CHAPTER 1

INTRODUCTION

In cellular biology, the involvement of the small metal-carrying proteins, metallothioneins (MTs), with the mitochondrion has not received much attention despite convincing evidence that such an association exists. The diversity of both the protein and organelle is perhaps the main reason why their association has not been properly recognised over the past 50 years of MT-related research. With the increased awareness of oxidative stress and oxidative damage in biological sciences, the mitochondrion has become the centre of many recent studies, especially those that focus on diseases. Conversely, MT-related investigations increasingly focus on the over-expression of MTs induced by reactive oxygen species (ROS) and their antioxidant properties. Hence, a link between MTs and the mitochondrion is already evident. However, the involvement of MTs in regulating levels of ROS produced in the mitochondrion to consequently protect against oxidative stress and related diseases has received relatively little attention, not to mention their involvement in oxidative phosphorylation (OXPHOS) deficiencies.

Van der Westhuizen *et al*. (2003) observed using a micro-array approach that the expression of various isoforms of MTs was significantly induced in various mutation associated complex I deficient fibroblast cell lines. This was followed by proving that MTs protect against increased oxidative stress and promote cell viability when OXPHOS are dysfunctional (Reinecke *et al.,* 2006). Furthermore, as will be discussed in Chapter 2, a large body of evidence describes the mostly protective role of MTs in various diseased states associated with oxidative stress-induced cell death. But is the main involvement of these proteins with mitochondrial function the scavenging of free radicals? Do they also play a role in mitochondrial energy metabolism regulation, where their role in metal homeostasis (e.g. in respiratory chain function) may come into play? The involvement of MTs in the mitochondrial function (i.e. energy metabolism) has been suggested after it was observed that MT knockout mice had a tendency to become moderately obese (Beattie *et al.,* 1998). Seeing that numerous enzymes require metal cofactors to function, this result indicates a possible role of MTs supplying metals to mitochondrial enzymes. An *in vitro* study also showed that mitochondrial aconitase accepts zinc from MTs but not $ZnCl_2$ (Feng *et al.,* 2005).

Hence, the view that MTs play a role in metabolism appears to be founded. Moreover, seeing that the mitochondrion is the centre of metabolism and that MTs donate zinc to proteins such as mitochondrial aconitase, a secondary role of MTs in the mitochondrion seems apparent in addition to their putative role in scavenging free radicals. But do these (and other similar) *in vitro* findings reflect the role of MTs *in vivo*? And if so, where in the metabolism do MTs have a most pronounced effect? In order to confirm whether MTs play a role in metabolism (specifically energy metabolism) and to find the pathways most involved, it was considered timely to initiate a metabolomics investigation using MT knockout mice. Interventions that specifically challenge mitochondrial function (energy metabolism) and disease were used to investigate the putative role of MTs in mitochondrial metabolism. This was done in an attempt to answer the above questions as well as the following more specific questions: are there any (vast) differences in the metabolism of MT knockout and wild type mice which are reflected in the metabolome during normal unchallenged conditions? Are there any differences in the metabolism of these mice, and what happens when mitochondrial metabolism is challenged with exercise and/or a high fat diet, or a more severe challenge such as a respiratory chain dysfunction? Can these differences (if any) emphasise the main regions that are affected by the absence of MTs in the MT knockout mice? Finally, can the results of this study give a more accurate picture of the involvement of MTs in mitochondrial metabolism regardless of this study's main aim to only emphasise important regions for further investigation?

This study has been designed to investigate the essential problem statements described above. Apart from the thesis document that follows, the study has thus far contributed to several conference proceedings (Annexure H) and a published peer-reviewed journal publication. It is important to note that in this study a major effort had to be taken to develop or modify research methodologies in the new field of metabolomics at the Centre for Human Metabonomics (NWU) where it was performed. A large part of the thesis is therefore used to describe these methodological developments, which in itself were considered a sub-study for exploring the rationale behind methodology choices, as well as advancement of the specialised discipline, albeit mostly localised to the centre where it was undertaken.

A comprehensive review on the involvement of MTs in mitochondrial function and disease is given in Chapter 2, which formed the basis of a review paper published in Current Protein and Peptide Science (11(4):292-309, 2010). In Chapter 3, the problem statement, aims and design of this study, which consisted of three sub-studies, are described. Chapter 4 entails the metabolomics investigation of the role MTs play in mitochondrial function when challenged with exercise and/or a high-fat diet. The investigation of the involvement of MTs in mitochondrial disease (complex I

inhibition) are described in Chapter 5, followed by a concluding chapter (Chapter 6). Chapters 4 and 5 are structured to first give the methodology of the respective sub-study, followed by the results and discussion section containing the metabolites that differed between the studied tissues of the experimental groups. The results of each tissue are discussed separately to allow a comprehensive view of the metabolic changes, in line with the untargeted nature of the metabolomics investigation. This is followed by a more combined interpretation linking all the tissue specific results to mitochondrial metabolism.

Annexure A contains a broad literature study on metabolomics technology and data mining methodology which forms the basis and rationale behind the selection and use of certain methods and techniques in this study. The selection and standardisation of metabolomics and data mining methods are given in Annexures B and C respectively. The development of a high-throughput metabolomics and data mining workflow for functional genomics type studies, such as the present, was mandatory to accomplish the goals set forth and to establish guidelines for future studies at the Centre for Human Metabonomics, NWU. A supplementary CD is included in Annexure G which contains all the figures and tables, the revised version of the review paper and a pdf version of this complete document.