HAEMODYNAMIC EFFECTS OF ADIPONECTIN ON PPAR–γ RECEPTORS IN DIABETIC AND NON-DIABETIC WISTAR KYOTO AND SPONTANEOUSLY HYPERTENSIVE RATS

SHERYAR AFZAL

UNIVERSITI SAISNS MALAYSIA
2017
HAEMODYNAMIC EFFECTS OF ADIPONECTIN ON PPAR–γ RECEPTORS IN DIABETIC AND NON-DIABETIC WISTAR KYOTO AND SPONTANEOUSLY HYPERTENSIVE RATS

by

SHERYAR AFZAL

Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

January 2017
DEDICATION

This thesis is dedicated to beloved Prophet Mohammad (peace be upon him). As regards all standards by which human greatness may be measured, we can say it for sure; there is no man greater than Him.
ACKNOWLEDGEMENT

First, and foremost, I would like to thank Almighty Allah for giving me the strength to complete my PhD studies. I would like to express my deepest gratitude and appreciation to my supervisor Prof. Dr Munavvar Zubaid Abdul Sattar for giving me the opportunity to work in this project. He stood beside me when I missed a family member, was always available as a friend and was exceedingly supportive even in times when I was going off in directions, and gave me all the support anytime without hesitation. I really appreciate his open-door policy to me and I have learned from him many lessons in life.

Second in line is Professor Emeritus Edward Johns, Department of Physiology, University College Cork, Ireland. Special thanks and appreciation go to him as I have no enough words to thank him. He has always allowed me complete freedom to do what I felt was relevant and has always been ready to infuse me with some of his characteristic optimism. I am indeed very fortunate to know a great scientist like him and have learned from him valuable things that I will appreciate the whole of my life. I would like to take this opportunity to thank my all laboratory colleagues, staff members and my fellow post graduate friends at Cardiovascular & Renal Physiology School of Pharmaceutical Sciences, Universiti Sains Malaysia for their support, love and friendship throughout my study period.

Last, but not the least, gratitude goes to my late father, Dr. Hameed Afzal and mother Surraiya Begum, who would have been proud of me. I remember their prayers and they have always followed me. I would like to thank all my family members; they have clung to me all my life. Special appreciation and thanks goes for my wife Saima
Sheryar for her patience throughout my studies and for her sacrifice, and love beyond anyone’s understanding, and my beautiful daughters Meerab Sheryar and Minsa Sheryar for their everlasting love and care. I will always appreciate their support and care.
# TABLE OF CONTENTS

ACKNOWLEDGMENT ii

TABLE OF CONTENTS iv

LIST OF TABLES xvii

LIST OF FIGURES xxvii

LIST OF ABBREVIATIONS xxxiv

ABSTRAK xxxviii

ABSTRACT xl

## CHAPTER 1: INTRODUCTION 1

1.1 Kidney 1

1.1.1 Anatomy of kidney 1

1.1.2 Physiology of kidney 3

1.2 Cardiovascular system 4

1.2.1 Anatomy of cardiovascular system 4

1.2.2 Heart and blood vessels 5

1.2.3 Physiology of cardiovascular system 6

1.3 Hypertension 8

1.3.1 Blood Pressure 9

1.3.2 Primary and secondary hypertension 9

1.3.3 Pathophysiology of hypertension 10

1.4 Diabetes 14
1.4.1 Diabetes and hypertension

1.4.2 Complications of diabetes in hypertension and oxidative stress

1.4.3 Diabetic nephropathy

1.4.4 Animal model of diabetic nephropathy

1.5 Oxidative stress

1.5.1 Reactive oxygen species

1.5.2 Nitric oxide, oxidative stress and hypertension

1.5.3 Regulation of blood pressure by the kidney

1.5.4 Hypertension and renal involvement

1.6 Experimental models of oxidative stress

1.6.1 Spontaneously hypertensive rat (SHR)

1.7 The Sympathetic nervous system (SNS)

1.7.1 The sympathetic nervous system and regulation of arterial pressure

1.7.2 Adrenoceptors

1.7.3 Renal α1-adrenoceptors subtypes

1.8 The renin-angiotensin system and its components

1.8.1 Inhibition of renin-angiotensin system

1.8.1(a) ACE inhibition

1.8.1(b) Angiotensin receptor blockade

1.9 Interaction between renin-angiotensin and sympathetic nervous systems

1.10 Adiponectin

1.10.1 Biosynthesis of adiponectin
1.10.2 Adiponectin forms and levels 48
1.10.3 Adiponectin receptors and Peroxisome Proliferator Activated receptor (PPAR) 50
1.10.4 Adiponectin regulation and metabolic syndrome 54
1.10.5 Role of adiponectin in diabetes and thiazolidinediones 58
1.10.6 Adiponectin, nitric oxide and endothelial function 63
1.10.7 Adiponectin and the antihypertensive drugs 68
1.10.7(a) Adiponectin and renin angiotensin aldosterone system (RAAS) 69
1.10.8 Adiponectin and the SNS 72
1.11 Research outline 73
1.11.1 Antioxidant potential of adiponectin 73
1.11.2 Effects of exogenous adiponectin, pioglitazone and irbesartan treatments 74
1.11.3 The effect of exogenous administration of adiponectin, irbesartan and pioglitazone on the adrenergic control of systemic and renal haemodynamics in diabetic and non diabetic normotensive and hypertensive rats 74
1.11.4 The interaction between renin-angiotensin, sympathetic nervous systems and peroxisome proliferator-activated receptor in the renal and systemic vasculature of adiponectin treatment alone and in combination with irbesartan or pioglitazone 75
1.12 Hypothesis 76
CHAPTER TWO: MATERIAL AND METHODS

2.1 Experimental Animals

2.1.1 General description

2.2 Experimental design

2.2.1 Renal haemodynamics study

2.2.2 Systemic vasopressor response study

2.3 Induction of Diabetes

2.4 Chronic treatment with Irbesartan, pioglitazone and adiponectin pre-treatment

2.5 Measurements

2.5.1 Physiological data (Basal and follow-up metabolic)

2.5.2 Measurement of conscious or non invasive blood pressure (NIBP)

2.6 Biochemistry of urine and plasma

2.6.1 Measurement of plasma and urine creatinine levels

2.6.1(a) Urine creatinine level

2.6.1(b) Plasma creatinine level

2.6.2 Creatinine clearance (mL/min/1000 gram of body BW)

2.6.3 Measurement of plasma and urine sodium and potassium levels

2.6.4 Calculation of renal functional parameters
2.6.4 (a) Urine flow rate
2.6.4 (b) Absolute urinary sodium and potassium excretion
2.6.4 (c) Fractional sodium and potassium excretion (FE x) (%)
2.6.4 (d) Urinary sodium to potassium ratio

2.6.5 Kidney Index

2.7 Histology of kidney tissue
2.7.1 Fixation of the tissue
2.7.2 Dehydration
2.7.3 Clearing
2.7.4 Embedding
2.7.5 Staining procedure

2.8 Measurement of in vivo oxidative stress and antioxidant markers
2.8.1 Plasma malondialdehyde
2.8.2 Total superoxide dismutase
2.8.3 Nitric oxide
2.8.4 Total anti-oxidant capacity
2.8.5 Plasma glutathione

2.9 Quantitative determination of adiponectin in rat plasma
2.9.1 Assay Procedure for plasma adiponectin concentration

2.10 Measurement of plasma triglycerides and lipoproteins (total cholesterol, LDL and HDL) level
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.11</td>
<td>Acute experiments: hemodynamic studies</td>
<td>108</td>
</tr>
<tr>
<td>2.11.1</td>
<td>Preparation and surgical procedure</td>
<td>108</td>
</tr>
<tr>
<td>2.11.2</td>
<td>Experimental protocol</td>
<td>110</td>
</tr>
<tr>
<td>2.11.2(a)</td>
<td>Systemic vasopressor response study</td>
<td>110</td>
</tr>
<tr>
<td>2.11.2(b)</td>
<td>Acute renal vasoconstrictor responses study</td>
<td>111</td>
</tr>
<tr>
<td>2.11.2(c)</td>
<td>Measurement of Pulse wave velocity</td>
<td>112</td>
</tr>
<tr>
<td>2.11.2(d)</td>
<td>Propagation distance</td>
<td>112</td>
</tr>
<tr>
<td>2.11.2(e)</td>
<td>Propagation time</td>
<td>112</td>
</tr>
<tr>
<td>2.11.3</td>
<td>Preparation of drugs (Vasoactive agents)</td>
<td>114</td>
</tr>
<tr>
<td>2.12</td>
<td>Data analysis</td>
<td>114</td>
</tr>
<tr>
<td>2.12.1</td>
<td>The metabolic, haemodynamics and functional parameters during the follow up study</td>
<td>114</td>
</tr>
<tr>
<td>2.12.2</td>
<td>Systemic vasopressor responses to Ang II and adrenergic agonists</td>
<td>115</td>
</tr>
<tr>
<td>2.13</td>
<td>List of chemicals</td>
<td>116</td>
</tr>
<tr>
<td>2.14</td>
<td>List of equipment</td>
<td>117</td>
</tr>
</tbody>
</table>

**CHAPTER 3: RESULTS**

3.1 General observations
   3.1.1 General observations in Streptozocotin-induced diabetic rats
3.2 In-vivo results of WKY (control), WKY+irbesartan, WKY+pioglitzone, WKY+adiponectin, WKY+irbesartan adiponectin
and WKY+pioglitazone+adiponectin

3.2.1 Body weight
3.2.2 Water intake
3.2.3 Urine flow rate
3.2.4 Blood glucose concentration
3.2.5 Systolic blood pressure, mean arterial pressure, diastolic blood pressure and heart rate
3.2.6 Plasma adiponectin and triglycerides
3.2.7 Plasma, urine creatinine and creatinine clearance (Cr.cl.)
3.2.8 Plasma and urinary sodium concentration
3.2.9 Absolute urinary sodium excretion (UNaV), fractional sodium excretion (FENa), urinary potassium and urinary sodium to potassium (UNa/UK) ratio
3.2.10 Pulse wave velocity (PWV), renal cortical blood perfusion (RCBP), oxidant and antioxidant biochemical markers
3.2.11 Systemic vasopressor responses study
   3.2.11(a) Acute vasopressor responses to adrenergic agonists and angiotensin II
3.2.12 Acute renal vasoconstriction experiments

3.3 In-vivo results of WKY (control), WKY diabetic, WKY diabetic irbesartan, WKY diabetic pioglitazone, WKY diabetic adiponectin, WKY diabetic Irbesartan+adiponectin and WKY
diabetic Pioglitazone+adiponextin treated groups

3.3.1 Body weight 126
3.3.2 Water intake 127
3.3.3 Urine flow rate 127
3.3.4 Blood glucose concentration 128
3.3.5 Systolic blood pressure, mean arterial pressure, diastolic blood pressure and heart rate 129
3.3.6 Plasma adiponectin and triglycerides 130
3.3.7 Plasma, urine creatinine and creatinine clearance (Cr.cl.) 131
3.3.8 Plasma and urinary sodium concentration 132
3.3.9 Absolute urinary sodium excretion and fractional sodium excretion 134
3.3.10 Urinary potassium and urinary sodium to potassium ratio 135
3.3.11 Kidney index 136
3.3.12 Pulse wave velocity and renal cortical blood perfusion 137
3.3.13 Plasma total superoxide dismutase and malondialdehyde 138
3.3.14 Plasma nitric oxide and total antioxidant capacity 139
3.3.15 Plasma glutathione 141
3.3.16 Systemic vasopressor responses study 141
   3.3.16(a) Noradrenaline 141
   3.3.16(b) Phenylephrine 142
   3.3.16(c) Methoxamine 143
   3.3.16(d) Angiotensin II 144
3.3.17 Acute renal vasoconstriction experiments 144

3.3.17(a) Renal cortical vasoconstrictor responses to alpha adrenergic agonists (noradrenaline, phenylephrine, methoxamine) 144

3.3.17(b) Angiotensin II (Ang II) 145

3.4 In-vivo results of results of WKY (control), SHR (control), SHR irbesartan, SHR pioglitazone, SHR adiponectin, SHR irbesartan+adiponectin and SHR Pioglitazone+adiponextin treated groups 146

3.4.1 Body weight 146

3.4.2 Water intake 147

3.4.3 Urine flow rate 147

3.4.4 Blood glucose concentration 148

3.4.5 Systolic and diastolic blood pressure 149

3.4.6 Mean arterial pressure and heart rate 150

3.4.7 Plasma adiponectin and triglycerides 152

3.4.8 Plasma creatinine, urine creatinine and creatinine clearance (Cr.cl.) 153

3.4.9 Plasma and urinary sodium concentration 154

3.4.10 Absolute urinary sodium excretion and fractional sodium excretion 155

3.4.11 Urinary potassium and urinary sodium to potassium ratio 157

3.4.12 Pulse wave velocity and renal cortical blood perfusion 158
3.4.13 Plasma total superoxide dismutase and malondialdehyde

3.4.14 Plasma nitric oxide and total antioxidant capacity

3.4.15 Plasma glutathione

3.4.16 Systemic vasopressor responses study
   3.4.16(a) Noradrenaline (NA)
   3.4.16(b) Phenylephrine (PE)
   3.4.16(c) Methoxamine (ME)
   3.4.16(d) Angiotensin II (Ang II)

3.4.17 Acute renal vasoconstriction experiments
   3.4.17(a) Renal cortical vasoconstrictor responses to adrenergic agonists (noradrenaline, phenylephrine and methoxamine)
   3.4.17(b) Angiotensin II (Ang II)

3.5 In-vivo results of WKY (control), WKY + diabetic, SHR + diabetic + irbesartan, SHR + diabetic pioglitazone, SHR + diabetic adiponectin, SHR + diabetic + irbesartan + adiponectin and SHR + diabetic + pioglitazone + adiponectin treated groups

3.5.1 Body weight

3.5.2 Water intake

3.5.3 Urine flow rate

3.5.4 Blood glucose concentration

3.5.5 Systolic and diastolic blood pressure

3.5.6 Mean arterial pressure and heart rate
3.5.7 Plasma adiponectin and triglycerides 174
3.5.8 Plasma creatinine urine creatinine and creatinine clearance 175
3.5.9 Plasma and urinary sodium concentration 177
3.5.10 Absolute urinary sodium excretion and fractional sodium excretion 178
3.5.11 Urinary potassium and urinary sodium to potassium ratio 180
3.5.12 Kidney Index 181
3.5.13 Pulse wave velocity and renal cortical blood perfusion 182
3.5.14 Plasma total superoxide dismutase and malondialdehyde 183
3.5.15 Total antioxidant capacity and plasma nitric oxide 185
3.5.16 Plasma glutathione 186
3.5.17 Systemic vasopressor responses study 187
  3.5.17(a) Noradrenaline (NA) 187
  3.5.17(b) Phenylephrine 188
  3.5.17(c) Methoxamine 189
  3.5.17(d) Angiotensin II 189
3.5.18 Acute renal vasoconstriction experiments 190
  3.5.18(a) Renal cortical vasoconstrictor responses to adrenergic agonists (noradrenaline, phenylephrine and methoxamine) 190
  3.5.18(b) Angiotensin II (Ang II) 191
3.6 Histology of renal tissues 192
CHAPTER 4: DISCUSSION

4.1  Animals and disease models

4.1.1  Spontaneously Hypertensive Rats (SHR)

4.1.2  Streptozotocin-induced diabetes model

4.1.3  Changes in physiological parameters

4.1.4  Changes in renal functional parameters, kidney index and histology of kidney

4.1.5  Changes in the baseline mean arterial pressure and renal blood flow

4.2  Blood glucose, water intake, urine flow rate and body weight

4.3  Blood pressure responses to adiponectin and combination of adiponectin with either irbesartan or pioglitazone

4.4  Effect of adiponectin, pioglitazone and irbesartan on heart rate

4.5  Adiponectin concentration in plasma and lipid profile

4.6  Pulse wave velocity and antioxidant changes

4.7  Drugs used in the systemic and renal vasoconstrictor experiments

4.7.1  Adrenergic agonists

4.7.2  The effect of irbesartan, pioglitazone, adiponectin and combined treatment of adiponectin on vasopressor and vasoactive responses to adrenergic agonists and Ang II and in systemic and renal vasculature of diabetic and non diabetic Wistar Kyoto Spontaneously hypertensive rats
4.8 Renal haemodynamics parameters 372

CHAPTER 5: CONCLUSIONS 377

REFERENCES 381

APPENDICES

LIST OF PUBLICATIONS

LIST OF CONFERENCE PRESENTATIONS
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1</td>
<td>Classification of blood pressure</td>
<td>9</td>
</tr>
<tr>
<td>Table 2.1</td>
<td>Grouping of experimental rats</td>
<td>80</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Preparation of blank, sample and standard for creatinine measurement in plasma and urine</td>
<td>87</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Sample assay protocol for MDA estimation</td>
<td>97</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Sample assay protocol for T-SOD estimation</td>
<td>99</td>
</tr>
<tr>
<td>Table 2.5</td>
<td>Sample assay protocol for NO estimation</td>
<td>101</td>
</tr>
<tr>
<td>Table 2.6</td>
<td>Sample assay protocol for T-AOC estimation</td>
<td>103</td>
</tr>
<tr>
<td>Table 2.7</td>
<td>Sample assay protocol for glutathione estimation</td>
<td>105</td>
</tr>
<tr>
<td>Table 2.8</td>
<td>Preparation of reagent for adiponectin measurement</td>
<td>106</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Body weight of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period</td>
<td>193</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>24 hours water intake during metabolic study of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan+adiponectin and Pioglitazone + adiponectin for 28 days period</td>
<td>194</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>Urine flow rate of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period</td>
<td>195</td>
</tr>
<tr>
<td>Table 3.4</td>
<td>Blood glucose concentration (mg/dl) of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days</td>
<td>196</td>
</tr>
<tr>
<td>Table 3.5</td>
<td>Systolic blood pressure of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period</td>
<td>197</td>
</tr>
<tr>
<td>Table 3.6</td>
<td>Diastolic blood pressure of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period</td>
<td></td>
</tr>
<tr>
<td>Table 3.7</td>
<td>Mean arterial blood pressure of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period</td>
<td></td>
</tr>
<tr>
<td>Table 3.8</td>
<td>Heart rate of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period</td>
<td></td>
</tr>
<tr>
<td>Table 3.9</td>
<td>Plasma Triglycerides and Lipoproteins (Total cholesterol, LDL, HDL) level of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin on day 28 of treatment period</td>
<td></td>
</tr>
<tr>
<td>Table 3.10</td>
<td>Plasma creatinine of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period</td>
<td></td>
</tr>
<tr>
<td>Table 3.11</td>
<td>Urine creatinine of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period</td>
<td></td>
</tr>
<tr>
<td>Table 3.12</td>
<td>Creatinine clearance of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period</td>
<td></td>
</tr>
<tr>
<td>Table 3.13</td>
<td>Plasma sodium concentration of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin,</td>
<td></td>
</tr>
</tbody>
</table>
Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.14 Urinary sodium concentration of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.15 Absolute urinary sodium excretion ($U_{Na}V$) of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.16 Fractional sodium excretion (FE Na%) of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days

Table 3.17 Urinary Potassium concentration of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.18 Urinary sodium to urinary potassium ratio of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.19 Body weight of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.20 24 hours water intake during metabolic study of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.21 Urine flow rate of WKY control and WKY diabetic groups
treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.22 Blood glucose concentration of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.23 Systolic blood pressure of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.24 Diastolic blood pressure of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.25 Mean arterial blood pressure of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.26 Heart rate of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.27 Plasma Triglycerides and Lipoproteins (Total cholesterol, LDL, HDL) level of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin

Table 3.28 Plasma creatinine of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period
Table 3.29 Urine creatinine of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period 221

Table 3.30 Creatinine clearance of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period 222

Table 3.31 Plasma sodium concentration of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period 223

Table 3.32 Urinary sodium concentration of WKY control and WKY groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period 224

Table 3.33 Absolute sodium excretion ($U_{Na}V$) of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days 225

Table 3.34 Fractional sodium excretion of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period 226

Table 3.35 Urinary potassium concentration of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period 227

Table 3.36 Urinary sodium to urinary potassium ratio of WKY control and WKY diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and 228
Table 3.37  
Body weight of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.38  
24 hours water intake during metabolic study of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.39  
Urine flow rate of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.40  
Blood glucose concentration (mg/dl) of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.41  
Systolic blood pressure of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.42  
Diastolic blood pressure of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.43  
Mean arterial blood pressure of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period
Table 3.44 Heart rate of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.45 Plasma Triglycerides and Lipoproteins (Total cholesterol, LDL, HDL) level of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin

Table 3.46 Plasma creatinine of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.47 Urine creatinine of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.48 Creatinine clearance of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.49 Plasma sodium concentration of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.50 Urine sodium concentration of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.51 Absolute sodium excretion of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period
adiponectin for 28 days period

Table 3.52 Fractional sodium excretion of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.53 Urinary potassium concentration of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.54 Urinary sodium to urinary potassium ratio of WKY control, SHR control and SHR groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.55 Body weight of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.56 24 hours water intake during metabolic study of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.57 Urine flow rate of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.58 Blood glucose concentration (mg/dl) of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.59 Systolic blood pressure of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone,
Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.60 Diastolic blood pressure of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.61 Mean arterial blood pressure of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.62 Heart rate of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.63 Plasma Triglycerides and Lipoproteins (Total cholesterol, LDL, HDL) level of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin

Table 3.64 Plasma creatinine of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.65 Urine creatinine of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.66 Creatinine clearance of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.67 Plasma sodium level of WKY, SHR control and SHR
diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.68  Urinary sodium concentration of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.69  Absolute sodium excretion ($U_{Na}V$) of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.70  Fractional sodium excretion of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.71  Urinary potassium concentration of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period

Table 3.72  Urinary sodium to urinary potassium ratio of WKY, SHR control and SHR diabetic groups treated with Irbesartan, Pioglitazone, Adiponectin, Irbesartan + adiponectin and Pioglitazone + adiponectin for 28 days period
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>Gross anatomy of kidney</td>
<td>3</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Gross anatomy of cardiovascular system of heart</td>
<td>8</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Pathophysiological mechanisms of hypertension</td>
<td>13</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Schematic representation of the involvement of oxidative stress induced by diabetes and the development of diabetic complications</td>
<td>20</td>
</tr>
<tr>
<td>Figure 1.5</td>
<td>Reactive oxygen species in the vascular system</td>
<td>24</td>
</tr>
<tr>
<td>Figure 1.6</td>
<td>Role of kidney and renin-angiotensin-aldosterone system in blood pressure control</td>
<td>29</td>
</tr>
<tr>
<td>Figure 1.7</td>
<td>Classification of adrenoceptors</td>
<td>35</td>
</tr>
<tr>
<td>Figure 1.8</td>
<td>Components of the renin–angiotensin system</td>
<td>38</td>
</tr>
<tr>
<td>Figure 1.9</td>
<td>Various effects produced by angiotensin II that lead to elevation in blood pressure</td>
<td>40</td>
</tr>
<tr>
<td>Figure 1.10</td>
<td>Domains and structure of adiponectin</td>
<td>47</td>
</tr>
<tr>
<td>Figure 1.11</td>
<td>PPAR-gamma agonists ameliorate insulin resistance and diabetes by both adiponectin-dependent and -independent pathways</td>
<td>54</td>
</tr>
<tr>
<td>Figure 1.12</td>
<td>Adipocytokines interact in a complex way to regulate vascular function and ultimately the development of cardiovascular diseases.</td>
<td>57</td>
</tr>
<tr>
<td>Figure 1.13</td>
<td>Intracellular signaling events that mediate the actions of adiponectin</td>
<td>67</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>Protocol for induction of diabetes in WKY and SHR groups</td>
<td>81</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Diagrammatic surgical set up for acute experiment.</td>
<td>110</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Presentation of propagation time and distance for measurement of pulse wave velocity</td>
<td>113</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Plasma adiponectin concentrations in WKY diabetic control and WKY diabetic treated rats</td>
<td>265</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>Kidney index of WKY diabetic control and WKY diabetic</td>
<td>266</td>
</tr>
<tr>
<td>Figure 3.3</td>
<td>Pulse wave velocity of WKY diabetic control and WKY diabetic treated rats</td>
<td>267</td>
</tr>
<tr>
<td>Figure 3.4</td>
<td>Baseline renal cortical blood perfusion of WKY diabetic control and WKY diabetic treated rats</td>
<td>268</td>
</tr>
<tr>
<td>Figure 3.5</td>
<td>Plasma total superoxide dismutase levels of WKY diabetic control and WKY diabetic treated rats</td>
<td>269</td>
</tr>
<tr>
<td>Figure 3.6</td>
<td>Plasma malondialdehyde levels of WKY diabetic and WKY control diabetic treated rats</td>
<td>270</td>
</tr>
<tr>
<td>Figure 3.7</td>
<td>Plasma nitric oxide levels of WKY diabetic and WKY control diabetic treated rats</td>
<td>271</td>
</tr>
<tr>
<td>Figure 3.8</td>
<td>Plasma total antioxidant capacity of WKY diabetic and control WKY diabetic treated rats</td>
<td>272</td>
</tr>
<tr>
<td>Figure 3.9</td>
<td>Plasma glutathione level of WKY diabetic and WKY control diabetic treated rats</td>
<td>273</td>
</tr>
<tr>
<td>Figure 3.10</td>
<td>The % change of MAP in response to graded doses of noradrenaline in WKY diabetic control and WKY diabetic treated rats</td>
<td>274</td>
</tr>
<tr>
<td>Figure 3.11</td>
<td>The overall % change of MAP in response to noradrenaline in WKY diabetic and WKY diabetic treated rats</td>
<td>275</td>
</tr>
<tr>
<td>Figure 3.12</td>
<td>The % change of MAP in response to graded doses of phenylephrine in WKY diabetic control and WKY diabetic treated rats</td>
<td>276</td>
</tr>
<tr>
<td>Figure 3.13</td>
<td>The overall % change of MAP in response to phenylephrine in WKY diabetic control and WKY diabetic treated rats</td>
<td>277</td>
</tr>
<tr>
<td>Figure 3.14</td>
<td>The % change of MAP in response to graded doses of methoxamine in WKY diabetic control and WKY diabetic treated rats</td>
<td>278</td>
</tr>
<tr>
<td>Figure 3.15</td>
<td>The overall % change of MAP in response to methoxamine in WKY diabetic control and WKY diabetic treated rats</td>
<td>279</td>
</tr>
<tr>
<td>Figure 3.16</td>
<td>The % change of MAP to graded doses in response to</td>
<td>280</td>
</tr>
</tbody>
</table>
angiotensin II in WKY control diabetic and WKY diabetic treated rats

Figure 3.17 The overall % change of MAP in response to angiotensin II in WKY diabetic control and WKY diabetic treated rats

Figure 3.18 The % change of RCBP to graded doses in response to noradrenaline in WKY diabetic control and WKY diabetic treated rats

Figure 3.19 The % change of RCBP to graded doses in response to phenylephrine in WKY diabetic control and WKY diabetic treated rats

Figure 3.20 The % change of RCBP to graded doses in response to methoxamine in WKY diabetic control and WKY diabetic treated rats

Figure 3.21 The overall % change of RCBP in response to noradrenaline in WKY diabetic control and WKY diabetic treated rats

Figure 3.22 The overall % change of RCBP in response to phenylephrine in WKY diabetic control and WKY diabetic treated rats

Figure 3.23 The overall % change of RCBP in response to methoxamine in WKY diabetic control and WKY diabetic treated rats

Figure 3.24 The % change of RCBP to graded doses in response to angiotensin II in WKY diabetic control and WKY diabetic treated rats

Figure 3.25 The overall % change of RCBP in response to angiotensin II in WKY diabetic control and WKY diabetic treated rats

Figure 3.26 Plasma adiponectin concentration in WKY, SHR control and SHR treated rats

Figure 3.27 Pulse wave velocity of WKY, SHR control and SHR treated rats

Figure 3.28 Baseline renal cortical blood perfusion of WKY, SHR control and SHR treated rats
Figure 3.29  Plasma total superoxide dismutase levels of WKY, SHR control and SHR treated rats

Figure 3.30  Plasma malondialdehyde levels of WKY, SHR control and SHR treated rats

Figure 3.31  Plasma nitric oxide levels of WKY, SHR control and SHR treated rats

Figure 3.32  Plasma total antioxidant capacity of WKY, SHR control and SHR treated rats

Figure 3.33  Plasma glutathione level of WKY, SHR control and SHR treated rats

Figure 3.34  The % change of MAP in response to graded doses of noradrenaline in WKY, SHR control and SHR treated rats

Figure 3.35  The overall % change of MAP in response to noradrenaline in WKY, SHR control and SHR treated rats

Figure 3.36  The % change of MAP in response to graded doses of phenylephrine in WKY, SHR control and SHR treated rats

Figure 3.37  The overall % change of MAP in response to phenylephrine in WKY, SHR control and SHR treated rats

Figure 3.38  The % change of MAP in response to graded doses of methoxamine in WKY, SHR control and SHR treated rats

Figure 3.39  The overall % change of MAP in response to methoxamine in WKY, SHR control and SHR treated rats

Figure 3.40  The % change of MAP in response to graded doses of angiotensin II in SHR and SHR treated rats

Figure 3.41  The overall % change of MAP in response to angiotensin II in WKY, SHR control and SHR treated rats

Figure 3.42  The % change of RCBP to graded doses in response to noradrenaline in WKY, SHR control and SHR treated rats

Figure 3.43  The % change of RCBP to graded doses in response to phenylephrine in WKY, SHR control and SHR treated rats

Figure 3.44  The % change of RCBP to graded doses in response to...
methoxamine in WKY, SHR control and SHR treated rats

Figure 3.45 The overall % change of RCBP in response to noradrenaline in WKY, SHR control and SHR treated rats

Figure 3.46 The overall % change of RCBP in response to phenylephrine in WKY, SHR control and SHR treated rats

Figure 3.47 The overall % change of RCBP in response to methoxamine in WKY, SHR control and SHR treated rats

Figure 3.48 The % change of RCBP to graded doses in response to angiotensin II in WKY, SHR control and SHR treated rats

Figure 3.49 The overall % change of RCBP in response to angiotensin II in WKY, SHR control and SHR treated rats

Figure 3.50 Plasma adiponectin concentration in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.51 Kidney index of WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.52 Pulse wave velocity of WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.53 Baseline renal cortical blood perfusion of WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.54 Plasma total superoxide dismutase levels of WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.55 Plasma malondialdehyde levels of WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.56 Plasma nitric oxide levels of WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.57 Plasma total antioxidant capacity of WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.58 Plasma glutathione level of WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.59 The % change of MAP in response to graded doses of noradrenaline in WKY, SHR diabetic control and SHR
diabetic treated rats

Figure 3.60 The overall % change of MAP in response to noradrenaline in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.61 The % change of MAP in response to graded doses of phenylephrine in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.62 The overall % change of MAP in response to phenylephrine in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.63 The % change of MAP in response to graded doses of methoxamine in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.64 The overall % change of MAP in response to methoxamine in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.65 The % change of MAP in response to graded doses of angiotensin II in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.66 The overall % change of MAP in response to angiotensin II in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.67 The % change of RCBP to graded doses in response to noradrenaline in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.68 The % change of RCBP to graded doses in response to phenylephrine in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.69 The % change of RCBP to graded doses in response to methoxamine in WKY, SHR diabetic control and SHR diabetic treated rats
Figure 3.70  The overall % change of RCBP in response to noradrenaline in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.71  The overall % change of RCBP in response to phenylephrine in SHR diabetic and SHR diabetic treated rats

Figure 3.72  The overall % change of RCBP in response to methoxamine in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.73  The % change of RCBP to graded doses in response to angiotensin II in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.74  The overall % change of RCBP in response to angiotensin II in WKY, SHR diabetic control and SHR diabetic treated rats

Figure 3.75  Light microscopy of renal tissue (5µm) of SHR diabetic control rats.

Figure 3.76  Light microscopy of renal tissue (5µm) of WKY diabetic control rats

Figure 3.77  Light microscopy of renal tissue (5µm) of SHR diabetic rats treated with adiponectin.

Figure 3.78  Light microscopy of renal tissue (5µm) of SHR diabetic rats treated with pioglitazone and adiponectin.

Figure 3.79  Light microscopy of renal tissue (5µm) from normal SHR rats treated with irbesartan

Figure 3.80  Light microscopy of renal tissue (5µm) from SHR diabetic rats treated with adiponectin and irbesartan
LIST OF ABBREVIATIONS

%  percentage
µg  micro gram
µL  microliter
µM  micro moles
ACRP  adipocyte complement related protein
ADP  adiponectin
AMPK  adenosine monophosphate-activated protein kinase
ANG II  angiotensin II
ANOVA  analysis of variance
APM1  adipose Most abundant gene transcript1
APPL  adaptor protein containing Pleckstrin homology domain, phosphotyrosine-binding domain and Leucine
ARB  angiotensin receptor blocker
AT1  angiotensin II (type 1) receptor
ATP  adenosine tri-phosphate
BMI  body mass index
BPU  blood perfusion unit
B.w  body weight
Cl.Cr  creatinine clearance
CRP  C-Reactive Protein
CVD  cardiovascular disease
DM  diabetes mellitus
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDCFs</td>
<td>endothelial-derived constrictors factors</td>
</tr>
<tr>
<td>EDRFs</td>
<td>endothelium-derived relaxing factors</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>eNOS</td>
<td>endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>FE&lt;sub&gt;Na&lt;/sub&gt;</td>
<td>fractional sodium excretion</td>
</tr>
<tr>
<td>FFA</td>
<td>free fatty acid</td>
</tr>
<tr>
<td>g</td>
<td>grams</td>
</tr>
<tr>
<td>GBP28</td>
<td>Gelatin binding Protein</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione</td>
</tr>
<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
</tr>
<tr>
<td>HMW</td>
<td>high Molecular Weight form</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>i.p.</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>Irb</td>
<td>irbesartan</td>
</tr>
<tr>
<td>LDL</td>
<td>low density lipoprotein</td>
</tr>
<tr>
<td>LMW</td>
<td>low- Molecular Weight</td>
</tr>
<tr>
<td>m/s</td>
<td>meter per second</td>
</tr>
<tr>
<td>MAP</td>
<td>mean arterial blood pressure</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen active protein kinase</td>
</tr>
<tr>
<td>MDA</td>
<td>malondialdehyde</td>
</tr>
<tr>
<td>ME</td>
<td>methoxamine</td>
</tr>
<tr>
<td>mg /dl</td>
<td>milligram per deciliter</td>
</tr>
<tr>
<td>mg/kg</td>
<td>milligram per kilogram</td>
</tr>
</tbody>
</table>

xxxv
mL
mL/min/kg
mmHg
mRNA
NA
NADPH
ng
NIBP
NO
NOS
Pcr.
PE
Pio
PKC
PPAR
PPAR-\(\gamma\)
PPAR-\(\alpha\)
PPAR-\(\beta\)
PWV
RAAS
RCBP
RNA
ROS
milliliter
milliliter per minute per kilogram
millimeter mercury
messenger Ribonucleic acid
noradrenaline
nicotinamide adenine dinucleotide phosphate oxidase
nano gram
non invasive blood pressure
nitric oxide
nitric oxide synthase
plasma creatinine
phenylephrine
pioglitazone
protein kinase C
peroxisome Proliferator Activated Receptor
peroxisome Proliferator Activated Receptor gamma
peroxisome Proliferator Activated Receptor alpha
peroxisome Proliferator Activated Receptor beta
pulse Wave velocity
renin angiotensin aldosterone system
renal cortical blood perfusion
ribonucleic acid
reactive oxygen species
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of mean</td>
</tr>
<tr>
<td>SHR</td>
<td>Spontaneously Hypertensive rats</td>
</tr>
<tr>
<td>SNS</td>
<td>sympathetic nervous system</td>
</tr>
<tr>
<td>T2DM</td>
<td>type 2 diabetes mellitus</td>
</tr>
<tr>
<td>T-AOC</td>
<td>total anti-oxidant capacity</td>
</tr>
<tr>
<td>TNF-a</td>
<td>tumor necrosis factor-alpha</td>
</tr>
<tr>
<td>T-SOD</td>
<td>total superoxide dismutase</td>
</tr>
<tr>
<td>TXA2</td>
<td>thromboxane A2</td>
</tr>
<tr>
<td>TZDs</td>
<td>thiazolidinedione</td>
</tr>
<tr>
<td>U/mL</td>
<td>units per millilitre</td>
</tr>
<tr>
<td>Ucr.</td>
<td>urinary creatinine</td>
</tr>
<tr>
<td>UFR</td>
<td>urine flow rate</td>
</tr>
<tr>
<td>U_{Na}V</td>
<td>absolute sodium excretion</td>
</tr>
<tr>
<td>VSMCs</td>
<td>vascular smooth muscle cells</td>
</tr>
<tr>
<td>WKY</td>
<td>Wistar Kyoto</td>
</tr>
<tr>
<td>δ</td>
<td>delta</td>
</tr>
<tr>
<td>ε</td>
<td>epsilon</td>
</tr>
</tbody>
</table>
KESAN HEMODINAMIK DARIPADA ADIPONECTIN TERHADAP
RESEPTOR PPAR-γ DALAM WISTAR KYOTO TIKUS DIABETES DAN
HIPERTENSI SECARA SPONTAN

ABSTRAK

Prevalens hipertensi dan diabetes semakin meningkat dengan kadar yang belum pernah berlaku sebelum ini di kedua-dua negara sedang membangun dan juga di negara maju. Adiponectin, yang merupakan suatu adipokin, adalah hormon protein yang menyederhana tindakannya dengan merangsang pelepasan nitrik oksida (NO) daripada endotelim vascular dan menyebabkan vasodilasi. PPAR-γ merupakan ahli reseptor diaktif proliferator peroksisom, yang merupakan pengawal atur positif bagi ekspresi gen adiponectin. Dalam kajian ini, irbesartan digunakan sebagai agonis PPAR-γ separa, Sebaliknya, pioglitazon bertindak sebagai ligan penuh bagi PPAR-γ dan menyebabkan peningkatan kepekatan adiponectin plasma. Kajian ini mengkaji antihipertensi, antidiabetes, potensi antioksidan dan hemodinamik renal adiponectin dengan agonis PPAR-γ dalam model diabetes dan bukan diabetes daripada tikus hipertensi. Di samping itu, kesan adiponectin dan gabungan rawatan daripada adiponectin dengan agomis PPAR-γ separa atau penuh pada α1-adrenoceptor menyebabkan perubahan vaskular renal dan sistemik daripada tikus hipertensi dan normotensi diabetes dan bukan diabetes turut dikaji. Diabetes jenis I diaruh menggunakan suntikan / injeksi intraperitoneal tunggal daripada streptozotocin pada dos 40 mg/kg berat badan yang dilarutkan dalam penampan natrium sitrat (pH 4.5). Satu set tikus normotensi (WKY) dan hipertensi (SHR) diberikan...
irbesartan, pioglitazone, adiponectin dan gabungan adiponectin dengan sama ada
irbesartan atau pioglitazon. Sementara itu, satu set lain yang tediri daripada tikus
normotensi bukan diabetes (WKY) dan tikus hipertensi (SHR) diberikan pola atau corak
rawatan yang sama. Tekanan darah (BP) dan parameter metabolisme diukur pada tikus.
Di samping itu, kelajuan gelombang nadi, perfusi darah kortikal renal, adiponectin
plasma, profil cecair dan elektrolit juga diukur semasa dan pada akhir kajian.
Selanjutnya, tikus diberi natrium pentobarbiton beranestetik dan dikurangkan min
tekanan arteri dan aliran darah kortikal renal diaruh melalui pemberian secara sistemik
dan intrarenal daripada noradrenalin, fenilefrin, metoksamin dan angiotensin II (Ang II).
Data, mean±SEM tertakluk pada ANOVA dengan signifikan pada P<0.05. Diabetes SHR
mempunyai BP yang lebih tinggi, adiponectin plasma yang rendah, ketidakfungsian renal
ditunjukkan dengan peningkatan kreatinin plasma, klearans kreatinin, kumuhan /ekskresi
natrium dalam bentuk pecahan dan mutlak. Rawatan dengan adiponectin sahaja dan
gabungan adiponectin dengan agonis PPAR-γ separa atau lengkap didapati
 mengurangkan BP, mengurangkan respons vaskular renal dan sistemik pada agonis α1-
adrenergic dan Ang II, memperbaiki stres oksidatif dan meningkatkan hemodinamik
renal dan fungsi kumuhan, justeru, ia memberikan suatu interaksi kompleks di antara
subjenis α1-adrenoceptor, reseptor ANG II dan adiponectin reseptor. Gabungan terapi
adiponectin dengan pioglitazone menggariskan peranan perlindungan reno daripada
adiponectin. Oleh itu, darjah sinergisme wujud di antara adiponectin dan agonis PPAR-γ
lengkap (pioglitazone).
HAEMODYNAMIC EFFECTS OF ADIPONECTIN ON PPAR-γ RECEPTORS IN DIABETIC AND NON-DIABETIC WISTAR KYOTO AND SPONTANEOUSLY HYPERTENSIVE RATS

ABSTRACT

The prevalence of hypertension and diabetes is mounting with unprecedented degree in both developing and advanced countries. Reduced plasma adiponectin concentrations have been found in hypertension and diabetes. Adiponectin, an adipokine, is a protein hormone which mediates its action by stimulating nitric oxide (NO) release from the vascular endothelium thereby causing vasodilation. PPAR-γ is a member of Peroxisome proliferator activated receptor, which is positive regulator of adiponectin gene expression. In this study irbesartan has been used as partial PPAR-γ agonist, by contrast, pioglitazone acts as full ligand for PPAR-γ, which causes increases in adiponectin plasma concentration. This study investigated the antihypertensive, antidiabetic, antioxidant potential and renal haemodynamics of adiponectin with PPAR-γ agonists in diabetic and non-diabetic model of rats. Besides, the effect of adiponectin and combined treatment of adiponectin with partial or full PPAR-γ agonists on the α1-adrenoceptor subtypes responsiveness in systemic and renal vasculature alteration of diabetic and non-diabetic normotensive and hypertensive rats was explored. Type 1 diabetes was induced using a single intra-peritoneal injection of streptozotocin at a dose of 40 mg/kg body weight, dissolved in sodium citrate buffer (pH 4.5). One set of diabetic normotensive (WKY) and hypertensive (SHR) rats received irbesartan, pioglitazone,
adiponectin and combination of adiponectin with either irbesartan or pioglitazone, while the other set of non-diabetic WKY and SHRs received the same pattern of treatment. Blood pressure (B.P) and metabolic parameters were measured in conscious rats. In addition pulse wave velocity, renal cortical blood perfusion, plasma adiponectin, lipid profile and electrolytes were also measured during and at the end of study. Moreover, rats were anaesthetized with sodium pentobarbitone and reductions in mean arterial pressure and renal cortical blood flow induced by systemic and intra-renal administration of noradrenaline, phenylephrine, methoxamine and angiotensin II (Ang II) were determined. Data, mean±SEM were subjected to ANOVA with significance at P<0.05. Diabetic SHRs had higher B.P, low plasma adiponectin, renal dysfunction marked by increased plasma creatinine, creatinine clearance, absolute and fractional sodium excretion. Treatment with adiponectin alone and combination of adiponectin with either partial or full PPAR-γ agonists reduced B.P, blunted systemic and renal vascular response to α₁-adrenergic agonists and Ang II, ameliorate oxidative stress and improved renal haemodynamics and excretory functions, thus signify a complex interaction between α₁-adrenoceptor subtypes, Ang II and adiponectin receptors. Moreover, combined adiponectin with pioglitazone underlie a reno-protective role of adiponectin, and signifies a degree of synergism exist between adiponectin and full PPAR-γ agonist (pioglitazone) treatment.
CHAPTER 1: INTRODUCTION

1.1  Kidney

1.1.1  Anatomy of kidney

In human beings, there is a pair of kidneys, one on each side of spine and located in the abdominal cavity called the retroperitoneal space. The exact location of kidneys in human beings is approximately at the vertebral level T12 to L3 (Walter and Boron, 2004). Each adult kidney weighs between 125 and 170 grams in males and between 115 and 155 grams in females. The kidney is approximately 11–14 cm in length, 6 cm wide and 4 cm thick. The left kidney is typically slightly larger than the right (Glodny et al. 2009).

Macroscopically each kidney has been described as a bean-shaped organ having both concave and convex surfaces. On the concave surface i.e., the medial side, there is a depression called hilum, at which the renal artery and nerve enters the organ, whereas the renal vein and ureter leaves the kidney (Marieb and Hoehn, 2007). In a cross section of the kidney, two major regions can be identified. The outer or superficial reddish brown region, which is granular in appearance, called cortex and inner or deep darker brown, appears striated is known as medulla. Nephrons, the urine-producing functional unit of the kidney, span the cortex and medulla (Shier, 2003). There are about 20 million nephrons in each kidney. The initial filtering portion of a nephron is made of glomerulus and Bowmans capsule collectively called renal corpuscle, located in the cortex, which is followed by renal tubule that passes from cortex deep into the medullary pyramids (Walter and Boron, 2004). The renal tubules consist of proximal convoluted tubule, loop of Henle and distal convoluted tubule. Kidneys receive blood from the respective
renal artery, left and right, which branches directly from the abdominal aorta. Each renal artery branches into segmental arteries, dividing further into interlobar arteries that penetrate the renal capsule and extend through the renal columns between the renal pyramids. Each arcuate artery supplies several interlobular arteries that feed into the afferent arterioles that supply the glomeruli (Walter and Boron, 2004). After filtration, the blood moves through a small network of venules that converge into interlobular veins. As with the arteriole distribution, the veins follow the same pattern, the interlobular veins provide blood to arcuate veins then back to interlobar veins that unite to form the renal vein that exits at the hilus of the kidney. Renal veins return the blood to inferior vena cava (Vander, 1995, Applegate, 2000, and Meyer et al. 2004).

The kidney and nervous system communicate via the renal plexus, whose fibres’ course along the renal arteries to reach the kidney. Input from the SNS triggers vasoconstriction in the kidney, thereby reducing renal blood flow. Interestingly kidney is devoid of input from the parasympathetic nervous system (Bard et al. 2003).
1.1.2 Physiology of Kidney

Kidneys contribute in whole-body homeostasis through the excretion of waste products of metabolism like urea and uric acid, regulation of acid base balance, electrolyte concentrations and extracellular fluid volume. Kidneys are also involved in the reabsorption of important nutrients like glucose and amino acids. The production of various hormones like erythropoietin and activation of vitamin-D also come under the functions of kidneys (Dantzler, 1989). Most of kidney's functions are completed by the simple mechanisms of filtration, reabsorption and secretion that take place within the nephron. Filtration occurs at the renal corpuscle. It is the process by which cells and large proteins are filtered from the blood to make the glomerular filtrate that eventually becomes urine. The kidney produces about 180 liters of ultra filtrate per day, while
reabsorbing a large percentage, allowing for the production of only approximately 2 liters of urine. Reabsorption is the transport of molecules from this ultra filtrate and into the blood. On the other hand, secretion is the reverse process, in which molecules are transported in the opposite direction, from the blood into the urine (Guyton, 1991b). One of the most vital functions that the normal kidneys perform is the long-term regulation of blood pressure. This regulation occurs through preservation of the extracellular fluid compartment, the size that depends on plasma sodium concentration through the activation of renin angiotensin-aldosterone system (Hall and Guyton, 2011). Kidneys work in conjunction with cardiovascular, endocrine and nervous system in order to maintain the blood pressure (Germann et al. 2005).

1.2 Cardiovascular system

1.2.1 Anatomy of cardiovascular system

The cardiovascular system distributes the blood and consists of the heart, blood and blood vessels. One of the essential components of cardiovascular system is the closed circulatory system that permits blood and lymph circulation in order to transport nutrients and waste products to and from cells in body (Dorland, 2011). In humans, the circulatory system includes the pulmonary circulation and systemic circulation. Pulmonary circulation is a loop through the lungs where blood gets oxygenated, whereas the systemic circulation provides oxygenated blood to the rest of the body (Guyton and Hall, 2006). The deoxygenated blood is brought back to the right atrium via the superior and inferior vena cava and follow through the tricuspid valve to the right ventricle, from where it is pumped through the pulmonary artery to lungs. Gaseous exchange occurs in the lungs and pulmonary vein returns the oxygenated blood to left atrium. Conversely,
systemic circulation transports oxygen rich blood away from the heart to the body except lungs from the left ventricle through aorta and bring back the oxygen-depleted blood back to the heart (Guyton and Hall, 2006).

### 1.2.2 Heart and blood vessels

The human heart is composed of cardiac muscle, which is an involuntary striated muscle tissue found only in this organ, and connective tissue. On the average human heart, beats 72 times per minute, roughly beats 2.5 billion times during an average sixty-five years of lifespan, and weighs approximately 300 to 350 grams (Kumar et al. 2005). The heart is muscular conical organ that lies between the lungs in the middle mediastinum and is enclosed in the pericardium. Heart is placed obliquely anterior to the vertebral column and behind the body of sternum so that 1/3 of it lies to the right and 2/3 lies to the left of median plane. It is enclosed in a double-walled sac called the pericardium. The superficial part of this sac is called the fibrous pericardium (Gavaghan, 1998). The outer wall of the human heart is composed of three layers. The outer layer is called the epicardium, or visceral pericardium since it is also the inner wall of the pericardium. The middle layer is called the myocardium and is composed of muscle that contracts. The inner layer is called the endocardium and is in contact with the blood that the heart pumps and it merges with the inner lining (endothelium) of blood vessels and covers the heart valves (Iles and Docherty, 2011).

The blood vessels refer to the closed system of tubes that transport blood to all parts of the body and back to the heart. The blood vessels consist of arteries, arterioles, capillaries, venules, and veins. The actual exchange of oxygen, carbon dioxide and waste
matter between the blood and the tissue fluid occurs in microscopically small vessels, called capillaries (Hall, 2010). Structurally the wall of arteries composed of three layers viz; tunica intima, tunica media and tunica adventitia. Tunica intima consists of an inner surface of smooth endothelium covered by a layer of elastic tissues. The tunica media is thicker in arteries and consists of smooth muscle cells mixed with elastic fibers. Tunica media of larger vessels is primarily composed of elastic fibers. Tunica adventitia of blood vessels is composed of collagenous and elastic fibres (Derrickson and Tortora, 2006, Human cardiovascular system, 2013). Progressive thinning of the vessel wall and a decrease in the size of the lumen results in the formation of arterioles that provides the most of the peripheral resistance (Tobian et al. 1961).

1.2.3 Physiology of Cardiovascular system

The heart acts as a functional syncytium and is divided into the right side and left side heart. Function of the right side of heart is to collect de-oxygenated blood, in the right atrium, from the body via superior and inferior vena cava and pump it to right ventricle, through the tricuspid valve into the lungs termed as pulmonary circulation. In the lungs there is oxygenation of blood through the passive process of diffusion. The left sided heart collects oxygenated blood from the lungs into the left atrium. From the left atrium the blood moves to the left ventricle, through the bicuspid valve, which pumps it out to the body via the aorta. On both sides, the lower ventricles are thicker and stronger than the upper atria. The muscle wall surrounding the left ventricle is thicker than the wall surrounding the right ventricle due to the higher force needed to pump the blood through the systemic circulation (Scott, 1986). The one complete beat of the heart that is one systole followed by one diastole is referred as one cardiac cycle. The duration of one
cardiac cycle in human beings is 0.8 seconds. Systole lasts for 0.3 seconds and diastole lasts for 0.5 seconds (Guyton and Hall, 2006). The heart is responsible for pumping blood throughout the blood vessels by repeated, rhythmic contractions and acts as a functional syncytium. Cardiac contractions are managed by specialized and self-excitatory conductive system of the heart. This conducting system consists of sinoatrial node or S-A node, in which the normal rhythmic impulse is generated; internodal pathway; A-V node; A-V bundle and right and left bundles of Purkinje fibers. Automacity is the process that can cause automatic rhythmical contractions and is best expressed in S-A node that is also known as pacemaker of the heart. Once a cardiac impulse is generated at the S-A node, it subsequently spread to all parts of heart through the conducting system resulting in rhythmic contractions and ventricles provide the major source of power for moving blood in the vascular system (Hall & Guyton, 2011). The cardiovascular system serve the needs of the tissue including transport of nutrients to tissue, transport of waste products away from tissues, hormones transport, maintenance of pH, thermoregulation, providing the necessary cardiac output and arterial pressure, preservation of fluid balance and to maintain homeostasis. The cardiovascular system works in concurrence with other body systems such as nervous, endocrine, renal and respiratory to maintain a suitable and steady environment.
1.3 Hypertension

Hypertension is a common worldwide health problem and about 40% of individuals had been diagnosed with this chronic illness in year 2008 (World Health, 2013). According to the Joint National Committee 7 (JNC 7), hypertension is defined as physician office systolic BP level of $\geq 140$ mmHg and diastolic BP of $\geq 90$ mmHg. The gray area between systolic BP of 120-139 mmHg and diastolic BP of 80-89 mmHg is defined as “prehypertension” (Chobanian et al. 2003).
1.3.1 Blood Pressure

Blood pressure is defined as the pressure exerted by the blood against any unit area of vessel wall (Guyton, 1991a). Blood pressure is the resultant of the activity of heart and blood vessels. Simply, blood pressure is equal to cardiac output multiplied by total peripheral resistance. The normal blood pressure ranges are systolic blood pressure 90-119 mmHg and diastolic blood pressure 60-79 mmHg (Chobanian et al. 2003). Many interrelated physiological mechanisms are involved in maintaining the blood pressure including sympathetic and parasympathetic nerves, baroreceptors, circulatory hormones and local auto-regulatory mechanisms. Derangement in these factors contributes to the elevation of blood pressure (Beevers et al. 2001).

Table 1.1: Classification of blood pressure

<table>
<thead>
<tr>
<th>Classification</th>
<th>Systolic BP (mmHg)</th>
<th>Diastolic BP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;120</td>
<td>And &lt;80</td>
</tr>
<tr>
<td>Pre-hypertension</td>
<td>120-139</td>
<td>Or 80-89</td>
</tr>
<tr>
<td>Stage 1 hypertension</td>
<td>140-159</td>
<td>Or 90-99</td>
</tr>
<tr>
<td>Stage 2 hypertension</td>
<td>≥ 160</td>
<td>≥ 100</td>
</tr>
</tbody>
</table>

BP = blood pressure (Human cardiovascular system), Data from National heart, lung and blood Institute: www.britannica.com/hypertension (accessed November, 2013).

1.3.2 Primary and secondary hypertension

Hypertension is divided into primary or essential and secondary hypertension. Primary hypertension is where there is rise of blood pressure due to unknown cause with a subsequent increased risk of cerebral, cardiac and renal complications (Messerli et al., 2007). Essential hypertension accounts for 90-95% cases of hypertension. The term secondary hypertension refers to remaining 5-10% cases of hypertension of known
origin. Primary hypertension, where there is no identifiable cause, is often a result of complex interactions between multiple environmental and genetic factors. On the other hand, identifiable causes of secondary hypertension include genetic syndromes, renal disease, renal vascular hypertension, Cushing syndrome, primary hyperaldosteronism, pregnancy, pheochromocytoma, hypercalcemia and medications (McPhee et al. 2010).

1.3.3 Pathophysiology of hypertension

Many pathophysiological mechanisms are involved in the genesis and maintenance of hypertension. However, extensive experimental and clinical data supports the view that impaired renal functions play a primary and vital role in the pathogenesis of hypertension as proposed by Guyton and Hall (Hall and Guyton, 2011). Pathophysiology is quite complex and multi-factorial, all these factors interact with each other through complex mechanisms that results in an increase of blood pressure, and related target organs damage (Feinleib et al. 1977, Longini et al. 1984). However, it has been suggested that the genetic causes of hypertension is uncommon in general hypertensive population. Moreover, the genetic predisposition can be expressed fully by the interaction of environmental and demographic factors (Oparil et al. 2003). Likewise, hypertension and type II diabetes mellitus coexist (Haffner et al. 1998). Similarly, metabolic syndrome also has been proposed in the pathogenesis of hypertension (Epstein et al. 1996). It has been proposed that over activity of SNS contributes to the development and maintenance of hypertension through increased cardiac output, increased vascular resistance and abnormal water retention (Mark, 1996). It has been reported that the vascular reactivity of hypertensive patients is greater as they exhibit greater vasoconstrictor responses to infused noradrenaline than normotensive persons (Ziegler et al. 1991).
Renal vasoconstriction, renal ischemia, local generation of reactive oxygen species and over production and activation of local angiotensin II act as stimuli for renal vasculopathy and hemodynamic effects leading to hypertension (Sealey et al. 1988). Vascular remodeling resulting in an increased vascular peripheral resistance is another feature of hypertension. Such that alteration in structure and functions of small arteries contribute to the elevation of blood pressure (Mulvany and Aalkjaer, 1990). Since the discovery of renin-angiotensin-aldosterone system (RAAS), its role in the pathogenesis of hypertension had been extensively studied and was found to be the key factor in the genesis and maintenance of hypertension. RAAS contributes in the elevation of blood pressure through multiple mechanisms including vasoconstriction of resistance vessels, release of aldosterone, renal sodium reabsorption, release of anti-diuretic hormone and augmenting of central sympathetic outflow. Angiotensin II (Ang II) has also been implicated in cardiac and vascular cell hypertrophy and hyperplasia through the activation of angiotensin type I receptors and by stimulating the release of growth factors and cytokines (McConnaughey et al. 1999). Similarly, aldosterone excess has been now considered as common contributing factor in hypertension (Lijnen and Petrov, 2000). Moreover, arterial stiffness and high levels of circulatory endothelin have also been proposed in the development of hypertension (Ergul et al. 1996).

The concept of oxidative stress and endothelial dysfunction contributing to the pathogenesis of hypertension has gained interest in recent years. Angiotensin II-induced activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase results in an increased production of oxidant superoxide anions (O2•¯). Increased O2•¯ production results in oxidative inactivation of nitric oxide (NO) and the resultant decreased bio-
availability of NO provides another mechanism of increased vasoconstriction and hypertension (Rajagopalan et al. 1996). Adipose tissue releases leptin, angiotensinogen and oxidized fatty acids to stimulate adrenal release of aldosterone via activation of the classic RAAS, as well as a non-classical pathway mediated by oxidized fatty acids. Leptin stimulates the central SNS, which in turn leads to renin release from the kidney. Activation of RAAS in other tissues contributes to renal and vascular dysfunction. Increased adipose tissue can lead to obstructive sleep apnoea (OSA), which can be treated by therapeutic weight loss or application of continuous positive airway pressure (cPAP). OSA leads to activation of the sympathetic nervous system (SNS), which activates RAAS in the kidney. Increased aldosterone can be reduced with mineralocorticoid receptor antagonists, (Figure 1.3).
Figure 1.3: Pathophysiological mechanisms of hypertension

(Adapted from DeMarco et al. 2014).

**Cpap**: continuous positive airway pressure; **OSA**: obstructive sleep apnoea,

**RAAS**: renin–angiotensin–aldosterone system; **SNS**: sympathetic nervous system,

**ARB**: angiotensin receptor blocker, **Ang II**: angiotensin II.
1.4 Diabetes

There has been an increase in the prevalence of diabetes mellitus over the past 40 years, both in the US and worldwide. The worldwide prevalence of diabetes in year 2000 was approximately 2.8% and is estimated to grow to 4.4% by the year 2030, that will lead to a rise in the number of diabetic patients from 171 million in year 2000 to 350 million in year 2030 (Wild et al. 2004).

Diabetes mellitus is one of the most important public health problems prevailing worldwide with a leading cause of death and huge economic burden. Until the early part of the 20th century, being diagnosed with diabetes used to mean certain death within few years due to the lack of proper therapeutic options. The revolutionary discovery of insulin allowed a control of this disease in terms of reduced mortality and morbidity. The advancement of science also enabled to undertake in-depth studies regarding the related intricate pathophysiology of diabetes and brought the adverse effect of this disease in forefront of medical and health research. The increasing prevalence and globalization of this disease has increased and is particularly observed in the recent decades (Kennedy and Zochodne, 2005).

Diabetes is defined as a chronic metabolic disorder that affects the metabolism of carbohydrates and other nutrients because of impaired insulin release and/or insulin resistance resulting in hyperglycemia (Tierney et al. 1996) Diabetes mellitus is classified into insulin dependent diabetes mellitus (IDDM) or type 1 diabetes and non-insulin dependent diabetes mellitus (NIDDM) or type 2 diabetes. The type 1 diabetes is mainly related to insulin deficiency in which a sequel of β-cell destruction is widely implicated.
in the pathogenesis of this type of diabetes. The type 2 diabetes may be caused due to either cellular insensitivity to insulin or insulin resistance and/or secretory dysfunction related to impaired β-cell function or the presence of non-functional β-cells. Type 1 diabetes mellitus which is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to a deficiency of insulin. It is either immune-mediated or idiopathic (Boon & Davidson, 2006). The majority of type 1 diabetes is of the immune-mediated in nature, where the loss of beta cells is due to autoimmune attack mediated by T-cell (Johns Hopkins Autoimmune Disease Research Center, 2007). On the other hand, Type II diabetes mellitus is characterized by the presence of insulin resistance or reduced insulin sensitivity, and with relatively reduced insulin secretion. This defective response of body tissues to circulating insulin involves insulin receptor in cell membranes (Rother, 2007). The exact cause and mechanism is not fully understood in type II diabetes mellitus. Certain risk factors are associated with increased incidence of diabetes such as obesity, as 55% of patient diagnosed with type 2 diabetes mellitus have central obesity. Other factors include ageing, increasing body mass and decrease demands of physical activity and family history (Kopelman, 2000). Genetics are strongly linked with both types of diabetes mellitus (Walley et al. 2006). The hyperglycemia due to poor glycemic control is common in overt diabetes and is associated with dysfunctions of different organs, particularly kidney, nerves, eye, heart and blood vessels. (Cooper et al. 2001).

1.4.1 Diabetes and hypertension

Hypertension and diabetes often coexist. Diabetics have increased prevalence of developing the hypertension. One prospective study (that included 12,550 adults),
indicates that the development of diabetes in hypertensive patients is 2.5 times greater as compared to normotensive subjects (National High Blood Pressure Education Program Working Group, 1994). Similarly, previous data suggests that there is increased prevalence of diabetes in hypertension and approximately 20% of hypertensives have coexisting diabetes (Contreras et al. 2000). Moreover, both diseases serve to induce and as well as exacerbate each other (Sowers and Epstein, 1995). Both hypertension and diabetes predispose to the development of cardiovascular disease (CVD) and renal disease as their major complications (Sowers, 2004). The co-existence of hypertension and diabetes in patients increases the risks of cardiovascular diseases by 75% (Adler et al. 2000). Diabetes mellitus & systemic hypertension promote the process of atherosclerosis, and their combination further increases this risk (Fuller, 1985).

1.4.2 Complications of diabetes in hypertension and oxidative stress

Hypertension and diabetes are associated with marked abnormalities of cardiovascular structure and functions. Hypertension and diabetes both can induce coronary heart disease, infarction, cerebrovascular accidents, nephropathy and retinopathy and peripheral vascular diseases (Bakris et al. 2000). Diabetes is commonly associated with both micro- and macro-vascular complications. These vascular complications are mainly accelerated in context of systemic hypertension. The underlying molecular mechanisms responsible for diabetic vascular complications are being elucidated. A large body of research is examining this topic and it appears that in case of diabetes, both metabolic and hemodynamic factors interact to stimulate the expression of cytokines and growth factors in the various vascular trees and contribute in the genesis of these complications.
Diabetes provides a distinct model of chronic vascular disease in which altered glycemic status of the body results in multiple organ dysfunctions. These diabetes-induced complications can divided into micro- and macro-vascular complications. Neuropathy, nephropathy and retinopathy are major diabetic micro-vascular complications of diabetes. The macro-vascular complication of diabetes is manifested as accelerated atherosclerosis that predisposes the patients to premature ischemic heart disease, increased risk for cerebrovascular disease and for severe peripheral vascular diseases. Several factors including metabolic, humoral & hemodynamic factors are believed to be involved in the pathogenesis of vasculopathy frequently observed in overt and poorly controlled diabetes (Wood et al. 1995).

Hypertension is a chronic medical condition and frequently remains asymptomatic until late in its course. It can be classified as essential (primary) and secondary hypertension. Essential hypertension is of unknown cause and constitutes about 90 to 95% of cases. Many pathophysiological factors contribute to the genesis of essential hypertension such as high sodium intake, inadequate dietary intake of potassium and calcium, increased secretion of renin, angiotensin II and aldosterone, overproduction of sodium-retaining hormones and vasoconstrictors, increased sympathetic activity and deficiencies of vasodilators (Wood et al. 1995).

In hypertension, production of cardiac and vascular ROS is increased. Increase in vascular oxidative stress has been observed in different models of experimental hypertension like angiotensin-II induced hypertension, Dahl salt-sensitive hypertension, obesity-associated hypertension, and SHR. Antioxidants and SOD mimetic decreased blood pressure and prevented the development of hypertension in animal models of
hypertension (Park et al. 2002). These beneficial effects point towards the role of ROS in the development of hypertension and vascular complications. In hypertension, antioxidants may improve endothelial function, regress vascular remodeling and reduce vascular inflammation.

Endothelial dysfunction is considered as the first step in the pathogenesis of micro and macro vascular complications of both diseases. Endothelium, the inner lining of blood vessels, releases certain chemical substances in response to acetylcholine. These substances are divided into two types according to the functions they perform (Wong et al. 2010). Endothelium-derived relaxing factors (EDRFs) including nitric oxide (NO), prostacyclin (PGI2) and endothelium-derived hyperpolarizing factors (EDHFs). All of them reduce the vascular tone. Opposite to the beneficial EDRFs endothelium also produces a vasoconstrictors substance called as endothelial-derived constrictors factors (EDCFs). Prostaglandin H2, thromboxane A2 (TXA2), leukotrienes, endothelin, and superoxide anions are included in this category (Vanhoutte, 2009). A critical balance is required between EDRFs and EDCFs in order to maintain the vascular health and function. Hypertension and diabetes tend to disturb this balance via either increasing or decreasing the production of one or both (Vanhoutte et al. 2009). Hyperglycemia associated with diabetes modifies the endothelial function through a numbers of complex mechanisms including oxidative stress (Laight et al. 2000), glycation of protein and lipids (Vlassara et al. 1992), and activation of protein kinase C (Hink et al. 2001). Similarly, the endothelial dependent vasodilatation is impaired in different animal models of hypertension including spontaneous hypertensive rats and renovascular hypertension (Quaschning et al. 2006). ROS formation can be a direct consequence of hyperglycemia.
Hyperglycemia induced endothelial cell mitochondrial overproduction of superoxide is involved in the pathogenesis of diabetes related complications. It has been proven that the inhibition of hyperglycemia induced overproduction of superoxide by manganese superoxide dismutase completely prevents advanced glycation end-product (AGE) formation, protein kinasae C activation (PKC) and the hexosamine pathway in endothelial cells (Brownlee, 2001, Nishikawa et al. 2000). Chronic hyperglycemia enhances the local activity of renin-angiotensin-aldosterone system (Nickenig et al. 1998), which leads to development of wall hypertrophy and fibrosis. Hyperglycemia can induce superoxide and lower SOD activity leading to impaired endothelium-dependent vasodilatation in diabetes (Du et al. 2003). Hyperglycemia is thought to increase the production of Ang II in various tissues, including blood vessel wall, kidney and heart. Ang II stimulates NAD(P)H oxidase via the AT1 receptor, leading to an increase in the tissue formation of reactive oxygen species (O2, OH and H2O2). Reactive oxygen species induce endothelial dysfunction reducing bioavailability of nitric oxide. Endothelial dysfunction leads to the release of endothelin and catecholamines that induce vasoconstriction (Giacchetti et al. 2005).
Figure 1.4: Schematic representation of the involvement of oxidative stress induced by diabetes and the development of diabetic complications
(Adapted from Giacchetti et al. 2005)

PAI-1: plasminogen activator inhibitor-1, VCAM: vascular cell adhesion molecule,
ICAM: intercellular adhesion molecule