# Type 2 diabetes as a redox disease

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Physical exercise has long been widely regarded as essential to human health.[1] Yet, we do not know how exercise-stressed skeletal muscle cells that generate reactive oxygen species such as hydrogen peroxide ($H_2O_2$) delay—if not prevent—the occurrence and severity of diseases such as type 2 diabetes (as well as dementias, cardiovascular disease, and some cancers). Also unexplained is the recent finding that metformin—the most commonly used drug to treat type 2 diabetes[2–4]—and physical exercise seem to be beneficial for several of the same diseases, including cancer, Alzheimer's disease, and cardiovascular disease.[5,6] New evidence[7] shows that combinations of short-term metformin treatment with single acute bouts of exercise do not, as generally expected, enhance insulin sensitivity. In fact, metformin alone can attenuate much of the oxidative effect of exercise.[7] The reason why exercise and metformin have opposing physiological consequences (oxidative *vs* reducing) has been shown by studies[8] that suggest that giving mice metformin increases synthesis of the transcription factor Nrf2, which controls the downstream synthesis of RNA molecules coding for major cellular antioxidant enzymes.

I postulate that diabetes, dementias, cardiovascular disease, and some cancers are accelerated—if not largely caused—by failure of the endoplasmic reticulum to generate sufficient oxidative redox potential for disulphide bonds to be formed. Physical exercise—by generating large numbers of reactive oxygen species—creates the oxidative redox potential needed to oxidise the free sulphhydryl groups of cysteine into the disulphide bonds used to stabilise the 3D conformation of physiologically active proteins. Compelling evidence that reductive redox potentials might be the molecular essence of type 2 diabetes first came to my attention in early 2013 when I learned from a 2009 German study that consumption of physiological amounts of the antioxidants vitamin C and vitamin E abrogated the capacity of physical exercise to make insulin more effective in lowering blood sugar concentrations.[7,9] This finding is supported by similar studies of other antioxidants in man.[10–12] Further suggestive evidence for the importance of an oxidative environment for promoting the action of insulin comes from patients with rare mutations impairing the production of antioxidant selenoproteins. Despite unequivocal evidence for a primary and severe deficiency of antioxidants including oxidative damage in tissues such as skin, these patients maintain supranormal insulin sensitivity even if they are obese.[13] Insulin resistance and type 2 diabetes might very well arise through insufficient supplies of key reactive oxygen species that normally oxidise key molecules controlling blood sugar concentrations.

The precise mechanism linking an oxidative environment with enhanced sensitivity to insulin in key tissues targeted by diabetes is still obscure. However, multiple studies[14] over the past decade report that the membranous sacs of the endoplasmic reticulum of insulin-resistant rodents contain much higher proportions of unfolded polypeptides and many fewer S–S bonds than does normal endoplasmic reticulum. Unlike almost all other cellular locations that have reducing redox potentials, normal endoplasmic reticulum has the oxidative redox potential necessary to form disulphide bonds. Only a third of all proteins are stabilised by disulphide bonds, with most using van der Waals' interactions and hydrogen bonds to generate their 3D shapes. Why only membrane-bound or secretory proteins require stabilisation by S–S bonds remains unclear. However, no uncertainty exists about the potential disease-causing consequences of lowering—if not stopping—S–S bond formation. How enzymes of the endoplasmic reticulum form the S–S bonds of secretory and membrane proteins is beginning to be understood.[15–17] Two structurally different oxidoreductive thiol enzymes have essential roles. Protein disulphide isomerases can directly insert the disulphide bond into target polypeptides. By so doing, they become reduced and unable to catalyse further S–S insertion until reoxidised by Ero1—a protein of a different disulphide oxidase protein family that contains flavin adenine dinucleotide. By oxidising protein disulphide isomerase, Ero1 becomes reduced and only resumes activity when it is reoxidised by passing electrons to molecular oxygen ($O_2$) which generates $H_2O_2$.

After these two thio-oxide reductases were identified, researchers began to investigate whether the insertion of S–S bonds into nascent proteins harms protein folding in patients with type 2 diabetes. The first such attempt was made in 2005 by Hungarian biochemists led by Gabor Nardaii.[18] They found that the polypeptide chain of protein disulphide isomerase in rat models of type 2 diabetes had relatively more reduced SH groups than did non-diabetic animals. By contrast, the polypeptides of Ero1 of diabetic rats had more oxidised S–S bonds than did non-diabetic rats. These results are compatible with diabetic cells having higher reductive redox potentials. Hopefully, these findings will stimulate a more thorough examination of S–S bond creation in human patients with type 2 diabetes.

Metformin actually seems to interfere with the beneficial effects of exercise in patients with diabetes.[7,9] This apparent paradox requires an explanation. Metformin has long been known to activate AMP-activated protein kinase (AMPK; the main cellular mediator of metabolic stress), but it does so indirectly.[19]

Its main—if not sole—molecular target is complex I of the mitochondrial electron transport system. Binding to complex I reduces ATP generation during electron transport by 30%. Mitochondrial AMP concentrations then increase to the level needed for significant activation of AMPK. AMPK then acts as an all-embracing molecular idler that quickly responds to low ATP concentrations by shutting down practically all anabolic metabolic pathways. Anabolic metabolism is then replaced by ATP-generating catabolic pathways that restore the cell's ability to quickly become growth oriented. Without the drastic redirection of metabolic pathways made possible by molecular stress responders, challenged cells can become acutely vulnerable to sudden changes in the supply of essential nutrients.[20]

The mechanism of action of metformin is being discovered. Kevin Struhl and colleagues have shown that metformin blocks the synthesis of the transcription factor NF-κB, which controls downstream synthesis of several molecules involved in the generation of inflammatory responses.[8] Inhibition of inflammation does not occur if metformin is added after the initial inflammatory signal. Equally important, metformin also activates synthesis of Nrf2, which controls the expression of many antioxidant enzymes.[8] These enzymes scavenge reactive oxygen species, stopping the cell-killing activities of inflammatory macrophages which, if unchecked, can bring about the apoptotic death of diabetic pancreatic β cells or destruction of the hippocampal nerve cells needed to form and maintain memories.

I postulate that the inflammatory responses now commonly thought to be at the heart of insulin resistance and type 2 diabetes are secondary to the unfolded protein responses resulting from failure to make disulphide bonds necessary to stabilise proteins' 3D conformation. If exercise does work through generating reactive oxygen species, thereby promoting formation of disulphide bonds, the most effective way to prevent and treat type 2 diabetes is probably through this approach rather than stopping later inflammatory responses. Exercise—not metformin—is already considered by much of the diabetic medical community as the most effective first route to lowering blood sugar concentrations.

In addition to its metabolic target tissues, insulin also affects the CNS, influencing not only appetite but also energy homoeostasis and even learning and memory.[21–23] Unsurprisingly, patients with type 2 diabetes have increased probability of developing dementia-like Alzheimer's disease. As Alzheimer's disease progresses, the endoplasmic reticulum of cells in stressed hippocampal regions increasingly accumulate unfolded proteins; these cells seem destined for apoptotic death. But if detected early enough, much of short-term loss of memory-forming capacity (the cardinal sign of early dementia) can be temporarily reversed by regular exercise.[24,25] Testing the effect of exercise on progression of Alzheimer's disease should be one of the highest priorities of medical research today. Experiments to assess whether metformin slows Alzheimer's disease progression are also needed.[26,27]

These observations might also be relevant to cancer.[28–30] For example, metformin kills cancer cells most effectively when AMPK does not become activated through phosphorylation by the liver cell kinase, LKB. At last we may have a plausible explanation for why cancer cells that have lost both copies of *p53* are much more susceptible to killing by metformin than are cells with *p53*. The inability of $p53^{-/-}$ cells to respond optimally to nutritional stress somehow causes apoptosis. Metformin's potential use as a broadly acting anticancer drug could depend on the development of new drugs designed to inactivate molecular cellular stress responders such as p53 and AMPK.

Much too little is known of how best to administer exercise as a treatment for type 2 diabetes. Are heightened heart rates needed to generate significant beneficial effects? Furthermore, we have little idea of how long intensive exercise should last (eg, 10 min *vs* 30 min *vs* 1 h). And are there limits to how long and intensively people should exercise before the production of reactive oxygen species leads to significant accumulations of antioxidants? Do most highly successful athletes generate reactive oxygen species in excess of what can be successfully scavenged by Nrf2-directed antioxidants? To date, antioxidants such as vitamin C and vitamin E have been frequently administered with the hope of promoting better athletic performance.[31,32] However, almost all such experiments have shown no positive effects. Many antioxidant supplements could lower intracellular concentrations of reactive oxygen species below those needed for normal disulphide bond formation.

Obtaining such data about exercise will not be easy. Studies will be difficult to initiate and expensive to complete, especially at a time when funding for intellectually more exciting medical research is under threat. Funding for such research into exercise would come most readily from a philanthropic billionaire. Financing these efforts is for the good of all peoples. Happily, the first super wealthy super athlete that I have approached, Sir Richard Branson, has responded positively to my request to consider providing financial support for a several-day conference at Cold Spring Harbor Laboratory to begin delineating in detail what an effective research programme for the science of exercise would cost. The first such meeting will occur in the first half of 2014.

Research devoted to quantification of the beneficial effects of mental exercise will require equally large sums of money. I am not alone in wanting reliable evidence to confirm the oft-heard assertion about the brain: "use it or lose it". My capacity to remain a full-time scientist at the age of 85 years has probably been much aided by regular exercise (singles tennis). But I may have been aided more by lifelong mental exercises and alleles I inherited from

my father and mother. Efforts to tease out the relative importance of these different factors cannot come too soon.

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**Conflicts of interest**

I declare that I have no conflicts of interest.

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