DNA variant associated with Leber hereditary optic neuropathy and high altitude Tibetans*. Proceedings of the National Academy of Sciences USA 109,7391-7396.
29. Wallace DC. 2013. *Bioenergetics in human evolution and disease: implications for the origins of biological complexity and the missing genetic variation of common diseases*. Philosophical Transactions of the Royal Society of London Series B 368, 20120267.
30. Fu W, O'Connor TD, Jun G, Kang HM, Abecasis G, Leal SM, Gabriel S, Rieder MJ, Altshuler D, Shendure J, Nickerson DA, Bamshad MJ; NHLBI Exome Sequencing Project, Akey JM. 2013. *Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants*. Nature 493, 216-220.
31. Eaton SB, and Cordain C. 1997. *Evolutionary aspects of diet: old genes, new fuels. Nutritional changes since agriculture*. World Reviews in Nutrition and Diet 81, 26-37.
32. <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5304a3.htm>.
33. Liu C, Yang Q, Hwang SJ, Sun F, Johnson AD, Shirihaï OS, Vasan RS, Levy D, Schwartz F. 2012. *Association of genetic variation in the mitochondrial genome with blood pressure and metabolic traits*. Hypertension 60, 949-956.
34. Malan L, Hamer M, Schlaich MP, Lambert GW, Ziemssen T, Reimann M, Steyn HS, Schutte R, Smith W, van Rooyen JM, Mels CM, Fourie CM, Uys AS, Malan NT. 2013. *Defensive coping facilitates higher blood pressure and early sub-clinical structural vascular disease via alterations in heart rate variability: the SABPA study*. Atherosclerosis 227, 391-397.
35. Vorster HH. 2002. *The emergence of cardiovascular disease during urbanisation of Africans*. Public Health Nutrition 5, 239-243.
36. Malan L, Hamer M, Reimann M, Huisman H, Van Rooyen J, Schutte A, Schutte R, Potgieter J, Wissing M, Steyn F, Seedat Y, Malan N. 2012. *Defensive coping, urbanization, and neuroendocrine function in Black Africans: the THUSA study*. Psychophysiology 49, 807-814.