Clinical pharmacological aspects of mitochondrial function in muscle

Diemen, M.P.J. van

CHAPTER VIII

GENERAL DISCUSSION

GENES, ANATOMY AND…ENERGY

Disease in Western medicine has been based on two fundamental principles: the anatomy of the human body and genetic inheritance. In 1543 Andries van Wesel (*1516-†1564), better known as Andreas Vesalius, described the human body as a corporeal structure filled with distinct and essential organs, arranged in a three-dimensional space. His groundbreaking, but for the time utmost controversial, work *De humani corporis fabrica,* is widely regarded as the foundation of modern anatomy and has led to the belief that tissue specific symptoms must be due to a tissue specific defect. A while later, Gregor Johann Mendel (1822-1884) established the ways of heredity around 1863, which would later be known as Mendelian inheritance of genetic information. Eventually, the theory was extended with genes and chromosomes and together this explained change in anatomy (either healthy or pathological variation) as being due to Mendelian inheritance and thus chromosomal. This also meant that any change, which could not be explained by chromosomal genetics, must be due to the environment.

Mendelian/anatomical medicine has been successful in explaining, diagnosing and treating acute diseases, but has failed to cure most of the chronic (age-related) diseases, which have become an ever-increasing burden on our global, aging society. The reason for this is that anatomy (structure) and genes (information) are only two out of three ingredients of life, the third being energy. Mitochondria provide over 90% of the cell's energy need and are required for everything we do (including writing this thesis). Mitochondria have their own DNA containing a set of genes encoding electron transport chain (ECT) proteins, which puts the mitochondria themselves in charge of our energy. Doug Wallace has been the pioneer in showing the importance of energy on disease. Looking from a bioenergetic point of view, with mitochondrial function at the center, all complex diseases and aging can be understood via the common pathophysiological mechanism 'mitochondrial dysfunction', the severity of which varies with the severity of the resulting disease.[1] Critical dysfunction of mitochondria is fatal at birth or during infancy while the natural course of accumulation of mitochondrial DNA mutations and the resulting gradual decrease in mitochondrial function is at least partly responsible for aging. A partial energy defect can be expected to specifically target organs with the highest energy demand. The brain only weighs 2% of total bodyweight, but uses 20% of the energy. Involvement of mitochondrial dysfunction in neurodegenerative and other age-related diseases is therefore not coincidental.

The central theme of this thesis was mitochondrial (dys)function of muscle and brain in aging and neurodegenerative disorders with the aim to explore several fundamental aspects of pharmacological treatment. Mitochondria are increasingly being investigated as a drug target[2-5] , however the translation of compounds from pre-clinical models into clinical trials is often unsuccessful.

Sarcopenia

> *'Last scene of all, That ends this strange eventful history, second childishness and mere oblivion; Sans teeth, sans eyes, sans taste, sans everything,'*

A common age-related disease is sarcopenia, or the decline in muscle mass and strength in the elderly population, which is part of the frailty syndrome. Sarcopenic elderly are prone to falling, often leading to a hip fracture and high mortality [6] . Mitochondria in muscle of sarcopenic elderly are dysfunctional and it is thought that mitochondrial dysfunction is a driver of the pathophysiology.[7-9] In **Chapter 2**, we explored mitochondrial function in pre-frail, sedentary elderly with the aim to further elucidate the etiological role of mitochondrial dysfunction in sarcopenia in order to identify strategies for prevention. We found that mitochondrial function was impaired in pre-frail elderly, when compared to healthy, active elderly, at multiple sites within the mitochondria and when measured *ex vivo* in muscle tissue and *in vivo* by phosphorous magnetic spectroscopy (31P-MRS) in the calf muscles. The findings confirm that mitochondrial dysfunction is a hallmark of pre-frailty and development of frailty. The results are important, first because such a comprehensive evaluation in the pre-frail elderly population was lacking and second because the results show that mitochondrial dysfunction precedes the frailty stage, during which (pharmacological) therapy has proven to be difficult.[10] As prevention is the best treatment, additional research should be performed to clinically evaluate the disease progression after early treatment of pre-frail elderly with interventions aimed at restoring mitochondrial function in the muscle.

An interesting find was that handgrip strength strongly correlated with the mitochondrial ECT complex activities measured in the muscle biopsy (see Table 8.1). Handgrip strength correlated even better to ECT complex activities than gold standard 31P-MRS of the calf muscle or quadriceps strength, even though this latter measurement was performed in the same leg as the muscle biopsy. Handgrip strength measurement can be easily performed in the outpatient clinic and is

thought to reflect the general condition of a person.[11-13] It has also been shown to predict outcome after several surgical procedures [11] and correlates to cognitive functioning in elderly.[14] These correlations are in accordance with the theory that disease and aging can be seen as a bodywide disturbance in bioenergetic status.[1]

There has been much speculation regarding the origin of the mitochondrial dysfunction in age-related diseases, including sarcopenia and frailty.[15] In our study, we used physical activity as a criterium amongst others to select pre-frail elderly subjects and their matched elderly controls. The pre-frail group had a mean energy expenditure of 392 metabolic equivalent (MET) minutes per week, which corresponds to less than 20 minutes of walking per day. In comparison, the mean energy expenditure in the active group was 6,508 MET minutes per week, which corresponds to 1 hour of vigorous exercise per day. This raises suspicion that a sedentary lifestyle could by itself cause mitochondrial dysfunction in skeletal muscle. This thought is supported by the fact that (resistance) exercise improves mitochondrial function in sarcopenia and frailty.[16] Even in neurodegenerative disorders, the effects on cognition are beneficial.[17] With obesity and an increasingly sedentary lifestyle on the rise, exercise appears to be more important than ever.

In **Chapter 3**, we established a model to predict recovery in mobility after a total knee arthroplasty (TKA) based on several pre-surgery functional measurements. Using wearable activity trackers to monitor patient's recovery after TKA, A multivariate regression analysis led to a positive correlation between the increase of the daily number of steps after TKA and baseline mitochondrial function (i.e. complex 5 abundancy in skeletal muscle), baseline activity (daily number of steps) and baseline grip strength. Combining these results, we formulated the following algorithm to predict the rate of recovery after TKA, based on baseline measurements:

Increase in daily number of steps = -112 + (0.02 × [activity before surgery]) + (0.2 x [CP5 abundancy]) + (3 × [grip strength])

Predicting and monitoring recovery after TKA is clinically important, because despite advances in technology and patient care, an estimated 20-25% of procedures have unsatisfactory results, with dissatisfaction in functional outcome ranging from 16-30%.[18-21] Having a better estimation of recovery might inform surgeons in how intense physical therapy should be and better manage patient's expectations. Measuring grip strength and the daily number of steps are easy to perform in a clinical setting with very low patient burden. Due to the increasing availability of modern smartphones, monitoring a patient's activity as part of postoperative recovery is now readily available. The built-in accelerometer and GPS are able to measure not only activity, but also the traveled distance and quality of walking (e.g. slow, fast and sitting), accurately. The data can automatically be shared online with the treating surgeon, providing objective data on mobility and still minimizing patient burden.

In **Chapter 4**, we demonstrated that simvastatin, a cholesterol lowering drug, can be used to sub-clinically lower mitochondrial function in a group of healthy middle-aged subjects, which can subsequently be reversed by simultaneous suppletion of ubiquinol, the reduced form of coenzyme Q10 (CoQ10). The purpose of the study described in Chapter 4 was to validate a human pharmacological challenge model which can be used to evaluate and prove the pharmacology of novel mitochondrial function enhancing compounds in the healthy subject stage of clinical drug development. In our study we managed to partially reverse the induced dysfunction. The reason for this is that simvastatin inhibits the biosynthesis of CoQ10, which passes electrons from ECT complex I and II to complex III but also directly inhibits complex III (explaining the partial reverse by CoQ10 suppletion). We believe that this makes the model potentially suitable for studies with future compounds that act on ECT complex I, II and III. This requires knowledge of the pharmacological mechanism of the compound beforehand in order to decide, whether the simvastatin POP model is a suitable model of efficacy. More POP models, targeting mitochondrial function in different ways, should be available for clinical studies. Although there are many known mitotoxic drugs like simvastatin, the problem is that for the model to work, the mitotoxic effects must also allow reversal by administration of another compound, like ubiquinol or the new drug under investigation. Unfortunately, most other candidates are too toxic. For instance, a small dose of cyanide is an excellent way to uncouple oxidative phosphorylation and to induce a significant amount of mitochondrial dysfunction, but not very suitable.[22]

In **Chapter 5**, we conducted a clinical trial with the novel mitochondrial function enhancing compound SBT-020 in a group of patients with mild to moderate Huntington's Disease (HD). SBT-020 optimizes the electron flow within the mitochondrial ECT by protecting cardiolipin from oxidation by radical oxygen species, similar to the related compound SS-31 (elamipretide).[2] Mitochondrial capacity was measured peripherally (in the calf muscle and in peripheral blood mononuclear cells (PBMCS)) and in the central nervous system (bio-energetic state in the visual cortex). Although the compound was safe during the multiple

ascending dose part (1 week) and the subsequent longer multiple dose part (4 weeks) and behaved well from a pharmacokinetic point of view, we did not observe clear pharmacological effects on mitochondrial function. HD is a complex and devastating disease, affecting motor, cognitive and psychiatric functioning.[23] There are strong indications in animals and man that mitochondrial dysfunction plays an important role in the pathophysiology of HD, induced via toxic accumulations of misfolded mutant Huntingtin (HTT) protein.[24] When the mitotoxic compound 3-nitropropionic acid is administered to mice, mitochondrial dysfunction is induced, and the animals start showing symptoms, typical for HD.[25] The same happens when mitochondrial function is chronically impaired in non-human primates.[26] In human HD patients, a disbalance in mitochondrial bio-energetics has been reported in the central nervous system (CNS), in the skeletal muscle and in circulating white blood cells.[27-29] Additionally, mutant HTT has been described to localize near mitochondria[30-32] and interact with its proteins.[33] Although mitochondrial dysfunction thus seems to be omnipresent in HD, the source of the symptoms clearly comes from atrophy of the striatum.[23] The striatum is a structure in the brain that is particularly energy demanding[34] and its cells are vulnerable to mitochondrial dysfunction, putting the bioenergetic theory of disease (discussed earlier) in practice. Thus, the reason for the lack of activity of this compound remains unclear

In **Chapter 6** we subsequently explored correlation between peripheral mitochondrial capacity (31P-MRS of the calf muscle), mitochondrial health in PBMCS (mitochondrial membrane potential ($\Delta\Psi m$)) and central mitochondrial bio-energetic state (31P-MRS of the visual cortex) within the same HD patients. We could not demonstrate a correlation between the peripheral and central variables or between the peripheral variables and clinical function, measured as the Unified Huntington's Disease Rating Scale (UHDRS) Total Motor Score (TMS). By contrast, the central mitochondrial bio-energetic state did show a significant correlation ($R = 0.48$, $p = 0.02$) to the TMS. This may be explained by the fact that mitochondria in skeletal muscle are known to have a higher reserve capacity than mitochondria in the striatum.[35] It has been shown that mutant HTT not only accumulates in the striatum, but also in skeletal muscle[27] , but the lack of correlation indicates that mitochondria in skeletal muscle and striatum are not affected to the same degree, even though both tissues are high in energy demand.[34,36] Mitochondrial function in skeletal muscle is also strongly improved by physical exercise such as walking or running, whereas striatal mitochondria are not likely to be influenced by any activity, physical or mental.[37]

Because the dysfunctional mitochondrion has been identified as a target in clinical trials for HD, it is relevant to use a method for mitochondrial function measurements that correlates to clinical function. Although *in vivo* 31P-MRS of the calf muscle has been proposed in other studies as a suitable marker for pharmacodynamic effects[27] , they do not appear to reflect clinical function. Repeat neurocognitive and motor testing (including the Stroop test, Single Digit Modalities Test and Tapping test) has shown to be sensitive to pick up changes over time that correlate with disease progression and thus offer greater value as pharmacodynamic measurements[38,39] , but are not a direct measurement of mitochondrial function. The central bio-energetic state of the visual cortex might be especially useful, because it reflects the bio-energetic state of neuronal mitochondria and correlates to clinical function.

The function of mitochondria within circulating PBMCS did also not correlate to the mitochondrial function in other tissues or clinical function. In the literature, PBMCS of HD patients have been observed to be dysfunctional and with a lower $\Delta\Psi m$ when compared to healthy volunteers.[40,41] However, we did not observe a significant difference between the patients and a group of healthy volunteers (data not published).

Due to the intravascular location, PBMCS have been evaluated as a tool for toxicology of existing and novel medications.[42] In **Chapter 7** we discussed mitotoxicity of commonly prescribed medications and methods to measure mitochondrial function *in vivo*. Mitotoxicity has only recently been recognized as an important mechanism of adverse drug effects and though to be the cause for withdrawal of previously approved medications, such as phenformin and buformin.[43] This has led more and more pharmaceutical companies to screen for mitotoxicity in addition to the standard toxicology screening. In preclinical studies, this is mainly done *in vitro*, using the SeaHorse or Oroboros respirometry devices. These devices assess the activity of the different ECT complexes by measuring the oxygen disappearance rate after adding a series of substrates, used by the complexes.[44] In clinical trials, however, *in vivo* or *ex vivo* methods are preferred due to the complexity of intra- and extracellular signals. The gold standard for measuring mitochondrial function *in vivo* has been 31P-MRS.[45] Although its primary outcome (the phosphocreatine recovery rate) is very reliable, the method requires specialized equipment and staff to operate. Several other (less burdensome and cheaper) techniques are available to measure mitochondrial function in the clinical setting, which we exploratorily used during the study with simvastatin in healthy volunteers (**Chapter 4**): the $\Delta\Psi m$ in PBMCS and oxygen consumption rate in thenar

muscle (using near-infrared spectroscopy (NIRS)) and the novel Protoporphyrin IX Triple State Lifetime Technique (PPIX-TSLT) technique which measures oxygen consumption in the skin. The PPIX-TSLT, a novel technique, makes use of the oxygen-dependent delayed fluorescence of protoporphyrin IX, a precursor protein in the heme-synthesis, which occurs within the mitochondria.[46] The oxygen disappearance rate indicates mitochondrial function.

The *ex vivo* measurement of the $\Delta\Psi m$ in PBMCS could only be performed in 8 subjects, but in this limited number did show an increase of the percentage of dysfunctional cells from 5.20% at baseline to 14.43% after 4 weeks of simvastatin use (CI95%, 2.416–16.056; p = 0.016). These results are consistent with previously observed effects from other mitotoxic medications, such as anti-retroviral medications, and highly useful as a screening tool for mitotoxicity in the clinical setting.[42]

Measuring oxygen consumption *in vivo,* both by NIRS and PPIX-TSLT, did not show an effect from simvastatin. Whereas NIRS measures the oxygen disappearance rate within the capillaries, which is not quite sensitive for mitochondrial dysfunction per se (ischemia triggers it as well), the PPIX-TSLT determines oxygen consumption only within the mitochondria.[46] PPIX-TSLT has been able to accurately measure the uncoupling of oxidative phosphorylation by cyanide when applied in low concentration to the skin[47] , providing a novel way to assess mitotoxicity with minimal exposure for the subject. The COMET is the latest evolution of the technique and a portable device for the clinical setting.

Future perspectives: development of mitochondrial function enhancing drugs

The future of pharmacological enhancement of mitochondria looks bright. More and more pharmacological companies have made mitotoxicity part of the pre-clinical toxicology screening and are investigating mitochondrial function enhancing compounds. The majority of the current clinical trials – listed on clinincaltrials.gov – are exploring the effect of existing medications in treatment of mitochondrial diseases or dysfunction, but also novel compounds are investigated (see Box 1). These developments are hopeful, but the translation of compounds from the pre-clinical phase into clinical trials is still largely based on clinical noticeable effects, such as disease severity. These outcomes are difficult to achieve even when the compound is pharmacologically active, due to the limited treatment time in early phase clinical trials. This is where the POP model with simvastatin will be useful. Mitochondria play an important role in the function of nearly all cells and unexpected or undesired pharmacology could therefore be potentially be severe. This requires early stage clinical trials with novel compounds to be conducted in healthy volunteers, instead of patient populations, to optimize safety. Proving the desired pharmacology during the same study using the POP model could provide a rational to further develop the compound or to end it, saving time and effort. Simvastatin affects mitochondrial function at different sites within the mitochondrial ETC, which makes it suitable for a broad range of mitochondrial function enhancing compounds (acting on complex I, II and III).[48,49] It is difficult to predict whether the POP model could be improved. By increasing the daily dose of simvastatin (for instance to a daily dose of 80mg), a more severe degree of mitochondrial dysfunction could be induced. This could provide more room for the novel medicine to reverse the dysfunction, but at the same time it does not reflect the low degree of mitochondrial dysfunction in age-related diseases and also would probably cause more muscle related adverse events in the healthy volunteers. However, prolonging the simvastatin administration period (for instance to 2 months) could maybe induce a more chronic form of mitochondrial dysfunction, more in line with age-related diseases.

Box 1 – Novel mitochondrial function enhancing compounds and their mechanism of action:

- Novel anti-oxidants: **EPI589, EPI-743, RP103, KH176,**
- Mitochondrial biogenesis: **RTA408, KL1333, SRT2104, TAK831**
- Improving mitophagy: **AMAZ02**
- Inhibiting NF-kappaB: **RG2133**
- Gene therapy: **GS010**

Future perspectives: mitochondrial dysfunction in neurodegenerative diseases

It has become clear that neuronal cells are influenced by a decrease in bio-energetic state resulting from mitochondrial dysfunction, proven by the fact that mitochondrial dysfunction is a common phenomenon in most of the neurodegenerative disorders. The question is therefore not if, but when efficacy of a mitochondrial function enhancing therapy in neurodegenerative disorders will be shown. Until recently, clinical outcome measures have been used to evaluate efficacy, which are notoriously difficult to influence in early stage clinical trials. The availability of *in*

vivo mitochondrial function specific biomarkers should make it easier to show efficacy. 31P-MRS of the visual cortex was an important first step, but contrary to 31P-MRS of the calf muscles, it merely evaluates the bio-energetic state and is not a robust reflection of true mitochondrial function. Due to the secluded nature of brain tissue, other techniques, such as the Near Infrared Spectroscopy and the Protoporphyrin 9 Triple State Lifetime Technique, are difficult to use. The future could lay in a specific biomarker in cerebrospinal fluid (CSF). A very recent mouse study showed the correlation between degenerative changes in the brain and FGF-21, which is a marker for mitochondrial stress, in CSF.[50] FGF-21 has previously been proposed to be a systemic marker for mitochondrial dysfunction[51-54] , and its role in human degenerative disorders should therefore be further explored.

Future perspectives: targeting mitochondria in skeletal muscle to influence body-wide metabolism

Just as mitochondria can no longer be regarded as solely powerhouses of the cell, skeletal muscle can no longer be seen solely as an organ to only grant us mobility. Apart from being able to move things through muscle contractions, skeletal muscle is a secretory organ and communicates with other organs – such as the liver, adipose tissue and the brain – through cytokines.[55] Termed myokines, these cytokines are released by myocytes on muscle contraction and play a role in the body-wide metabolism, including counteracting the pro-inflammatory effect of adipokines (cytokines secreted by adipocytes).[56,57] Exercise (aerobic or non-aerobic) results in a healthy adipokine-myokine balance, whereas a sedentary lifestyle results in the opposite.[56] The effects of exercise (or the lack of) also become clear in a clinical setting: an active lifestyle with plenty of exercise significantly reduces the chance on medical conditions such as cardiovascular pathologies, diabetes, certain types of cancer, depression, neurological disorders or stroke.[58-60] A sedentary lifestyle increases the chance on such conditions.[61-64] Combine this with an excessive calorie intake and the odds are exacerbated by the resulting obesity.[65,66] One could actually argue that doctors should have the option to prescribe physical activity with the same ease as medications. A declining muscle mass due to physical inactivity (eventually leading to sarcopenia) might therefore have influences on the beneficial effects of skeletal muscle on metabolism due to a myokine – adipokine disbalance. Recently, a low level of the circulating myokine irsin was proposed to be a predictive biomarker for sarcopenia.[67] Given the close relationship between inactivity, sarcopenia and skeletal muscle mitochondrial dysfunction, the effects of improving mitochondrial function go further than local effects in skeletal muscle alone. Again, physical activity will play an important role, but it is not always feasible or even possible to pursue an active lifestyle, as is obvious in for instance the recovery period after surgery or during hospitalization. Therefore, pharmacologically improving mitochondrial function in skeletal muscle to induce the beneficial effect on metabolism could be an alternative to physical exercise. In other words, improving mitochondrial function in sarcopenic elderly should thus aim to improve the myokine-adipokine balance, leading to a general improvement in body-wide metabolism and bio-energetic state. Future clinical studies in this field should thus expand the scope from focusing on separate organs to a holistic view of the body in terms of bio-energetics. *In vivo* methods to measure mitochondrial function, such as 31P-MRS, will be of essence. Also, the correlation between myokines and mitochondrial function in different organs should be studied. Skeletal muscle makes up about 30-40% of our body weight, so let's put it to work!

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TABLE 1 **Pearson correlations.** Correlations between mitochondrial function and clinical function measurements in pre-frail and active elderly.

	Handgrip	31P-MRS	Quadriceps strength	IPAQ
Complex 1	R = 0.64 P = 0.002	R = – 0.44 P = 0.04	R = 0.52 P = 0.01	R = 0.66 P = 0.001
Complex 2	R = 0.42 P = 0.06	R = 0.16 P = 0.49	R = 0.34 P = 0.13	R = – 0.06 P = 0.78
Complex 4	R = 0.62 P = 0.003	R = – 0.48 P = 0.03	R = 0.47 P = 0.03	R = 0.56 P = 0.008
Complex 5	R = 0.66 P = 0.001	R = – 0.44 P = 0.05	R = 0.64 P = 0.002	R = 0.57 P = 0.007