



<http://researchspace.auckland.ac.nz>

ResearchSpace@Auckland

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage.

<http://researchspace.auckland.ac.nz/feedback>

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form.

Investigation of diabetic cardiomyopathy and its treatment by copper chelation

Sarah Glyn-Jones



**A thesis submitted in partial fulfilment of the requirements for the
degree of Doctor of Philosophy**

The University of Auckland

2007

Abstract

Diabetes mellitus is estimated to affect approximately 7% of the populations living a western lifestyle. Of the multiple etiologies associated with diabetes, heart failure is the most common cause of death. A specific type of heart disease called diabetic cardiomyopathy is thought to be partially responsible. At this time, no one specific treatment is available for diabetic cardiomyopathy due to the wide variety of possible complex molecular changes, including metabolic disturbances, myocardial fibrosis, LV hypertrophy, and increased ROS production.

Abnormal copper metabolism in diabetes has been proposed to form part of the pathway that leads to diabetic cardiomyopathy. Our group have shown that treatment with the copper (Cu^{II}) chelator, triethylenetetramine, ameliorates the effects of diabetes on the heart at both the functional and molecular level.

This thesis aimed to further these studies by increasing our understanding of the mechanism of triethylenetetramine action on the diabetic heart. This was primarily achieved through the use of microarray technology but included the use of a range of molecular experimental techniques.

During this investigation it was determined that the most suitable microarray platform for our studies was the Affymetrix GeneChip® system. Using this system we identified more than 1600 gene changes associated with diabetes in the left ventricle wall. A disproportionate number of significant messenger RNA transcript changes were associated with the mitochondria and further investigation of these genes revealed changes associated with perturbed lipid metabolism and increased oxidative stress.

A second study investigated the molecular mechanisms underpinning improved cardiac function in the left ventricle of the heart from diabetic and sham animals treated with triethylenetetramine. There was an observed decrease in diabetic cardiac tissue triglyceride towards normal, possibly through improvement of the structure and stability of the mitochondria. Only a small number of changes in gene expression were detected after triethylenetetramine treatment using microarray technology, and none were detected using real time-quantitative PCR.

The final aim of this thesis was to understand the absorption and excretion of triethylenetetramine by both sham and diabetic animals. Our study found differences in the ability of diabetic animals to absorb and metabolise triethylenetetramine compared to sham animals. Also, the length of exposure was found to be an influencing factor in triethylenetetramine metabolism.

Acknowledgments

Firstly, I would like to acknowledge my supervisor Garth Cooper for the opportunity he has provided me in putting this project together. I am appreciative of his encouragement and insight, and for teaching me that the more you know, the less you really know.

I am especially grateful to Anthony Philips. Without his guidance, this project would not have gone forward or been as interesting.

I would like to thank the people of the Level Five office, both past and present, members of the Cooper group and fellow SBS PhD students for providing an important support network that enabled me to complete this thesis.

I would like to express deep gratitude to the statisticians in my life, Mik Black who was there in the beginning to show me the fundamentals of microarray stats and Kathy Ruggerio who unfortunately had an office close enough to mine for me to nag her on a regular basis, but was kind enough to teach me anyway.

I would like to recognize Rosemary, Lily, Silvana, Stan, Nancy and Vernon for their assistance with the animal work. You were life savers!

I would like to acknowledge the financial support of the Foundation of Research, Science and Technology and the University of Auckland. I would also like to recognise the financial contribution of ProteMix Corporation Limited.

To my family, thank you for helping me out when I was a little bit worried and listening to me even though you didn't really understand.

And finally, the biggest thanks of all to Ivan, for having to live with me and my thesis.

Table of Contents

Abstract	i
Acknowledgments	ii
Table of Contents	iii
List of Figures	x
List of Tables.....	xii
Abbreviations.....	xiv
Chapter 1 General Introduction.....	1
1.0 Diabetes.....	1
1.1 Diabetic cardiomyopathy	1
1.1.1 Definition	1
1.1.2 Diabetic heart function assessed in animal models.....	3
1.1.3 Dysregulation of energy metabolism in the heart	3
1.1.3.1 Energy metabolism in the normal heart	4
1.1.3.2 Energy metabolism in the diabetic heart	5
1.2 Molecular mechanisms underlying diabetic complications	6
1.2.1 Pathway 1: An increase in Polyol-pathway flux resulting in a reduction in Glutathione	7
1.2.2 Pathway 2: Increased intracellular formation of advanced glycation end-products.....	8
1.2.3 Pathway 3: Activation of Protein Kinase C	9
1.2.4 Pathway 4: Increased flux through the Hexosamine pathway.....	10
1.2.5 The common element	11
1.3 Treatment of diabetic cardiomyopathy.....	12
1.3.1 Antioxidant therapies	13
1.3.2 Triethylenetetramine (TETA) as a treatment for diabetic cardiomyopathy	13
1.3.2.1 Current understanding of TETA pharmacology.....	15
1.4 Copper homeostasis	16
1.4.1 Normal copper homeostasis	16
1.4.1.1 Copper uptake into the cell.....	17
1.4.1.2 Copper distribution within cells.....	18
1.4.2 Copper and oxidative stress	19
1.4.3 Defective copper metabolism in diabetes	20
1.4.4 Copper and the development of cardiomyopathy	21
1.5 Thesis Objectives	22
1.6 Experimental Approach	23
1.6.1 Streptozotocin-induced diabetic animal model	23
1.6.2 Microarrays	25
1.6.2.1 Fabrication of microarrays.....	25
1.6.2.2 Microarray general experimental outline	27
1.6.2.3 Minimum Information About a Microarray Experiment (MIAME).....	28
1.7 Summary	29

Chapter 2 Materials and Methods	30
2.1. Animal model	30
2.1.1 Administration of Triethylenetetramine (TETA).....	30
2.1.2 Metabolic cage 24hr urine collection.....	31
2.2 Isolation of RNA from the left ventricle of a rat heart	31
2.2.1 Tissue collection.....	31
2.2.1.1 Surgical procedure (Studies One and Two)	31
2.2.1.2 Perfusion and removal of left ventricle	32
2.2.2 RNA Isolation	33
2.2.2.1 RNA isolation from tissue using Qiagen MIDI Kit protocol.....	33
2.2.2.2 RNA isolation from tissue using a combined TRIZOL/Qiagen MINI method (Study Two).....	34
2.2.2.3 Assessment of RNA Quality/Quantity.	34
2.3 Microarray Analysis.....	35
2.3.1 RNA.....	35
2.3.2 Ramaciotti Rat 10K Combo Slides.....	35
2.3.2.1 cDNA synthesis and labelling.....	36
2.3.2.2 Short blocking protocol for Eppendorf Creative – Epoxy	36
2.3.2.3 Hybridisation.....	37
2.3.2.4 Post-hybridisation washing.....	37
2.3.2.5 Scanning.....	37
2.3.3 Amersham Codelink.....	38
2.3.3.1 cRNA synthesis from total RNA	38
2.3.3.2 Hybridisation.....	38
2.3.3.3 Washing.....	38
2.3.3.4 Scanning.....	39
2.3.4 Agilent	39
2.3.4.1 cRNA synthesis from total RNA	39
2.3.4.2 Hybridisation.....	40
2.3.4.3 Washing.....	40
2.3.4.4 Scanning and analysis of slides	40
2.3.5 Affymetrix	40
2.3.5.1 cDNA synthesis	40
2.3.5.2 Synthesis of biotin-labelled cRNA	41
2.3.5.3 Target hybridisation	41
2.3.5.4 Washing, staining and scanning.....	42
2.4 Real-time quantitative PCR validation	43
2.5 Histology	44
2.5.1 Indirect <i>in situ</i> immunofluorescence labelling of collagen.....	44
2.5.2 Nile red staining of lipid in frozen left ventricle heart sections	44
2.5.3 Transmission electron microscopy (TEM) ultrastructural analysis	45
2.5.4 Imaging	45
2.5.4.1 Confocal imaging of collagen III	45
2.5.4.2 Confocal Imaging of cardiac tissue lipid (Nile red).....	46
2.6 Serum biochemistry	46
2.6.1 Albumin	47
2.6.2 Alkaline phosphatase (ALP).....	47
2.6.3 Alanine aminotransferase (ALT)	47

2.6.4 Aspartate aminotransferase (AST)	47
2.6.5 Calcium	48
2.6.6 Chloride	48
2.6.7 Cholesterol	49
2.6.8 Creatinine.....	49
2.6.9 Ferroxidase	49
2.6.10 HDL cholesterol.....	50
2.6.11 Iron	50
2.6.12 Non-esterified (free) fatty acids (NEFA).....	51
2.6.13 Phosphate	51
2.6.14 Potassium	51
2.6.15 Sodium	52
2.6.16 Total bilirubin.....	52
2.6.17 Total protein	53
2.6.18 Triglyceride.....	53
2.6.19 Urea	53
2.7 Determination of TETA, monoacetylated-TETA (MAT) and diacetylated-TETA (DAT) levels using an HPLC based methodology	54
2.7.1 Reagents	54
2.7.2 Protocol	55
2.7.3 Sample dilution.....	55
2.7.4 HPLC protocol.....	55
2.8 Graphite Furnace - Atom Absorption Spectroscopy (GF-AAS) analysis of copper, zinc, manganese or iron levels in 24hr rat urine	56
2.8.1 Sample preparation.....	56
2.8.2 Instrument details and specific settings for the detection of Cu, Zn, Mn or Fe.....	57
2.8.3 Analysis by GF-AAS.....	57
2.9 Flame ionisation detection thin-layer chromatography (latroscan) determination of lipid in heart tissue.....	58
2.9.1 Lipid extraction	58
2.9.2 Rod spotting and latroscan run.....	58
2.9.3 Quantification of lipid.....	59
2.10 Cardiac mitochondria functional assays.....	60
2.10.1 Isolation of cardiac mitochondria	60
2.10.2 Enzyme functional assays	60
2.10.2.1 Citrate synthase (CS, E.C.4.1.3.7).....	61
2.10.2.2 L3-hydroxyacyl CoA:NAD ⁺ oxidoreductase (HOAD, E.C.1.1.1.35).....	61
2.10.2.3 Carnitine palmitoyl transferase (CPT, E.C. 2.3.1.21).....	61
2.10.2.4 Isocitrate dehydrogenase (IDH-NADP ⁺ , E.C. 1.1.1.42).....	61
2.11 Inductively coupled plasma – mass spectrometer (ICP-MS) Analysis	62
2.11.1 Method detection limits and analytical range.....	63
2.12 Statistical Analysis	63
2.12.1 General	63
2.12.2 Microarray statistics	63
2.12.2.1 Agilent.....	63
2.12.2.2 Amersham	63
2.12.2.3 Affymetrix.....	64
2.12.3 GSEA	64

2.12.4 Mixed model statistics	66
2.12.4.1 Iatrosan data	66
2.12.4.2 RT PCR data	66
2.12.5 Dose-response data	67
2.12.5.1 Split-plot in time ANOVA or ANCOVA	67
2.12.5.2 General linear model ANOVA or ANCOVA	67
2.12.5.3 Repeated measures ANOVA (Mixed Linear Model)	68
2.12.5.4 Regression analysis	68

Chapter 3 Pilot study to assess suitability of three commercially available microarray platforms for assessment of changes in gene expression 69

3.1 Introduction	69
3.1.1 Hybridising the probe to the array	70
3.1.2 Washing and staining of slides	70
3.1.3 Spot finding	70
3.1.4 Single versus competitive hybridisation	71
3.1.5 Cost and ease of use	72
3.2 Results	72
3.2.1 Spotted arrays from Clive & Vera Ramaciotti Centre for Gene Function Analysis	72
3.2.2 Amersham, Agilent and Affymetrix commercially available microarray systems	74
3.2.2.1 Amersham Codelink	75
3.2.2.2 Agilent	77
3.2.2.3 Affymetrix	80
3.2.3 Definition of significant change in gene expression	82
3.2.4 Inter-platform variability	84
3.3 Discussion	88
3.3.1 Overview of the three commercially available systems	88
3.3.2 Correlation between Affymetrix and Agilent Systems	90
3.4 Conclusions	91

Chapter 4 Assessment of differences in the transcriptome between STZ-diabetic and sham LV heart tissue at sixteen weeks 92

4.1 Introduction	92
4.2 Results	93
4.2.1 Characterisation of the STZ-diabetic model at sixteen weeks	93
4.2.2 Microarray changes in gene expression	96
4.2.2.1 Mitochondrial energy utilisation	97
4.2.2.2 Diabetic complications as a consequence of excess ROS	101
4.2.3 Real time quantitative PCR (RT-qPCR) validation of microarray results	106
4.3 Discussion	107
4.3.1. Metabolic Inflexibility	108
4.3.1.1 Carbohydrate metabolism	108
4.3.1.2 Lipid metabolism	109
4.3.2 Hypothesis for damage caused in heart by ROS	113
4.3.2.1 Reduction in GSH	113

4.3.2.2 Increased intracellular formation of AGEs.....	113
4.3.2.3 Activation of PKC.....	114
4.3.2.4 Increased flux through the Hexosamine pathway	114
4.3.2.5 The common element.....	115
4.3.2.6 Relationship between fatty acid oxidation, ROS generation and mitochondrial function	116
4.4 Conclusions	117

Chapter 5 Characterization the physiological effects on and metabolism of TETA-disuccinate in diabetic and sham rats after eight weeks of treatment119

5.1 Introduction.....	119
5.2 Results.....	120
5.2.1 Experimental design	120
5.2.1.1 Stability study of TETA-disuccinate in water	121
5.2.2 Physiological characterization.....	122
5.2.2.1 Blood glucose	122
5.2.2.2 Total body weight	123
5.2.2.3 Heart weight	127
5.2.3 Serum biochemistry	128
5.2.3.1 Liver function tests	129
5.2.3.2 Lipid markers	129
5.2.3.3 Renal function.....	130
5.2.3.4 Metal ion homeostasis.....	130
5.2.4 TETA and metabolite analysis	133
5.2.4.1 Serum	134
5.2.4.2 Urine	136
5.2.5 Levels of trace metals, copper, zinc, iron and manganese in 24hr urine	144
5.2.5.1 Changes in metal ion excretion with the onset of diabetes.....	144
5.2.5.2 Change in metal ion excretion with TETA treatment over time.....	146
5.2.5.3. Correlation between urine levels of TETA, metabolites and trace metals	149
5.3 Discussion.....	152
5.3.1 Physiological characteristics	152
5.3.1.1 Total body and heart weight.....	152
5.3.1.2 Serum biochemistry.....	153
5.3.2 TETA and metabolite levels	153
5.3.3 Levels of trace metals, copper, zinc, iron and manganese in 24hr urine	154
5.3.4 Relationship between TETA, MAT and metal levels in the serum and urine of treated animals.....	155
5.4 Conclusion	156

Chapter 6 Molecular changes in the left ventricle of diabetic and sham animals after eight weeks treatment with a high dose of TETA-disuccinate	157
6.1 Introduction	157
6.2 Results	158
6.2.1 Summary of results from Chapter Five for untreated and treated animals	158
6.2.2 Microarray analysis	159
6.2.2.1 Selection criteria	159
6.2.2.2 Analysis of changes in gene expression	160
6.2.2.3 Gene Set Enrichment Analysis	165
6.2.3 RTqPCR	169
6.2.4 Histology	171
6.2.4.1 Collagen III and myocyte histology	172
6.2.4.2 Nile Red staining for lipid content	173
6.2.4.3 TEM scanning of muscle fibres	175
6.2.5 Flame-ionisation detection thin-layer chromatography (Iatroscan)	176
6.2.6 Mitochondrial functional analysis	178
6.2.6.1 Citrate synthase	178
6.2.6.2 CPT	179
6.2.6.3 HOAD	180
6.2.6.4 IDH-NADP+	181
6.3 Discussion	182
6.3.1 Gene expression analysis	182
6.3.2 ECM structure in the LV	185
6.3.3 Changes in fuel metabolism	186
6.4 Conclusions	188
 Chapter 7 Final Discussion and Conclusions	 190
7.1 Thesis findings	190
7.1.1 Primary aim of Thesis	190
7.1.2 Summary of main findings	190
7.1.2.1 Pilot study to assess suitability of three commercially available microarray platforms for assessment of changes in gene expression	190
7.1.2.2 Assessment of differences in the transcriptome between STZ-diabetic and sham LV heart tissue at sixteen weeks	191
7.1.2.3 Physiological and molecular changes in the left ventricle of diabetic and sham animals after eight weeks treatment with TETA-disuccinate	193
7.1.2.4 Characterization of the metabolism of TETA-disuccinate by diabetic and sham rats after eight weeks of treatment and its relationship to metal ion excretion	198
7.2 Potential mechanism of TETA	201
7.3 Limitations of the current studies	204
7.3.1 Study design	204
7.3.2 Microarray technology	204
7.3.2.1 Statistical analysis	204
7.3.2.2 Oligonucleotide length	208

7.4 Future experiments	209
7.4.1 Microarray analysis of LV heart tissue at different doses of TETA.....	209
7.4.2 Radioassays.....	210
7.4.2.1 Analysis of β -oxidation	210
7.4.2.2 TETA distribution	210
7.4.3 Effects of insulin treatment combined with TETA treatment.....	211
7.4.4 Effects of TETA treatment on the liver	211
7.5 Concluding summary	212
 Appendix 1: Bioanalyzer Analysis of RNA samples (Melbourne)	 214
1.i Bioanalyzer Analysis.....	214
 Appendix 2: Statistical Analysis of latroscan Data	 215
2.i Exclusion criteria.....	215
2.ii Statistical analysis of latroscan TG data with and without excluded animals ..	216
2.iii Pearson's correlation analysis of lipid level and final body weight	217
 Reference	 218

List of Figures

Figure 1.1 AGE formation pathways.....	8
Figure 1.2 Representative molecular structures of TETA and its metabolites MAT and DAT.....	16
Figure 1.3 Chemical structure of streptozotocin (STZ).....	24
Figure 1.4 Schematic of a general microarray experiment.....	27
Figure 3.1 Ramaciotti 10K Combo microarray slides	73
Figure 3.2 Amersham CodeLink UniSet Rat 1 Bioarrays	76
Figure 3.3 Agilent 22K Rat Oligo Microarray	78
Figure 3.4 Affymetrix Rat 230 2.0 GeneChip.....	81
Figure 3.5 Venn diagram of the distribution of significant genes, using a 1.5-fold cut off, of the subset of genes that are common between all three systems.....	83
Figure 3.6 Venn diagram of the distribution of significant genes, at a $P < 0.05$ cut-off, of the subset of genes that are common between all three systems.....	84
Figure 3.7 Plot of all fold-change values from Agilent slides plotted against all fold-change values from Affymetrix slides	85
Figure 3.8 Initial correlation analysis of 825 probes common to the Agilent and Affymetrix microarray systems to be used in probe match analysis	86
Figure 3.9 Correlation between sequence matched probes of the Agilent and Affymetrix microarray systems.....	87
Figure 3.10 Correlation between Agilent and Affymetrix, fold-change values with $P < 0.0588$	
Figure 4.1 Gross characterisation of STZ diabetic model.....	94
Figure 4.2 Heart weight and heart weight/body weight comparison	95
Figure 4.3 Pie chart illustrating the global distribution of genes in rat LV myocardium with expression significantly altered by diabetes	97
Figure 4.4 Schematic representation of fuel-metabolic pathways affected by diabetes in the heart.....	112
Figure 5.1 General experimental design.....	120
Figure 5.2 Stability of TETA-disuccinate in milliQ water	122
Figure 5.3 Analysis of changes in weight as a result of STZ or saline injection	124
Figure 5.4 Analysis of changes in weight as a result of TETA administration	126
Figure 5.5 Effects of TETA-disuccinate treatment on absolute heart weight with and without animal ID # 42	128
Figure 5.6 Serum copper and ferroxidase Levels.....	131
Figure 5.7 Iron levels in serum	132
Figure 5.8 Zinc levels in serum.....	133
Figure 5.9 TETA levels in 16 week terminal serum	134
Figure 5.10 MAT levels in 16 week terminal serum.....	135
Figure 5.11 TETA in urine over Week 10 and 15 time points	138
Figure 5.12 Levels of MAT in 24hr urine at 10 and 15 weeks	139
Figure 5.13 Levels of DAT in 24hr sham urine at Week 10 and 15	140

Figure 5.14 Levels of DAT in 24hr sham and diabetic urine at Week 10.....	141
Figure 5.15 Percentage of unmetabolised TETA in urine.....	143
Figure 5.16 Metal ion levels six weeks after injection with STZ or saline	145
Figure 5.17 Change in iron and manganese excretion combined over the Week 10 and 15 24hr collection corrected for body weight	147
Figure 5.18 Copper excretion over time (Weeks 10 and 15).....	148
Figure 5.19 Zinc excretion over time (Weeks 10 and 15).....	149
Figure 6.1 Confocal image analysis of collagen III levels in the LV.....	172
Figure 6.2 Confocal Image analysis of lipid levels in the LV.....	174
Figure 6.3 Representative TEM images of LV myocardium	176
Figure 6.4 Citrate synthase.....	179
Figure 6.5 CPT activity in isolated mitochondria.....	180
Figure 6.6 HOAD activity in isolated mitochondria	181
Figure 6.7 IDH activity in isolated mitochondria.....	182
Figure 1.i Bioanalyzer results for pooled RNA samples	214
Figure 2.i Diabetic untreated group weight analysis	215
Figure 2.ii Correlation analysis between animal tissue TG level and final body weight...	217

List of Tables

Table 1.1 Three stages of diabetic cardiomyopathy	2
Table 2.1 Block design	31
Table 2.2 Solutions for cleaning of equipment and surfaces, heart perfusion	32
Table 2.3 Ramaciotti hybridisation solution for Hybrislip covers.....	37
Table 2.4 Ramaciotti solutions for washing of epoxy slides post-hybridisation	37
Table 2.5 Affymetrix GeneChip® hybridisation cocktail.....	42
Table 2.6 Affymetrix stain components.....	43
Table 2.7 GF-AAS settings	57
Table 2.8 Instrumental operating parameters for ICP-MS	62
Table 2.9 ICP-MS detection limits	63
Table 3.1 Amersham slide comparison	76
Table 3.2 Diabetes vs. sham top 10 genes	77
Table 3.3 Agilent slide comparison.....	79
Table 3.4 Diabetes vs. sham top 10 genes	79
Table 3.5 Affymetrix slide comparison.....	82
Table 3.6 Diabetes vs. sham top 10 genes	82
Table 3.7 Overview of all three systems.....	89
Table 4.1 Biochemical markers in serum from STZ-diabetic and sham rats	95
Table 4.2 Genes involved in carbohydrate metabolism (as defined by their GO annotation) whose expression in LV myocardium was significantly altered by diabetes.....	98
Table 4.3 Genes associated with lipid metabolism (as defined by their GO annotation) whose expression was significantly altered in diabetic LV	100
Table 4.4 Genes involved in GSH metabolism	102
Table 4.5 Expression changes in genes associated with activation of PKC.....	103
Table 4.6 Expression changes of genes associated with the Hexosamine pathway.....	104
Table 4.7 Genes that play roles in oxidative stress (as defined by their KEGG pathway description) whose expression was significantly altered in diabetic LV	105
Table 4.8 Comparison between gene expression differences determined by RT-qPCR and microarray methods	107
Table 5.1 Amounts of water and food consumed and urine excreted during 24hr	121
Table 5.2 Heart weight and heart weight body weight measurements	127
Table 5.3 Indicators of liver function	129
Table 5.4 Lipid levels in serum	129
Table 5.5 Indicators of renal function in serum biochemical markers.....	130
Table 5.6 Average TETA-disuccinate intake (24hr urine collection).....	136
Table 5.7 Correlation analysis in the urine of sham animals	150
Table 5.8 Correlation analysis in the urine of diabetic animals.....	151
Table 5.9 Correlation analysis in the serum of sham animals	151
Table 5.10 Correlation analysis in the serum of diabetic animals.....	152
Table 6.1 Physiological changes	158

Table 6.2 Serum biochemistry	159
Table 6.3 Copper and Zinc excretion.....	159
Table 6.4 Genes involved in carbohydrate metabolism (as defined by their GO annotation) whose expression in LV myocardium was significantly altered by diabetes.....	161
Table 6.5 Genes associated with lipid metabolism (as defined by their GO annotation) whose expression was significantly altered in diabetic LV	161
Table 6.6 Expression changes in genes associated with activation of PKC.....	162
Table 6.7 Genes that play roles in oxidative stress (as defined by their KEGG pathway description) with expression significantly altered in diabetic LV	162
Table 6.8 Sham untreated vs. sham-treated, top 15 genes (based on unadjusted P-value)	162
Table 6.9 Diabetic untreated vs. diabetic-treated, top 15 genes (based on unadjusted P-value).....	163
Table 6.10 Gene expression changes common to both treated vs. untreated comparisons	164
Table 6.11 GSEA comparison summary	166
Table 6.12 Gene-sets identified as significantly correlated with the diabetic group	167
Table 6.13 Gene-sets identified as significantly correlated with the sham group	167
Table 6.14 Gene-sets identified as significantly correlated with the diabetic group	167
Table 6.15 Gene-sets identified as significantly correlated with the diabetic group	168
Table 6.16 Sham vs. diabetic comparison.....	170
Table 6.17 Diabetic-treated vs. diabetic comparison.....	171
Table 6.18 Sham-treated vs. sham comparison	171
Table 6.19 Percentage area of Collagen III staining.....	173
Table 6.20 Percentage of Nile red (lipid) area staining.....	175
Table 6.21 Lipid classes determined by Iatroscan analysis.....	177
Table 7.1 Literature review of microarray studies looking at changes in gene expression in the diabetic state.....	206
Table 2.i Covariance parameter estimates (all data).....	216
Table 2.ii Mixed model ANOVA table (Type III error) all data.....	216
Table 2.iii Covariance parameter estimates (rat 8, 42, 145 and 168 data excluded)	216
Table 2.iv Mixed model ANOVA table (Type III error), rat 8, 42, 145 and 168 data excluded)	216

Abbreviations

ACC	Acetyl-CoA carboxylase
AGE	Advanced glycation end products
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
Angptl4	Angiopoeitin-like protein 4
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
AP-1	Activating protein 1
AST	Aspartate aminotransferase
AT II	Angiotensin II
ATP	Adenine triphosphate
ATP7A	Cation-transporting P-type ATPase 7A
ATP7B	Cation-transporting P-type ATPase 7B
ATX1	Anti-oxidant protein 1
BSA	Bovine serum albumin
BW	Body weight
°C	Temperature (degrees celsius)
CAD	Coronary artery disease
CCCP	Carbonyl cyanide <i>m</i> -chlorophenylhydrazone
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate
cmH ₂ O	Centimeters of water (pressure)
CoA	Coenzyme A
COX	Cytocrome oxidase
Cox17	Cytochrome oxidase assembly, subunit17
CPT	Carnitine palmytoyl transferase
cRNA	Complementary ribonucleic acid
CS	Citrate synthase
CTGF	Connective tissue growth factor
CTR	Copper transport protein
Cu	Copper
CV	Coefficient of variation
CVD	Cardiovascular disease
Cy-3/5	Cyanine-3/5
Cytb5	Cytochrome b5 (mitochondrial)
Da	Peptide mass (Dalton)
DAG	Diacylglycerol
DAT	<i>N</i> ₁ , <i>N</i> ₁₀ -diacetyltriethylenetetramine
DCM	Diabetic cardiomyopathy
DEPC	Diethylpyrocarbonate
DTT	Dithiothreitol
dNTP	Deoxyribonucleotide triphosphate
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
ECM	Extracellular matrix
EDTA	Ethylenediamine tetraacetic acid
ES	Enrichment score
ET	Endothelin
FA	Fatty acid
FAO	Fatty acid oxidation
FAT	Fatty acid transporter
FDR	False discovery rate
Fe	Iron
FFA	Free fatty acid
FMOC	9-Fluorenylthoxycarbonyl chloride

g	Mass (gram)
g	Acceleration due to gravity
γGCS	γ-glutamylcysteine synthetase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GF-AAS	Graphite furnace - atom absorption spectroscopy
GFAT	Glutamine:fructose-6-phosphate amidotransferase
GEO	Gene expression omnibus
GlcNAc	UDP- <i>N</i> -acetylglucosamine
GLUT	Glucose transporter
GO	Gene ontology
GSEA	Gene-set enrichment analysis
GSH	Glutathione
GST	Glutathione S-transferase
HAD	Hexamethylenediamine dihydrochloride
hCTR	Human copper transport protein
HDL	High density lipoprotein
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HF	Heart failure
HNO ₃	Nitric acid
H ₂ O	Water
H ₂ O ₂	Hydrogen peroxide
HOAD	L3-hydroxyacyl CoA:NAD ⁺ oxidoreductase
HPLC	High performance liquid chromatography
hr	Time (hour)
HW	Heart weight
HW/BW	Heart weight/body weight ratio
ICP-MS	Inductively coupled plasma-mass spectrometer
IDH	isocitrate dehydrogenase
IgG	Immunoglobulin G
IL-1β	Interleukin-1β
KEGG	Kyoto encyclopaedia of genes and genomes
LC-MS	Liquid chromatography-mass spectrometry
LPL	Lipoprotein lipase
LV	Left ventricle
MAT	<i>N</i> ₁ -acetyltriethylenetetramine
MCD	Malonyl-CoA decarboxylase
MES	Maximum enrichment score
μg	Mass (microgram)
mg	Mass (milligram)
MIAME	Minimum information about microarray experiments
min	Time (minute)
M	Amount of substance per litre (molar)
mm	Length (millimetre)
μM	Amount of substance per litre (micromolar)
mM	Amount of substance per litre (millimolar)
μl	Volume (microlitre)
ml	Volume (millilitre)
MMLV	Moloney Murine Leukemia virus
mol	Amount of substance
mmol	Amount of substance
MMP	Matrix metalloproteinase
Mn	Manganese
mRNA	Messenger ribonucleic acid
MRI	Magnetic resonance imaging
MT	Metallothionein
mtDNA	Mitochondrial deoxyribonucleic acid
MVEC	Microvascular endothelial cell

NAD ⁺	Nicotinamide adenine dinucleotide
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NCBI	National Center for Biotechnology Information
NF- κ B	Nuclear factor- κ B
nm	Length (nanometre)
NO	Nitric oxide
NOD	Non-obese diabetic mouse
NOS	Nitric oxide synthases
NTP	Nucleotide triphosphate
OCT	Optimum cutting temperature
O/N	Overnight
ONOO ⁻	Peroxynitrite
OXPHOS	Oxidative phosphorylation
PAI-1	Plasminogen activator inhibitor-1
PARP	Poly-(ADP-ribose) polymerase
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDH	Pyruvate dehydrogenase
PDK	Pyruvate dehydrogenase kinase
PDP	Pyruvate dehydrogenase phosphatase
PEPK	Phosphoenolpyruvate carboxykinase
PET	Positron-emission tomography
PKC	Protein kinase C
PPAR	Peroxisome proliferator-activated receptor
RAGE	Receptor of AGE
REML	Restricted maximum-likelihood
RNA	Ribonucleic Acid
RNase	Ribonuclease
ROS	Reactive oxygen species
rpm	Revolutions per minute
RT	Room temperature
RT-qPCR	Real time quantitative polymerase chain reaction
s	Time (second)
SAPE	Streptavidin-phycoerythrin
SCO	Synthesis of cytochrome oxidase
SDS	Sodium dodecyl sulphate
SHR	Spontaneously hypertensive rat
snRNP	Small nuclear ribonucleoprotein
SOD	Superoxide dismutase
STZ	Streptozotocin
TBS	Tris buffered saline
TCA	Tricarboxylic acid cycle
TEM	Transmission electron microscope
TETA	Triethylenetetramine
Tfam	Transcription factor A, mitochondrial
TG	Triglyceride
TGF- β 1	Transforming growth factor - β 1
TIE	TGF- β 1 inhibitory elements
TIGR	The Institute for Genomic Research
TOC	Tri-functional β -oxidation complex
TNF- α	Tumour necrosis factor- α
TXN	Thioredoxin
UCP	Uncoupling protein
UV	Ultraviolet
VLDL	Very low-density lipoprotein
yCCS	Yeast copper chaperone for superoxide dismutase
ZDF	Zucker diabetic fatty rat
Zn	Zinc