ROLE OF PROINFLAMMATORY AND ANTI-INFLAMMATORY ADIPOKINES IN METABOLIC DISEASES

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Ph.D

The Hong Kong Polytechnic University

2018

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Role of Proinflammatory and Anti-inflammatory Adipokines in Metabolic Diseases

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

July 2017

Certification of Originality

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material which has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

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Rashmi Supriya (Name of Student)

Dedication

To my mother and father with lots of love and respect

Abstract

The prevalence of metabolic disorders such as metabolic syndrome (MetS) and diabetes is increasing worldwide. Type 2 diabetes is projected to be the 7th leading cause of death by 2030, as estimated by the World Health Organization. The signs and symptoms of type 2 diabetes are not always obvious, but it is often diagnosed during a routine check-up because the symptoms are often mild and develop gradually over many years. Symptoms include central obesity, elevated fasting plasma glucose, elevated blood pressure, high serum triglycerides, and low high-density lipoprotein levels. The clustering of these medical conditions is sometimes referred to as prediabetes or MetS and leads to diabetes, cancer or cardiovascular disorders.

The interactions between the components of the clinical phenotype (central obesity, insulin resistance, hypertension and dyslipidaemia) of metabolic disorders might contribute to the development of a proinflammatory state. Adipokines (secreted by adipose tissue) and cytokines (secreted by macrophages) offer links between metabolism and inflammation. Researchers propose that anti-inflammatory and proinflammatory adipokine equilibrium is necessary to prevent metabolic disorders.

Therefore, the present thesis aims to explore the unique roles of adipokines in differentiating the risk factors of metabolic diseases. In addition, it also aims to determine the role of adipokines as a biomarker for worsening conditions of metabolic diseases. This thesis was designed to investigate the role of proinflammatory and anti-inflammatory adipokines in metabolic disorders via

four studies. In the first study, the distinctive influence of central obesity on other cardiometabolic risk factors of MetS was investigated by adipokine profiling. In the second study, the consequences of hypertension and obesity were differentiated based on proinflammatory and anti-inflammatory adipokine profiles. Further, in the third study, the role of adipokines as indicators was explored to determine the beneficial role of a one-year yoga intervention in MetS subjects with high-normal blood pressure. Finally, in the last study, the critical role of adipokines in diagnosing worsening type 2 diabetic conditions was investigated by determining the pathogenesis of doxorubicin (an anticancer drug) in diabetic muscle in which insulin signalling and muscle atrophy markers were not indicative of any exacerbation.

Central obesity is always emphasized in metabolic syndrome (MetS). This study examined the circulatory adipokines in adults with different cardiometabolic characteristics to reveal the interacting influence of central obesity with other MetS cardiometabolic risk factors, including hyperglycaemia, hypertriglyceridemia, dyslipidaemia and hypertension**.** Eighty-three blood samples were selected from an archived sample pool of 1,492 Hong Kong Chinese adults who were previously screened for MetS according to the guideline of the United States National Cholesterol Education Program Expert Panel Adult Treatment Panel III criteria[1]. Insulin and 11 adipokines, namely, visfatin, chemerin, plasminogen activator inhibitor-1, resistin, C-C motif chemokine ligand 2, interleukin-6, interleukin-8, interleukin-10, tumour necrosis factor-α, leptin, and adiponectin, were assessed. Our generalized estimating equation (GEE) analyses revealed significant interaction effects between central obesity and the cluster of the other 4 MetS cardiometabolic risk factors

on TNF-α, adiponectin, and leptin ($P < 0.05$). TNF-α, adiponectin and leptin differentiate and demonstrate the interacting influence of central obesity with other MetS cardiometabolic risk factors.

Central obesity and hypertension are common risk factors for metabolic syndrome and cardiovascular diseases. The prevalence of myocardial infarctions has been reported to be higher in adult women when compared to adult men in the United States. Additionally, obese females have 6 times whereas obese males have only 1.5 times more chance to develop hypertension compared to their non-obese counterparts. The interacting influence on adipokines from central obesity and hypertension is largely unknown. Adipokines have been proposed as the links between obesity and hypertension, and this study aimed to differentiate the effects of central obesity and hypertension by examining the circulatory profile of adipokines including adiponectin, PAI-1, leptin, and TNF-α. A total of 387 women aged 58 ±11 years (selected from a pool of 1492 Hong Kong Chinese adults), who were previously screened for metabolic syndrome[1] were examined with a 2 x 2 factorial design for central obesity (waist circumference ≥ 80 cm) and hypertension (≥ 140/90 mmHg). Subjects with hyperglycemia, hypertriglyceridemia, and dyslipidemia were excluded to eliminate their confounding effects. Our generalized estimating equation (GEE) analyses revealed significant interaction effects between central obesity and hypertension on the serum abundances of adiponectin and TNF-α. Significant main effects of central obesity were observed on the increases in PAI-1 and leptin. In conclusion, blood profiling of adiponectin and TNF-α reveals the complications of the interaction of central obesity with hypertension in middle-aged and older women, suggesting the

existence of inter-relationship between these two-common cardiovascular risk factors MetS is a cluster of conditions - visceral obesity, dyslipidaemia, hyperglycaemia, and hypertension.

Most people with MetS have been diagnosed with hypertension. MetS is associated with the development of diabetes mellitus, stroke, and cardiovascular diseases. Interestingly, the prevalence of elevated blood pressure among people with MetS has been reported as high as 85%. Our previous study indicated that subjects with MetS showed a significant decrease in the waist circumference and a decreasing trend in blood pressure after 1-year of yoga intervention. Therefore, this study was designed to further investigate the effect of yoga intervention on MetS subjects with high normal blood pressure by exploring modulations in pro-inflammatory adipokines (leptin, chemerin, visfatin and plasminogen activator inhibitor-1 or PAI-1) and anti-inflammatory adipokine (adiponectin). 97 Hong Kong Chinese individuals aged 57.6 ± 9.1 with MetS and high-normal blood pressure (systolic pressure ≥ 130 mmHg or diastolic pressure ≥ 85 mmHg) MetS (defined by United States National Cholesterol Education Program Expert Panel Adult Treatment Panel (NCEP ATP III) guidelines[2]), were randomly assigned to control (n = 45) and yoga group (n = 52). Subjects in control group were not given any intervention but were contacted monthly to monitor their health status. Subjects in yoga group underwent a yoga training program with three 1-hour yoga sessions weekly for 1 year. Serum harvested were assessed for adipokines including leptin, chemerin, visfatin, PAI-1, and adiponectin. Generalized estimating equation (GEE) was used to examine the interaction effect between 1-year of time (pre vs. post) and intervention (control vs. yoga) on adipokines and MetS

risk factors. The results of this study revealed significant interaction effects between time and intervention on leptin, chemerin and adiponectin. Main effect of intervention was observed on PAI-1. These results demonstrated that 1-year yoga training decreased pro-inflammatory adipokines (leptin, chemerin, and PAI-1) and increased anti-inflammatory adipokine (adiponectin) in adults with MetS and high-normal blood pressure. These findings support the beneficial complementary role of yoga in managing MetS by favorably modulating the circulatory adipokines.

The anti-cancer agent doxorubicin (DOX) has been demonstrated to worsen insulin signalling, engender muscle atrophy, trigger proinflammation, and induce a shift to anaerobic glycolytic metabolism in skeletal muscle. The myotoxicity of DOX in diabetic skeletal muscle remains largely unclear. The fourth study examined the effects of DOX on insulin signalling, muscle atrophy, pro-/anti-inflammatory microenvironments, and glycolysis metabolic regulation in the skeletal muscle of db/db diabetic and db/+ non-diabetic mice. DOX had no effects on insulin signalling markers (Glut4, pIRS1Ser636⁄639, and pAktSer473) or muscle atrophy markers (muscle mass, MuRF1, and MAFbx) in diabetic muscle. However, DOX exposure resulted in the enhancement of a proinflammatory favouring microenvironment (as indicated by TNF-α, HIFα, and pNFκBp65), accompanied by a diminished anti-inflammatory favouring microenvironment (as shown by IL15, PGC-1α, and pAMPKβ1Ser108). The metabolism in diabetic muscle was shifted to anaerobic glycolysis after DOX exposure as demonstrated by our analyses of PDK4, LDH, and pACCSer79. The results showed that there might be a link between inflammatory modulation and the dysregulation of aerobic glycolytic metabolism in DOX-injured diabetic

skeletal muscle. These findings help to understand the pathogenesis of DOX-induced myotoxicity in diabetic muscle.

Therefore, we conclude that adipokines (proinflammatory and anti-inflammatory) play an important role as a biomarker in metabolic disorders. Knowledge about the modulation of pro- and anti-inflammatory adipokines with changes in the major risk factors for MetS, including obesity, hypertension, high triglycerides, low high-density lipoprotein, and high fasting blood glucose, might help in differentiating the inflammatory and metabolic molecular pathways that cause metabolic diseases. Additionally, the modulation (proinflammatory decreases and anti-inflammatory increases) of adipokines contributes to understanding the beneficial effects of yoga in subjects with high-normal blood pressure and MetS in whom other risk factors of MetS are not indicative of the beneficial effects of yoga. Further, the modulation (proinflammatory increases and anti-inflammatory decreases) of adipokines helps to understand and detect the worsening effects of doxorubicin injection in diabetic skeletal muscle in which insulin signalling and muscle atrophy markers are not indicative of the exacerbation. Although numerous effects of adipocytokines have been reported in recent studies, further investigation of their signalling pathways is still needed to understand how they are eventually integrated. It will be important to focus on how adipocytokine signalling participates with intracellular cascades triggered by other factors in the immune cells. Understanding the mechanism of action of adipocytokines may be crucial in the development of novel therapeutic approaches for treating metabolic disorder-induced inflammatory diseases.

Publications Arising from this Thesis

Journal

Supriya R, Tam BT, Pei XM, Lai CW, Chan LW, Yung BY and Siu PM, Doxorubicin Induces Inflammatory Modulation and Metabolic Dysregulation in Diabetic Skeletal Muscle. Frontiers in Physiology (2016). DOI: 10.3389/fphys.2016.00323

Supriya R, Yu AP, Lee PH, Lai CW, Cheng KK, Yau SY, Chan LW, Yung BY, Siu PM. Yoga training modulates adipokines in adults with high-normal blood pressure and metabolic syndrome. (Manuscript accepted in Scandinavian Journal of Medicine & Science in Sports)

Supriya R, Tam BT, Yu AP, Lee PH, Lai CW, Cheng KK, Yau SY, Chan LW, Yung BY, Siu PM. Circulatory adipokines exhibit the influence of interaction of central obesity with other cardiometabolic risk factors of metabolic syndrome. (Manuscript under revision in Plos One)

Supriya R, Yung BY, Yu AP, Lee PH, Lai CW, Cheng KK, Yau SY, Chan LW, , Siu PM. Complications of the interaction of central obesity and hypertension on circulatory adipokines in adult women. (Manuscript under revision inFrontiers in endocrinology)

Conference

Supriya R, Tam BT, Pei XM, Lai CW, Chan LW, Yung BY and Siu PM, Doxorubicin Induces Inflammatory Modulation and Metabolic Dysregulation in Diabetic Skeletal Muscle, Immunometabolism, 2016, Hong Kong

Supriya R, Yu AP, Lee PH, Lai CW, Chan LW, Yung BY and Siu PM, Title: Effect of 1-year Yoga on adipokine profile in Chinese adults with high-normal blood pressure and metabolic syndrome, 22nd Annual Congress European College of Sports Science, MetropolisRuhr 2017, Germany

Acknowledgments

All praises to GOD, the creator of the universe, who blessed me with the knowledge and enabled me to complete this thesis.

I would like to thank my chief supervisor, Prof. Yung for his guidance and advice since I started my PhD. He has been my scientific mentor and an understanding supervisor who always helped me in need. My deepest gratitude also goes to my project supervisor, Dr. Parco Siu. I am obliged to him for accepting me as one of his team members and guiding me. I am very thankful to him for his quick, extensive review of my manuscripts irrespective of how late or early I sent him papers. He never allowed me to suffer because of his busy schedule and he always reverted before time to meet the deadlines. He always provided all the resources including the analytical support and adipokines expertise. I am very thankful to him for his patience and motivation throughout my studies.

I would also like to express thanks to Dr. Lawrence for being there always to co-supervise me with his team members. My gratitude also goes to Dr. Chien Ling Hung, who co-supervised me initially and took care of me like her younger sister. I will always be obliged to her as she also trained me for the basic lab skills. I am also very thankful to the head of the department, Prof. Yip for his time to clarify my doubts without delay.

I am indebted to my friend and colleague Mr. Sawaid Abbas. He was always there to support me through his statistical, scientific, and moral suggestions. It is not enough to thank him as without him, I would have never been able to complete this thesis.

I would also like to acknowledge my research fellows for maintaining friendly environment. Bjorn is one of the best teachers, who provided me the lab skills and basic rules for the research life. Angus, another team member was also there for me in clarifying my doubts. I would also thank Felix, my fellow researcher, who always participated in the discussion with me for solving the problems related to research.

My mother suffered from cancer a lot in my PhD journey. I was not with her when she needed me most. She never complained about her deadly pain to keep me going in my research work. I love you mom, and I will always think of your courage before complaining about my hard times. Finally, my thesis would not have been possible without the support of my family and my love.

Rashmi SUPRIYA

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List of Abbreviations

AHA	American Heart Association
11β-HSD1	Hydroxysteroid Dehydrogenase Type 1
AACE	American Association of Clinical Endocrinology
ACC2	Acetyl-Coenzyme A (Coa) Carboxylase 2
AGE	Advanced Glycation End Product
AKT	Protein Kinase B
AMPK	5' AMP-Activated Protein Kinase
ANS	Autonomic Nervous System
ATP	Adenosine Triphosphate
Bpdia	Blood Pressure Diastolic
Bpsys	Blood Pressure Systolic
CCL-2	C-C Motif Chemokine Ligand 2
CHD	Coronary Heart Disease
cm	Centimeters
CON	Control
CVD	Cardiovascular Diseases
DECODE	Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe
DOX	Doxorubicin
ELISA	Enzyme-Linked Immunosorbent Assay
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GC	Glucocorticoids (GC)

GEE	Generalized Estimating Equation
GLUT4	Glucose Transporter Type 4
GSK3β	Glycogen Synthase Kinase 3 Beta
HDL	High Density Lipoprotein
HIF	Hypoxia-Inducible Factors
HPA	Hypothalamic-Pituitary-Adrenal Axis
IDF	International Diabetes Federation
IGFBP	Insulin-Like Growth Factor Binding Protein
IL-15	Interleukin 15
IL-10	Interleukin 10
IL-6	Interleukin 6
IOTF	International Obesity Task Force
IRS1	Insulin Receptor Substrate 1
IVF	In Vitro Fertilization
LDH	Lactate Dehydrogenase
MetS	Metabolic Syndrome
mg/dl	Milligram Per Deciliter
MI	Myocardial Infarction
miRNAs	Micro Rnas
mmol/L	Millimole Per Liter
mRNAs	Messenger Rnas
mTOR	Mechanistic Target of Rapamycin
NADPH	Nicotinamide Adenine Dinucleotide Phosphate

NCEP ATPIII	National Cholesterol Education Program Adult Treatment Panel **III**
NEFA	Nonesterified Fatty Acids
NFKβ	Nuclear Factor-Kb
NHANES	National Health and Examination Survey
NIH	National Institute of Health
NO	Nitric Oxide
OGTT	Oral Glucose Tolerance Test
OSPHOS	Oxidative Phosphorylation
PAI-1	Plasminogen Activator Inhibitor-1
PDK4	Pyruvate Dehydrogenase Lipoamide Kinase Isozyme 4
PGC-1α	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha
PVDF	Polyvinylidene Difluoride
RAS	Renin Angiotensin System
RBP4	Retinol Binding Protein 4
ROS	Reactive Oxygen Species
SD	Standard Deviation
SEM	Standard Error Mean
SHBG	Sex Hormone-Binding Globulin
sTNF-α-R	TNF-A Receptor
T2D	Type 2 Diabetes
TCA cycle	Tricarboxylic Acid Cycle

TGs	Triglycerides
TNF-α	Tumour Necrosis Factor Alpha
TZD	Thiazolidinedione
WHO	World Health Organization
WHR	Waist-To-Hip Ratio
WS	Waist Circumference

Chapter 1

Introduction

1.1. Introduction

Metabolic diseases, such as metabolic syndrome (MetS), comprise a collection of risk factors, including central obesity, hypertension, dyslipidaemia, hypertriglyceridaemia, and insulin resistance, that together lead to increased risks of cardiovascular disease, type 2 diabetes mellitus (T2D), and a plethora of cancer types, including breast cancer and colorectal cancer [1]. MetS increases the risk of T2D by five-fold and cardiovascular disease by three-fold [3,4]. MetS has been emphasized as a major socioeconomic problem worldwide because its burden, along with its risk factors, such as insulin resistance, central obesity, hypertension and dyslipidaemia, are apparent across all ethnicities. For instance, an 8-year study from the United States involved mature individuals (n=3,323) of mostly Caucasian ethnicity who did not have cardiovascular disease or T2D at baseline. The study reported that according to the National Cholesterol Education Program:Adult Treatment Panel III (NCEP:ATP III) definition of MetS [1], MetS prevalence in both women and men increased by approximately 50% over the 8 years. The prevalence of MetS was increased from 12.5 to 23.6% in women and 21.4 to 33.9% in men. The risk of cardiovascular disease and T2D was also reported to be increased in correlation with the increase in MetS prevalence in both sexes. At the end of their 8-year study, they reported that 107 individuals had chronic heart disease, 174 individuals had cardiovascular disease, and 178 people had T2D [5].

Central obesity [2,6] and hypertension [7,8] have often been emphasized as the causes underlying the pathogenesis of metabolic diseases, including MetS and T2D. According to the IDF consensus worldwide definition of MetS published in 2006, obesity contributes to hypertension, high serum cholesterol, low high-density lipoprotein (HDL)-cholesterol and hyperglycaemia [9–11]. In contrast, the European Society of Hypertension-European Society of Cardiology Guidelines and the Seventh Report of the Joint National Committee emphasize the importance of identifying MetS when treating patients with hypertension [12,13]. Currently, there is a lack of certainty in defining MetS. Therefore, an internationally acceptable definition for MetS is now compulsory to avoid misunderstandings and to help achieve the earliest diagnosis and intervention possible. Early diagnosis of MetS could alleviate the increased risk of many associated problems, such as cardiovascular disease, T2D and cancer.

Metabolic dysregulation has been acknowledged as the cause of most of the diseases, including MetS, T2D [14] and cancer [15]. Biomarkers aid in the diagnosis and management of many pathological states when there are no evident clinical signs or obvious anatomic abnormalities [16]. Therefore, metabolic disease biomarkers may provide a relatively easy, minimally invasive means of detecting those people who are at a higher risk for developing MetS and its subsequent complications. Adipose tissue, in addition to being a primary energy depot in the human body, functions as an important endocrine organ that secretes bioactive factors, collectively known as adipokines. Adipokines regulate appetite, insulin signalling, endothelial functions, and inflammation at both the tissue and systemic levels [17]. An abnormal adipokine profile may contribute to MetS and thus other MetS-related diseases. An epidemiological

link has been well established between adiposity, central obesity, and hypertension [18,19]. As obesity starts to develop, adipocytes increase in size due to a positive energy balance. The enlarged adipocytes begin to secrete proinflammatory adipokines, such as leptin [20], IL-6 [21], TNF-α [22], CCL-2 [22] and chemerin. These proinflammatory adipokines are classic macrophage (M1) stimuli, inducing inflammation and a shift from alternate macrophages (M2) to M1 macrophages in obese adipose tissue at an early stage of obesity. With increasing adiposity, obese adipose tissue activates CD8+ T cells, which recruit monocytes into the adipose tissue to activate proinflammatory M1 macrophages. Furthermore, a large number of migrated M1 macrophages, which are different from the original adipose tissue resident macrophages, overwhelm the anti-inflammatory effects of the M2 macrophages and promote systemic inflammation, thus causing MetS [23,24]. Similar to central obesity, the role of adipokines in contributing to hypertension has been recognized several times [25]. Hypertension has been reported to be strongly associated with increased visceral adiposity [26]. Subcutaneous adipose depot secretions, including leptin and adiponectin, have been shown to predict the development of hypertension [27]. For instance, leptin has been reported to upregulate the renin-angiotensin system (RAS) and mediate angiotensin II-elicited hypertension in rats fed a high-fat diet for 3 weeks [28]. Furthermore, increased levels of PAI-1, another proinflammatory adipokine, have been associated with RAS and cardiovascular risk factors, such as hypertension, obesity, insulin resistance, and diabetes [29,30].

Researchers suggest that a single adipokine is not strong enough to induce MetS. Instead, the interaction between proinflammatory and anti-inflammatory

adipokines causes a systemic metabolic abnormality [31]. Thus, adipokine equilibrium is necessary to prevent MetS [31]. Unbalanced adipokine equilibrium has also been reported in pathological states in which clinical signs are not evident. For instance, the prevalence of MetS was reported to be 1.49 times higher when low adiponectin (an anti-inflammatory adipokine) and high retinol binding protein 4 (a proinflammatory adipokine) levels were combined, compared to subjects with low retinol binding protein 4 and high adiponectin levels [31]. Several lines of evidence also demonstrate that fluctuations in the adipokine equilibrium may also indicate the positive impact of any intervention. For example, a randomized controlled trial of a one-year yoga intervention in industrial workers (n = 48, aged 30-58 years), revealed a significant decrease in IL-1 β (a proinflammatory adipokine) and a significant increase in IL-10 (an anti-inflammatory adipokine) in the yoga group. The authors showed that the control group showed no significant change in either IL-1 β or IL-10 levels [32]. Therefore, each adipokine has a particular role in maintaining the delicate equilibrium between pathophysiological and protective impacts.

1.2. Significance of this thesis

In this thesis, we profiled a panel of the most studied adipokines to further determine their unique functions in differentiating the two main clinical manifestations of MetS, including central obesity and hypertension. The results of the thesis also propose that the adipokine panel may be used as a biomarker for identifying the protective outcome of yoga interventions in worsening metabolic conditions and may be useful for diagnosing worsening pathological conditions of metabolic diseases, such as T2D after doxorubicin (DOX) (an anti-cancer drug) administration.

The implications of metabolic diseases are complex because the risk for the sequelae of metabolic diseases varies among individuals. For instance, some adults with obesity are at a comparatively low risk of developing imminent disease [33,34]. On the other hand, several ethnicities have a higher risk for developing chronic diseases linked to obesity [35,36]. Further, ethnicity-inclusive means of risk identification are needed among adults to accurately target treatment for future disease and to motivate patients and their families to make lifestyle improvements. One of the major points of significance of this thesis is its specificity to Hong Kong Chinese adults. Several lines of evidence indicate that ethnic differences in metabolic diseases may result in the variances in circulating adipokines and inflammatory markers [37]; therefore, the results of this thesis are not general, and this thesis is important for Hong Kong Chinese adults. Specific targeting and exhaustive treatment efforts may help in avoiding the future sequelae of metabolic diseases among all ethnicities.

Currently, a universally accepted defined diagnostic criterion for defining MetS is lacking. Nearly all the studies focusing on MetS are difficult to interpret as

they did not specify which three or more MetS risk factors were used for the selection of their subjects [34,38]. The results of the first study of this thesis are of great importance, as the study design allowed us to make an explicit interpretation solely on the individual and interaction effects of central obesity and a cluster of four cardiometabolic risk factors for MetS, including hyperglycaemia, hypertriglyceridemia, high blood pressure and reduced high-density lipoprotein cholesterol. The results support the notion that particular attention is needed for considering central obesity with other MetS cardiometabolic risk factors.

Few pieces of research have attempted to note subtle differences between the sequelae observed in lean individuals with hypertension versus obese people [39,40]. Adipokines have long been presented as the link between obesity and hypertension [41]. The results of the second study of this thesis are significant because the study design allowed us to explicitly interpret the individual and interaction effects of central obesity and hypertension without the confounding effects other MetS risk factors, including hyperglycaemia, hypertriglyceridemia, and reduced high-density lipoprotein cholesterol. In addition to the design, the ethnic and sex specificity of this study makes the results more reliable and specific for Hong Kong Chinese female adults. The results of this study are of foremost importance as adipokine modulation was shown to be exacerbated when the effect of obesity interacted with the consequences of hypertension. Therefore, the results indicate that using an ethnic-specific adipokine panel as a circulation biomarker may be useful for diagnosing the worsening of obesity by including hypertension among different ethnicities.

Favourable effects of a short-term intensive yoga (90 minutes/day for 15 days) programme have been demonstrated to cause changes in body mass index, waist-hip circumference and total cholesterol, as well as improve postural stability and increase hand grip strength, in obese subjects [42]. The beneficial effects of yoga (1 year) in MetS subjects have also been indicated by a reduction in abdominal obesity [2]. Nevertheless, the results of the third study of this thesis suggest that the beneficial effects of yoga in MetS subjects with high-normal blood pressure are not clinically evident, consistent with the notion that MetS identification should be emphasized when treating patients with hypertension [13,43]. The results are significant as adipokines were shown to be favourably regulated after yoga intervention in MetS subjects with high-normal blood pressure. Therefore, the results propose that an adipokine panel as a circulation biomarker may be useful for identifying the protective outcomes of interventions, even in worsening metabolic conditions in which clinical outcomes are not obvious.

The effects of doxorubicin (an anticancer drug) on skeletal muscle have been demonstrated to blunt insulin signalling, stimulate muscle atrophy, spread proinflammatory factors, and cause a shift to anaerobic glycolysis in normal skeletal muscle [44–47]. The myotoxicity of doxorubicin and the responsible molecular mechanisms in diabetic muscle have not been investigated comprehensively. The fourth study of this thesis notes the important role of adipokines in understanding the pathogenesis of doxorubicin in diabetic muscle when insulin signalling and muscle atrophy markers are not indicative of any exacerbation. Therefore, the results propose that an adipokine panel as a

skeletal muscle biomarker may be useful for diagnosing the worsening of metabolic conditions when clinical outcomes are not obvious.

1.3. Study objectives

1. To investigate the distinctive influence of central obesity, separate from other cardiometabolic risk factors of MetS by adipokine profiling in Hong Kong Chinese adults who have been diagnosed with MetS under NCEP ATP III criteria.
2. To investigate the distinctive influence of central obesity (based on NCEP ATP III criteria) separate from hypertension (based on the Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure) by profiling proinflammatory and anti-inflammatory adipokines in Hong Kong Chinese adults.
3. To investigate the protective effects of a one-year yoga intervention on the manifestations of MetS and the adipokine profiles in Hong Kong Chinese adults with MetS and high-normal blood pressure.
4. To investigate the mechanisms and the role of adipokines in skeletal muscle in diagnosing the worsening of T2D after doxorubicin injection in diabetic muscle.

Chapter 2

Literature Review

2.1. Metabolic diseases

Metabolic diseases are the diseases that occur when the usual metabolic processes of the body are disrupted. These diseases may be acquired (such as diabetes) or congenital (such as phenylketonuria) [48]. MetS and type 2 diabetes are the two most common metabolic diseases and are the focus of this thesis.

2.1.1. Metabolic syndrome

Metabolic syndrome (MetS) refers to the accumulation of the core manifestations of the syndrome, including increases in circulating triacylglycerol and low-density lipoprotein (LDL)-cholesterol (dyslipidaemia), dysregulated glucose homeostasis, visceral obesity, and elevation of arterial blood pressure (BP). MetS is also called pre-diabetes as it leads to the development of type-2 diabetes mellitus (T2D) and cardiovascular disease (CVD) [49]. To better understand the underlying pathophysiology, we must better understand the central features of MetS and their correlations among themselves. Currently, there is no universally accepted definition of MetS. A comprehensive, universally accepted definition for MetS would facilitate research to develop new insights into pharmacologic and treatment approaches.

2.1.1.1. Components of metabolic syndrome

There are five manifestations of MetS, including dyslipidaemia, dysregulated glucose homeostasis, visceral obesity and elevated arterial blood pressure. These manifestations are correlated with each other. For instance, insulin resistance has a strong correlation with visceral and abdominal subcutaneous fat [50]. The accumulation of lipids in insulin-resistant muscles leads to fatty liver disease and dyslipidaemia [51]. Additionally, insulin resistance is associated with high levels of triglycerides in muscles and high blood pressure [52,53]. Lipoprotein metabolism and blood pressure have been reported to be substantially influenced by genetic variations and are thus expressed variably in response to obesity and insulin resistance. Each risk factor of MetS is regulated through both acquired and genetic factors. For instance, lipoprotein metabolism is controlled by genetic variations; therefore, significant variance in the expression of dyslipidaemia has been observed in response to insulin resistance and obesity [54]. The following paragraphs will provide a detailed description of each of the manifestations of this syndrome.

Triglycerides

Chemically, triglycerides are triesters of glycerols and fatty acids. The triglycerides cannot be absorbed by the duodenum. Therefore, triglycerides need to break down into monoglycerides (one fatty acid and one glycerol) or diglycerides, to be absorbed by the duodenum. The fatty acids are released when the easter bond is hydrolyzed by pancreatic lipase. This process is called lipolysis where triglycerides are split into free fatty acids by the action of lipases and bile in the intestine [55]. They are then moved to the enterocyte cells which

are the absorptive lining of cells in the intestines. Then subsequently in the enterocytes, triglyceride fragments rebuilt triglycerides and are packaged together with proteins and cholesterol to form chylomicrons. Chylomicrons are then excreted from the cells, collected by the lymph system and then elated to the large blood vessels adjacent to the heart before being ejected in the circulation. Many tissues can seize the chylomicrons as a source of energy. Such as, liver cells can synthesize as well as store triglycerides. In liver, glucagon (hormone) signals lipases to break down the triglycerides into fatty acid. Our brain uses glycerol component of the broken triglycerides by the process of gluconeogenesis [56]. Triglycerides are the major component of very low-density lipoprotein and chylomicrons. Triglycerides are the transporter of fat and are energy sources and hence plays an important role in metabolism. They contain more energy (38 Kilojoule/gram) as compared to carbohydrates (17 Kilojoule/gram) [55]. High levels of triglycerides in the bloodstream of the human body have been linked to atherosclerosis, CVD, T2D and other metabolic diseases [57]. Nevertheless, the relative impact of raised levels of triglycerides compared to that of the ratio between low-density lipoprotein (LDL): High-density lipoprotein (HDL) is still being investigated. It is suspected that the risk of the metabolic disorders may be accounted by a strong inverse relationship between triglyceride level and HDL-cholesterol level [56]. In circulation, triglycerides support the bidirectional transfer of fats and glucose between the liver and body cells. High level of triglycerides in circulation has been strongly associated with the risk of coronary heart diseases [58]. The inverse relationship between high triglycerides and insulin action has been observed in skeletal muscle of both animal and human models [59]. The results

indicate the possible relationship between triglycerides and T2D. Individuals with obesity, and habits of smoking, drinking alcohol and consuming high-carbohydrate diets are likely to have higher blood triglycerides. The America Heart Association (AHA) recommendation for an optimal triglyceride level is ≤100 mg/dL (1.1 mmol/L) to improve heart health. The National Cholesterol Education Program (NCEP) has set physiological interpretation of the triglyceride levels in circulation after 8 to 12 hours of fasting as presented in Table 2.1.

Table 2.1: Physiological interpretation of the triglycerides level in circulation.

Level		**Interpretation**
mg/dL	mmol/L	
<150	<1.70	Normal range – low risk
150–199	1.70–2.25	Slightly above normal
200–499	2.26–5.65	Some risk
500 or higher	>5.65	Very high – high risk

Source: [60]

High-Density Lipoprotein

Lipoprotein contains a central core of a hydrophobic lipid comprised of triglycerides and esters which are covered in a hydrophilic coat of polar phospholipid, free cholesterol, and apolipoprotein. Lipoproteins are particles made up of lipid and protein, responsible for transporting cholesterol in the bloodstream. High-density Lipoproteins (HDL) are one of the five types of lipoproteins. HDL are also called "good cholesterol" as it transports cholesterol from body cells to the liver for their breakdown and excretion. It is demonstrated that high level of HDL in the blood contributes to good health as it has a protective function against coronary heart disease and CVD [61]. Epidemiological

evidence indicates that HDL level is inversely correlated with coronary heart disease incidence and the mortality rates of CVD [62]. Also, HDL can prevent LDL, which is also one of the types of lipoprotein and called "bad cholesterol." LDL transports cholesterol to body cells from the liver for oxidative modification. HDL exerts its protective effect against metabolic disorders by preventing LDL [63]. The NCEP, American Heart Association (AHA) and National Institutes of Health (NIH) guidelines for physiological interpretation of the HDL level in circulation after 8 to 12 hours of fasting are mentioned below in Table 2.2.

Table 2.2: Physiological interpretation of the high-density lipoprotein level in circulation.

Level		**Interpretation**
mg/dL	mmol/L	
<40 for men, <50 for women	<1.03	Low HDL cholesterol, heightened risk for heart disease
40–59	1.03–1.55	Medium HDL level
>60	>1.55	High HDL level, optimal condition considered protective against heart disease

Source: [64–67]

Fasting glucose level

Metabolic homeostasis in the body is maintained via the regulation of blood glucose levels. Glucose is a 6-carbon sugar that undergoes cellular respiration to provide energy for humans and animals. Glucose is transported from the intestine or liver to cells throughout the body via the bloodstream. Glucose is made available for cell absorption through the action of insulin (secreted from pancreas). Blood sugar levels outside the normal range may be an indicator of

metabolic disturbances. A persistently high level of glucose is called hyperglycaemia, and low levels are referred to as hypoglycaemia. T2D is characterized by hyperglycaemia [68]. In the human body, glucose is stored as glycogen and can be converted back to glucose when needed. This conversion of glucose to glycogen is controlled by insulin. Insulin deficiency leads to type I diabetes (T1D), while insensitivity or resistance to insulin causes T2D. The Diabetes Prevention Trial-Type 1 demonstrated that mortality due to coronary heart disease (CHD) and risk of developing large-vessel disease have a positive and significant correlation with subjects with impaired glucose tolerance [69]. During the body's normal homeostatic mechanism, blood sugar levels range from 4.4 to 6.1 mmol/L (79.2 to 110 mg/dL) when measured while fasting [70]. The normal blood glucose level (tested while fasting) for non-diabetics should range between 3.9 and 5.5 mmol/L (70 to 100 mg/dL). In humans, the mean normal blood glucose level should be approximately 5.5 mmol/L (100 mg/dL); nevertheless, this level fluctuates throughout the day. Blood sugar levels for those without diabetes and who are not fasting should be below 6.9 mmol/L (125 mg/dL) [71]. The blood glucose target range for diabetics is less than 10 mmol/L (180 mg/dL) after meals and ranges between 5.0–7.2 mmol/l (90–130 mg/dL) before meals, according to the American Diabetes Association [70].

Blood pressure

The pressure exerted upon the arterial walls by the circulation is called blood pressure. Systolic blood pressure is the pressure exerted when the heart pumps blood during heart beats, whereas diastolic blood pressure is the pressure exerted when the heart is relaxed between heart beats. High blood

pressure commonly occurs due to ageing. It is believed that the lifetime risk of developing high blood pressure for individuals of age 55 or above is 90% [72]. High blood pressure can cause complications such as CVD, stroke, and diabetes. However, diet control and treatment of high blood pressure can reduce the mortality rates associated with diabetes, diabetic retinopathy, and other diabetes-related complications. The risk of cardiovascular disease increases gradually when the blood pressure goes above 115/75 mmHg [73]. Studies suggest that people with low arterial pressures have much better long term cardiovascular health [74]. The classification of blood pressure adopted by the American Heart Association for adults who are 18 years and older is shown in Table 2.3.

Table 2.3: Classification of blood pressure for adults

Category	systolic, mmHg	diastolic, mmHg
Hypotension	< 90	< 60
>60	>1.55	High HDL level, optimal condition considered protective against heart disease
Desired	90–119 90–129	60–79 60–84
Prehypertension (high normal)	120–139 130–139	80–89 85–89
Stage 1 hypertension	140–159	90–99
Stage 2 hypertension	160–179	100–109
Hypertensive urgency	≥180	≥110
Isolated systolic hypertension	≥ 160	< 90

Source: [75–77]

Waist circumference

The waist is the portion of the abdomen between the rib cage and hips [78]. The waist circumference measurement indicates abdominal obesity. Excess abdominal fat is a risk factor for developing CVD and other metabolic diseases. The National Heart, Lung, and Blood Institute classifies the risk of obesity-related diseases as high when men have a waist circumference greater than 102 cm (40 inches) and when women have a waist circumference greater than 88 cm (35 inches) [79]. Central obesity or abdominal obesity is an important parameter of MetS. It refers to intra-abdominal obesity, which involves the accumulation of fat within the peritoneum and around the viscera [80]. In general, obesity leads to high blood pressure, high LDL-cholesterol, low HDL cholesterol and hyperglycaemia. It is also strongly related to CVD risk. Among various types of obesity, central obesity has the strongest correlation with MetS risk factors [81]. According to the International Diabetes Federation, visceral obesity can be measured by waist circumference and defined using the guidelines shown below in Table 2.4 that are based on gender and ethnic groups.

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Table 2.4: Specific value of waist circumference for different ethnic groups

Ethnic group	Gender	Waist circumference(cm)
Europoids	male	≥ 90
	female	≥ 80
South Asians, Chinese	male	≥ 90
	female	≥ 80
South Asians, Japanese	male	≥ 85
	female	≥ 90
Ethnic South and Central Americans	male	Uses South Asians recommendations
	female	Uses South Asians recommended data
Sub-Saharan Africans	male	Uses Europeans recommended data
	female	Uses Europeans recommended data
Eastern Mediterranean and Middle East	male	Uses Europeans recommended data
	female	Uses Europeans recommended data

Source: [82]

2.1.1.2. Lack of consensus for the definition of MetS

Gerald M Reaven was the first to define the concept of MetS; he assumed that it was a dominant feature in the development of CVD and T2D because it caused insulin resistance [83]. Later, many international organizations and expert groups, such as the European Group for the Study of Insulin Resistance (EGIR), the World Health Organization (WHO), the National Cholesterol Education Program Adult Treatment Panel III (NCEP:ATPIII), the International Diabetes Federation (IDF), the American Association of Clinical Endocrinology

(AACE), and the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI), have endeavoured to incorporate all the manifestations to define MetS. All expert groups or international organizations apply the criteria differently to identify active MetS, but they all agree on the same components of MetS for identifying MetS; these components include the following: obesity, insulin resistance, dyslipidaemia, and hypertension (Table 2.5).

Table 2.5: MetS definition by different international organizations and expert groups

INTERNATIONAL ORGANIZATIONS AND EXPERT GROUPS	MANIFESTATIONS CRITERIA	REFERENCE
World Health Organization	Insulin resistance is defined as T2D or impaired fasting glucose (IFG) (> 100 mg/dl) or impaired glucose tolerance (IGT), plus two of the following: 1. Abdominal obesity (waist-to-hip ratio >0.9 in men or >0.85 in women, or body mass index (BMI) >30 kg/m^2. 2. Triglycerides 150 mg/dl or greater, and/or high-density lipoprotein (HDL)-cholesterol <40 mg/dl in men and <50 mg/dl in women. 3. Blood pressure (BP) 140/90 mmHg or greater. 4. Microalbuminuria (urinary albumin secretion rate 20 µg/min or greater, or albumin-to-creatinine ratio 30 mg/g or greater).	84
European Group for the Study of Insulin Resistance	Insulin resistance defined as insulin levels >75th percentile of non-diabetic patients, plus two of the following: 1. Waist circumference 94 cm or greater in men, 80 cm or greater in women. 2. Triglycerides 150 mg/dl or greater and/or HDL-cholesterol <39 mg/dl in men or women.	85

	3. BP 140/90 mmHg or greater or taking antihypertensive drugs. 4. Fasting glucose 110 mg/dl or greater.	
National Cholesterol Education Program Adult Treatment Panel III (NCEP:ATPIII)	Any three or more of the following: 1. Waist circumference >102 cm in men, >88 cm in women 2. Triglycerides 150 mg/dl or greater. 3. HDL-cholesterol <40 mg/dl in men and <50 mg/dl in women. 4. BP 130/85 mmHg or greater. 5. Fasting glucose 110 mg/dl* or greater.	1
American Association of Clinical Endocrinology	Insulin glucose tolerance (IGT) plus two or more of the following: 1. BMI 25 kg/m2 or greater. 2. Triglycerides 150 mg/dl or greater and/or HDL-cholesterol <40 mg/dl in men and <50 mg/dl in women. 3. BP 130/85 mmHg or greater.	86
International Diabetes Federation (IDF)	Central obesity (defined as waist circumference but can be assumed if BMI >30 kg/m^2) with ethnicity-specific values,* plus two of the following: 1. Triglycerides 150 mg/dl or greater. 2. HDL-cholesterol <40 mg/dl in men and <50 mg/dl in women.	87

	3. BP 130/85 mmHg or greater. 4. Fasting glucose 100 mg/dl or greater. *To meet the criteria, waist circumference must be: for Europeans, >94 cm in men and >80 cm in women; and for South Asians, Chinese, and Japanese, >90 cm in men and >80 cm in women. For ethnic South and Central Americans, South Asian data are used, and for sub-Saharan Africans and Eastern Mediterranean and Middle East (Arab) populations, European data are used.	
American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI)	Any three of the following: 1. Waist circumference 102 cm or greater in men, 88 cm or greater in women. 2. Triglycerides 150 mg/dl or greater. 3. HDL-cholesterol <40 mg/dl in men and <50 mg/dl in women. 4. BP 130/85 mmHg or greater. 5. Fasting glucose 100 mg/dl or greater	88
Consensus definition (incorporating IDF and AHA/NHLBI	Any three of the following: 1. Elevated waist circumference (according to population and country-specific definitions). 2. Triglycerides 150 mg/dl or greater. 3. HDL-cholesterol <40 mg/dl in men and <50 mg/dl in women.	6

	4. BP 130/85 mmHg or greater. 5. Fasting glucose 100 mg/dl or greater.	

Currently, the two most widely used definitions are those of the IDF and NCEP:ATP III. Both focus explicitly on waist circumference, which is a surrogate measure of abdominal/central obesity. In contrast, the WHO, AACE, and EGIR focus particularly on insulin resistance. Specifically, IDF criteria for diagnosing MetS include central obesity plus any of the two following factors: insulin resistance, dyslipidaemia, and hypertension. According to the IDF, central (abdominal) obesity is found to be independently associated with each of the other MetS components, especially insulin resistance [89,90]. Obesity is probably an independent risk factor for coronary heart disease, and it plays a significant role in exacerbating coronary heart disease risk factors, including hypertension and dyslipidaemia [91].

However, the setting of waist size cut-offs for different ethnic groups to define obesity is the biggest problem faced by both the WHO and NCEP:ATPIII. This can be explained by the decreased risk of T2D at lower levels of obesity in Asian people compared to European people. The IDF recommends that the threshold for waist circumference to define abdominal obesity in people of European origin be ≥94 cm for men and ≥80 cm for women. In contrast, the AHA/NHLBI recommends cut-off points of ≥102 and ≥88 cm for men and women, respectively. Additionally, the IDF has proposed ethnic/racial-specific cut-offs for unifying the criteria of MetS among all ethnic groups. Still, in western countries, a 14-cm difference compared to the current criteria for abdominal

obesity in both males and females is still under debate. The confusion in defining MetS leads to uncertainty in classifying men with MetS as having an increased cardiometabolic risk and fewer diagnoses of MetS in women. Regardless of the definition used, prevalence estimates for MetS have been similar for any specified population, but outlying individuals have been identified [92]. The reason for this is the different focus of each definition, from the obesity-centric IDF definition to the glucocentric WHO definition to the collection of risk factors for CVD by the NCEP: ATPIII definition. Considering the escalating epidemic of T2D and CVD worldwide, the need for identifying one universal definition to identify MetS patients accurately has become imperative. Although there are a substantial number of controversies regarding the presence of MetS as a disease, this combination of various metabolic aberrations has been widely acknowledged as a screening tool for recognizing subjects with an elevated risk of cardiovascular disease. With the continuing research in this area, researchers aim to soon have a basic clinical definition to categorize subjects at high risk of metabolic problems and cardiovascular disease [6,81].

2.1.1.3. Prevalence of MetS worldwide

The prevalence of MetS has been growing over the past decades and has reached epidemic proportions, as indicated by the increasing percentage of MetS from 24.9 % in 1998 to 31.3% in 2007 in Korea [93]. In the United States, the prevalence of MetS was 23.7% in adults in 1988–1994, which was similar to that in Europeans [94]. In Asia, variations in the prevalence of MetS have been seen. For instance, the prevalence of MetS was 21.9% in Thailand, 49.4% in Malaysia [93,94], 29.3% in middle-aged Chinese men and 26.8% in Hong Kong professional drivers [95,96]. In a cross-sectional study, 3988 urban Chinese

middle-aged men (age = 40-74 years) without T2D were studied. They reported that the prevalence of MetS was 18.36%, 18.63% and 29.34% according to the ATP III, IDF and ATP III-modified criteria, respectively [95]. According to the consensus criteria for Chinese individuals, the prevalence of MetS (unadjusted for age) was 32.5% for women and 35.1% for men. A study also reported that females tend to have a higher MetS prevalence compared to men, and the prevalence of MetS increases with age for men over 60 years old and women over 70 years old [97]. Interestingly, the prevalence of MetS was reported to increase progressively from 12.1% in individuals aged 32–45 years to 45.4% in individuals aged ≥75 years [98]. A study demonstrated that the percentage of MetS had increased nearly 13% (from 9.6% in the 1990s to 23% in 2000s) in 10 years in Hong Kong [99]. Therefore, these data indisputably illustrate that MetS is a frightening global public health concern.

2.1.1.4. Epidemiology of MetS

The epidemiology of MetS according to the various definitions used depends on the criteria used and the composition of the population (ethnicity, race, age, and sex) [100]. Despite the criteria used, MetS prevalence is high and increasing in western countries, perhaps because of the obesity epidemic [101,102]. The National Health and Examination Survey (NHANES) in 2003-2006 reported that approximately 34% of people were diagnosed with MetS based on the NCEP: ATPIII revised criteria [103]. There have been differences in the age-adjusted estimates of MetS using the various definitions within three NHANES cohorts, including 1988-1994, 1999-2002 and 2003-2006 (Table 2.6). The estimated MetS prevalence has increased by 5% in the past 15 years according to the revised NCEP:ATPIII criteria. The WHO criteria, which are more restrictive,

provide the same estimated MetS prevalence. On the other hand, the IDF criteria, which adopt a comparatively lower cut-off for the waist circumference, report a higher prevalence estimate [104]. Regardless of the different prevalence estimates according to the various definitions of MetS, an enormous proportion of the population has been reported to have an elevated risk of developing CVD and T2D. For instance, the age-adjusted prevalence and unadjusted age prevalence of MetS increased from NHANES (1999-2006: 29% to 34.2%) to NHANES-III (1988-1994: 27.9% to 34.1%). However, it remained similar in the last NHANES cohorts (1999-2002 and 2003-2006 cohorts). In countries other than Africa and Europe, a greater prevalence of MetS has been reported based on the IDF criteria compared to the NCEP: ATPIII criteria [105–108]. However, a similar prevalence of MetS in the Iranian population was reported based on ATPIII and IDF criteria (33.2% and 32.1%, respectively) [109].

Table 2.6: Age-adjusted prevalence of MetS within NHANES cohorts**.**

	Number of Subjects	**NCEP ATP III 2001**	**NCEP ATP III revised**	**WHO**	**IDF**
NHANES 1988-1994	8,814	23.7%			
NHANES 1988-1994	8,608	23.9%		25.1%	
NHANES 1988-1994	6,436	24.1%	29.2%		
NHANES 1999-2002	1,677	27.0%	32.3%		
NHANES 1999-2002	3,601		34.6%		39.1%
NHANES 2003-2006	3,423		34%		

NHANES = National Health and Examination Survey; ATPIII = National Cholesterol Education Program Adult Treatment Panel III; WHO = World Health Organization; IDF = International Diabetes Federation.

According to NHANES (2003-2006 cohort), the prevalence of MetS was reported to be increased with age. The results indicated that MetS was prevalent in 16% of females and 20% of males under 40, in 37% of females and 41% of men between 40-59, and in 54% of women and 52% of males equal to or greater than 60 [103]. The trend for a higher MetS prevalence with progressing age was similar in other populations, too [92,110,111].

The prevalence of MetS increases even more with BMI increases. In the NHANES 2003-2006 cohort, overweight males and obese females were

respectively found to be 6 times and 5.5 times more likely to meet the criteria for MetS compared to underweight and normal weight individuals. Additionally, obese males and females were respectively found to be 32 times and 17 times more likely to meet the criteria for MetS compared to underweight and normal weight individuals [103]. MetS prevalence has rapidly increased in developing countries, from 9.8% in urban North Indian males to 42% in urban Iranian females [112]. Increases in prevalence are observed irrespective of the criteria used and are likely due to lifestyle transitions from traditional to western. The changes cause significant effects on body metabolism and composition and result in an increase in body mass index and abdominal obesity, as well as an increase in dyslipidaemia.

2.1.1.5. Causes and pathophysiology of metabolic syndrome

Different factors, such as genetics, a high-fat diet, an insufficient amount of physical activity, smoking, alcohol consumption and inadequate sleep, lead to MetS [113–118]. Genetically, chromosomes 1, 2 and 16 are reported to be linked to all MetS components in Hong Kong Chinese people [119]**.** Family histories of diabetes, age, smoking, and education have been significantly associated with the risk of MetS [95]. Regardless of advances in pathophysiology and descriptions of risk factors that pre-dispose individuals to MetS, many important characteristics and aspects remain unclear. The great disparity in susceptibility and age at inception in individuals with very similar MetS risk profiles indicates a major interaction between environmental factors and genetics [120]. Apart from insulin resistance (IR) and obesity, other factors also play a significant role in the pathophysiology of MetS. For example, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS), chronic

stress, renin-angiotensin-aldosterone system (RAS) activity, increases in cellular oxidative stress and intrinsic tissue glucocorticoid actions, adipokines, and molecules such as micro-RNAs may be involved in the pathogenesis of MetS.

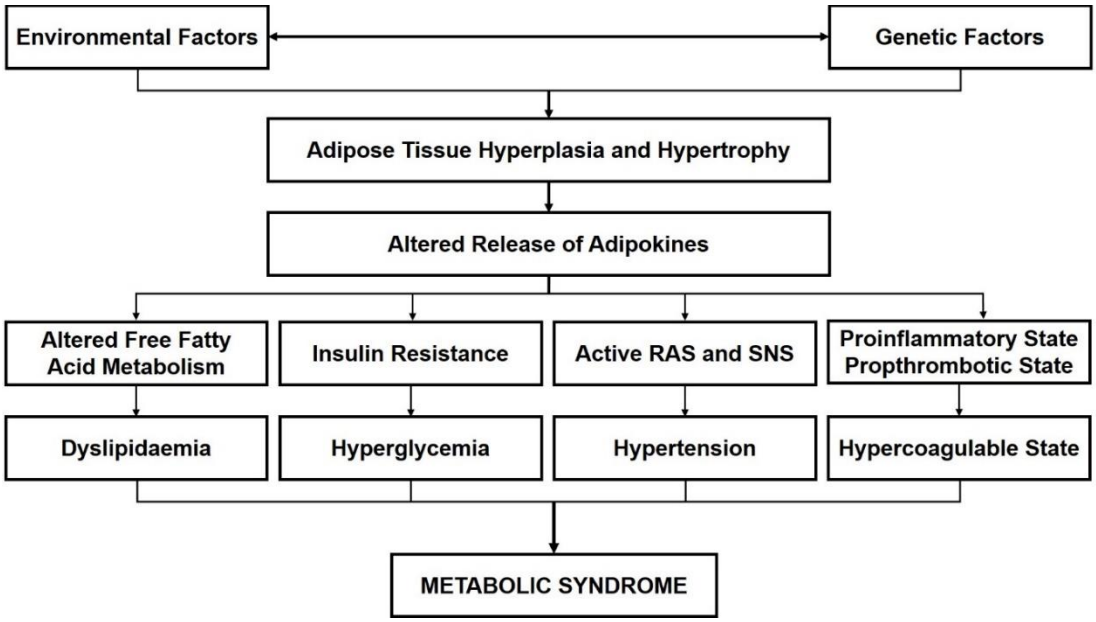

Figure 2.1: Schematic presentation of pathogenesis of meatbolic syndrome

Source: (Modified from Kaur J. A comprehensive review on MetS. Cardiol Res Pract. 2014; 2014:943162.)

There is an urgent need to precisely define MetS components due to the necessity to correctly detect individuals at substantial risk for T2D and CVD. Each of the components of MetS is involved in conferring risk for T2D and CVD. Notably, increased LDL, high triglyceride and decreased HDL levels in the circulation are individually associated with cardiovascular risk [121]. Furthermore, IR significantly increases the risk of developing T2D [122]. In addition, central obesity has been reported in numerous studies to be connected with an increased risk of T2D and CVD [123]. Independent of the diagnostic criteria used, several lines of epidemiological evidence have confirmed that people with MetS

are at a higher risk for CVD [124]. There is increasing awareness of MetS in recent years because CVD and T2D are believed to be the primary outcomes of MetS since patients with MetS have greater cardiovascular mortality and morbidity [125]. Several lines of evidence indicate that patients with MetS have increased occurrence of CVD, stroke, and diabetes by 1.5- to 3-fold when compared to individuals without MetS [126]. The risks of developing CVD and T2D are increased 2-fold and 5-fold, respectively, in MetS patients when compared to healthy individuals [127]. It has been estimated that MetS accounts for 6–7% of all-cause mortality, 12–17% of CVD cases, and 30–52% of T2D cases.

In contrast, the Casale Monferrato and PROSPER studies in older people did not show any association between an increased risk of CVD and MetS [126,128]. Due to these contradictions, numerous recent studies aimed to investigate which of the definitions of MetS is best related to CVD risk so that it can be implemented in clinical practice. A meta-analysis study recommended that the WHO definition is associated with a slightly greater risk of CVD than the ATPIII criteria [129]. Interestingly, the first large-scale multi-ethnic international investigation (the INTERHEART study) demonstrated that the incidence of MetS is associated with a more than 2.5-fold increase in acute myocardial infarction (MI) risk by using either the WHO or IDF criteria [130]. The assessment of MetS risk for MI is greater compared to the risk determined by using the sum of individual constituent risk factors [131]. An INTER-HEART study reported that the number of components of MetS increases the risk of MI. Similarly, other studies also demonstrated that incident coronary heart disease (CHD) and atherosclerosis increase with an increase in the number of MetS risk factors

[132–135]. It is well known that the incidence of MetS increases T2D risk and is associated with a five times higher risk for the occurrence of T2D [136].

Even though the existence of MetS can predict T2D and CVD risk, it cannot evaluate the specific risk, as a substantial part may be attributed to other factors, such as age, gender, smoking or lifestyle. In particular, higher concentrations of inflammatory markers (such as high-sensitivity C-reactive protein (hs-CRP)) are found in females compared to those in men, possibly because women have an increased accumulation of abdominal fat [137,138]. Furthermore, factors other than the five existing components of MetS, such as small dense oxidized LDL, endothelial dysfunction, insulin resistance, a proinflammatory state and prothrombotic tendencies that are also essential elements and determine imminent cardiometabolic risk have been neglected. Indeed, a significant amount of information on cardiometabolic risk has been provided by markers of a proinflammatory state, such as γ-glutamyltransferase (γ-GT), hs-CRP, uric acid, apoE, apoB, and fibrinogen, and the associated dysfunction of HDL and apolipoprotein A-I (apoA-I) [139,140].

2.1.1.6. Conditions related to metabolic syndrome without epidemiological confirmation

Chronic stress: dysregulation of hypothalamic pituitary axis and metabolic syndrome:

Chronic hypersecretion of stress intermediaries, for instance, cortisol, in persons with a genetic disposition who are exposed to a permissive atmosphere may result in visceral fat accumulation [141,142]. Furthermore, hypercortisolemia is involved in various pathophysiologies, such as anxiety,

insomnia, depression, chronic pain, obesity, fatigue syndromes, MetS, hypertension, atherosclerosis, and T2D, and their cardiovascular, autoimmune inflammatory and osteoporosis consequences [143]. Further, alterations in hormones may also lead to the hypersecretion of reactive insulin, sarcopenia, increasing visceral obesity dyslipidaemia, hypertension, and T2D [144]. Stress-related hypersecretion of IL-6 with adipose tissue-released inflammatory cytokines and hypercortisolemia lead to blood hypercoagulation and the increased production of acute phase reactants leading to MetS [145,146].

Additionally, intracellular glucocorticoid (GC) levels are regulated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). 11β-HSD1 converts inactive cortisone to cortisol, and its expression in tissue-specific alterations has been focused on recently. Its activity in obesity and IR has also been studied. In obesity (global), the activity of 11β-HSD1 is impaired, as evaluated by urinary corticosteroid metabolite analysis [147,148]. Selective inhibitors of 11β-HSD1 are in development, and a few reports with good results in rodents that show improvements in their metabolic profiles indicate its involvement in MetS [149].

An association between inflammatory cytokines, visceral fat, stress hormones, and insulin resistance and sleep apnoea has recently been reported [150,151]. Visceral obesity and insulin resistance, affected by environmental and genetic factors, may be the main culprit causing sleep apnoea. Further, they may accelerate metabolic abnormalities, perhaps through the progressive increase of cytokines and stress hormones (such as noradrenaline, IL-6, and TNF-α) [152].

Another system, the circadian CLOCK, in addition to the stress hypothalamic-pituitary axis (HPA) and its end effectors, glucocorticoids, may also be involved in the pathogenesis of MetS. Remarkably, most of the metabolic phenotypes related to dysregulation of the HPA axis and circadian CLOCK system overlap [153].

Cellular oxidative stress/renin-angiotensin-aldosterone system and metabolic syndrome

Numerous studies suggest that inflammation, nitric oxide (NO), and oxidative stress also play important roles in the pathophysiology of hypertension, MetS, and T2D [154,155]. Activation of the renin-angiotensin-aldosterone system (RAS) has been linked with an increase in reactive oxygen species (ROS) in skeletal muscle and cardiovascular tissues and various tissues [156,157]. Activation of the RAS is also reported in the development of IR [158]. Upregulation of angiotensin II (a key player in the RAS) promotes the production of ROS in various tissues, including skeletal muscle and vascular smooth muscle, and causes IR in patients with MetS [156,158]. Additionally, genetic knockout of the angiotensin II type 1 receptor and antagonists of the angiotensin II type 1 receptor efficiently diminish the accumulation of lipids in the liver [159,160].

Micro RNAs and metabolic syndrome

Micro RNAs (miRNAs) are important in regulating many biological processes, including metabolic integration, adipocyte differentiation, appetite regulation and IR [161]. The exact mechanism of action remains unknown, but it has been shown that miRNAs may regulate cellular gene expression at either the transcriptional or post-transcriptional level. They either suppress the translation

of protein-coding genes or cleave target messenger RNAs (mRNAs) to cause degradation [162,163]. Interestingly, a phase I clinical trial has already been successfully conducted in which miRNA-122 was blocked in vivo by using antagomirs, which are cholesterol conjugated antisense oligonucleotides [164]. Additional studies are still needed to explore the potential of miRNAs as innovative biomarkers and therapeutic mediators of MetS.

Fetal/developmental basis of metabolic syndrome

Several lines of evidence in both animal and human studies suggest that the nutritional, metabolic, hormonal and early postnatal conditions of mothers may permanently reprogram the physiology and structure of their offspring [165–167]. Interestingly, the possible effect of in vitro fertilization (IVF) on the prevalence of MetS risk factors has been studied, but the results are confusing [168,169]. Therefore, more studies of the metabolic profiles of children conceived by IVF with longer follow-ups are essential to draw any conclusions.

2.1.1.7. Obesity as one of the primary manifestations of metabolic syndrome

Prevalence, pathogenesis, and measurement of obesity

According to the WHO, individuals with a body mass index ≥30 are considered obese. Obesity is major health problem worldwide, occurring in approximately 13% of the world's adult population. The prevalence of obesity has more than doubled since 1980. In 2014, 1.9 billion adults (39%) were found to be overweight, and 600 million (13%) of these were obese. Approximately 41 million children were overweight or obese in 2014. Over 1/3 of the citizens (ages 18 to 64) of Hong Kong with a body mass index ≥20 were considered

overweight or obese and were diagnosed with obesity [170]. Obesity is a major health concern worldwide [171]. The prevalence of central obesity is observed to be higher in women when compared to men [172]. Additionally, it has been discovered that central obesity increases with age, especially in female subjects [170].

Obesity (specifically, abdominal obesity) has been considered to play a significant role in the development of MetS [154,173]. A sedentary lifestyle, physical inactivity, and unhealthy dietary behaviours cause obesity and then MetS [174–176]. These factors promote chronic positive energy balance resulting in adipose tissue expansion to store excess energy. Adipose tissue functions as an important endocrine organ that secretes bioactive factors, collectively known as adipokines. Adipokines regulate appetite, insulin signalling, endothelial functions, and inflammation at both the tissue and systemic levels [177]. The NCEP ATP III considers central obesity to be the leading cause of the rising incidence of MetS. Obesity contributes to high serum cholesterol, low HDL cholesterol, hypertension, and hyperglycaemia, and it is associated with higher CVD risk. Central/abdominal obesity has been especially correlated with metabolic risk factors. Superfluous adipose tissue releases several products that apparently aggravate these MetS risk factors. These include cytokines, nonesterified fatty acids (NEFAs), plasminogen activator inhibitor-1 (PAI-1), leptin and adiponectin. Elevated levels of plasma NEFAs burden the liver and muscle with lipids, leading to insulin resistance. A proinflammatory state and excess cytokine expression may be indicated by high C-reactive protein (CRP) levels and obesity. Low adiponectin levels have been correlated with worsening MetS risk factors, whereas increased PAI-1 levels contribute to a prothrombotic

state. NCEP:ATP III guidelines define MetS as an accumulation of the metabolic complications of obesity [54].

When excess fat is deposited around the stomach and abdomen, it will result in central obesity or abdominal obesity. According to the NCEP:ATP III, central obesity is defined as a waist circumference larger than 102 centimetres (cm) for males and 88 cm for females. Since different ethnicities have various measurements and standards, the panel has been adjusted to differentiate patients more accurately. For instance, the modified Asian criteria of the NCEP:ATP III or the Asian-Pacific waist circumference is more commonly used in Asian studies. The waist circumferences for defining central obesity were modified to 90 cm for males and 80 cm for females [178,179].

The presence of central obesity is determined by measuring the waist circumference of an individual. One study compared the correlation of visceral fat and MetS risk factors with waist circumference and waist-to-hip ratio (WHR). When compared with the WHR, the waist circumference was found to have better a correlation with the accumulation of visceral fat, MetS components, fasting and post-glucose insulin levels, and cardiovascular risk [89]. It was also confirmed that waist circumference is a significant predictor of health risks related to obesity in the absence of body mass index (BMI) measurements [180]. The cut-off points of waist circumference might not apply to all ethnic groups due to the differences in abdominal tissue composition and different relations with MetS risk factors among the various ethnic groups. When compared with Caucasians, higher morbidity is shown in Asians at lower waist circumference cut-off points [181]. Therefore, the cut-off points for waist circumference need to be adjusted according to ethnicity.

Associated risks of obesity

Obesity has been associated with chronic diseases, such as metabolic disease and cardiovascular disease. A study based on data from 774 men who participated in the Kuopio Ischaemic Heart Disease Risk Factor Study with 4 years of follow-up demonstrated that abdominal obesity was associated with the accelerated progression of atherosclerosis, and these findings have been supported by other scholars [182]. Another study based on 5881 subjects (3177 women and 2704 men) who participated in the Framingham Heart Study described the relationship between body mass index and the incidence of heart failure [183]. Obese subjects with a BMI ≥30 were found to have a doubled risk of suffering heart failure than subjects with an average BMI of 18.5 to 24.9. Another study correlated obesity with an increased risk of premature death [184]. Obesity has also been proposed as one of the major risk factors of metabolic disorders, such as T2D and MetS. However, due to the different effects of obesity on various metabolic pathways, the latter study was not able to differentiate a more specific pathway over which obesity had metabolic control.

2.1.1.8. Obesity linked hypertension

Obesity increases the threat of the development of high blood pressure. This association has been the subject of numerous recent reviews [185,186]. The potential mechanisms through which obesity may lead to elevated blood pressure are discussed in the following paragraph.

Pathophysiologic mechanisms

The Guyton hypothesis states that sustained hypertension can occur only when the relationship between natriuresis (excretion of sodium in urine) and arterial

pressure is abnormal. The theory that increased arterial pressure will result in increased sodium excretion that results in blood pressure lowering has been proposed. Even though the evidence to support this theory is widespread, it must not be stated that hypertension results from kidney disease because various hormones can change the pressure-natriuresis relationship in kidneys that are functioning normally. For example, hypertension and renal tubular sodium reabsorption due to the action of aldosterone occur even in people who have healthy functioning kidneys [187].

Obesity has been associated with hypertension, increased blood flow, cardiac output, and vasodilatation. Even if the cardiac index (cardiac output/body weight) does not increase, the rate of glomerular filtration and cardiac output can increase. It has also been suggested that the retention of renal sodium also increases and leads to hypertension [186,188]. The mechanisms considered accountable for obesity-related hypertension natriuresis curves include the activation of the RAS, structural variations in the kidney, hyperinsulinaemia, and dysregulation of adipokines, such as adiponectin and leptin. The sympathetic blockade prevents obesity-linked hypertension in both animals and humans [186,188,189]. Similarly, leptin, which is a satiety-inducing hormone secreted from fat cells that activates the sympathetic nervous system, can cause hypertension and enhance thermogenesis. Sympathetic blockade can prevent leptin-induced hypertension. The sympathetic activation effects on obesity-linked hypertension seem to be related to the initiation of renal nerve traffic. Subsequently, disruption of the pressure-natriuresis relationship as a loss of nerve supply in the kidneys prevents the development of hypertension in obesity-related hypertension animal models [186,188–190]. Additionally, the

hypothalamic leptin-melanocortin pathway is an important modulator of weight, and hyperleptinemia contributes to high sympathetic outflow. Recent investigations of the melanocortin pathway conclude that mutations in the melanocortin 4 receptor cause hypertension in men, thus demonstrating that the hypothalamic leptin-melanocortin pathway directly regulates weight and hypertension [191,192].

The RAS is activated in hypertension with the upregulation of circulating angiotensin II and renin-angiotensinogen, regardless of increases in renal sodium retention [186,193]. The cause of RAS activation is not well documented, but the involvement of adipose tissue is evident because adipose tissue produces angiotensinogen [194]. Additionally, during obesity, an increase in renin activity could be the outcome of increased sympathetic activity. Several lines of evidence indicate that an increase in angiotensin II directly increases the reabsorption of sodium in renal tubules and stimulates the synthesis of aldosterone (sodium-retaining hormone) [194]. In the same way, obesity has been associated with hyperinsulinaemia [190,195]. Insulin may enhance the tubular reabsorption of sodium and may elevate arterial pressure under some circumstances. The role of the RAS in hyperinsulinemia is indirectly supported by the effects of peroxisome proliferator-activated receptor gamma agonists and angiotensin-converting enzyme inhibitors on blood pressure [196–198].

A lesser known endocannabinoid system may also play a role in obesity-linked hypertension. Obesity increases the levels of endocannabinoids both in tissues and in the circulation [196,198]. Notably, the agonists (rimonabant and taranabant) of cannabinoid receptor 1 ameliorate obesity-associated metabolic disorders,

thus suggesting a role for endocannabinoids in obesity-linked hypertension [196,198].

Finally, following obesity, structural changes in the kidney appear to be important. The pressure exerted by fat deposits around the kidneys, in combination with increased abdominal pressure due to abdominal obesity, has been proposed as a supplementary cause of disordered renal sodium reabsorption. Deposition of glycoproteins in the renal medulla may also contribute to dysregulated renal sodium reabsorption [186,193]. Furthermore, obesity causing hyperfiltration sets the stage for further glomerular and renal function loss and increases in arterial pressure [186,193].

2.1.1.9. Current therapeutics approaches for MetS

The fundamental risk factors that promote the development of MetS are obesity, physical inactivity, and an unhealthy/atherogenic diet. The guidelines for managing the individual components of MetS focus on lifestyle modifications (weight loss and physical activity) as the first-line therapy. The NCEP ATP III announced the concept of MetS in its cholesterol guidelines to highlight the requirement of more exhaustive lifestyle changes to prevent CVD in patients at higher risk. The most common therapeutic strategies for treating MetS are discussed below.

Lifestyle modifications

Almost 70% of the US population can be classified as being physically inactive. Regular exercise has been publicized to improve numerous metabolic risk factors and has been associated with a decrease in the risk of developing various chronic diseases. For these reasons, physical inactivity must be

considered to be a significant contributor to the development of MetS [199]. Current physical activity guidelines suggest practical, moderate and regular routines for exercise. The standard recommendation for daily exercise is a minimum of 30 minutes of physical activity at a moderate intensity. Additionally, other beneficial effects of increasing physical activity have been reported [199]. Recommendations that may help in maintaining a regular exercise schedule include the following: avoiding sedentary activities, incorporating multiple short bouts of activity (10 to 15 minutes of brisk walking), performing simple exercises on simple equipment such as treadmills, and including regular exercise into daily schedules (e.g., jogging, biking, swimming or golfing). Additional exercise is even more effective for controlling weight [199]. According to WHO, the global recommendations on health for adults aging between 18-64 years, physical activity includes transportation, occupational, household chores, games, play, sports or planned exercise, in daily context. The recommendations for improving muscular fitness, cardiorespiratory, bone health, and to reduce the risk of non communicable diseases and depression includes: at least 150 mins / week or 75 mins/week of moderate-intensity aerobic physical activity or vigorous-intensity aerobic physical activity respectively [200].

Due to the relation between MetS and physical inactivity, managing MetS should include the initiation of a programme of regular physical activity. Researchers reviewed several clinical trials that showed that the combination of increased physical activity and weight loss could reduce the progression new-onset diabetes by nearly half over a period of several years in people with MetS. However, whether this combination of weight reduction and regular exercise reduces CVD risk has not been tested in controlled clinical trials.

Nevertheless, epidemiological data provide evidence that weight reduction and exercise have favourable effects on CVD risk factors [199].

Yoga as an effective life style modification

Yoga is instrumental in Indian philosophy and has been a traditional spiritual practice for ages [201]. Traditionally, yoga is a complex intervention as it combines physical activity, breathing exercises, meditation and healthy and ethical lifestyle advice. Even though the ultimate goal of traditional yoga is uniting the body, mind, and spirit, it has become a popular way to encourage mental and physical well-being [201,202]. In Europe and North America, yoga is most often associated with breathing techniques (Pranayama), physical postures (asanas), and meditation (Dhyana) that place changing levels of demand on physical and mental practises [201]. In Europe and North America, yoga has gained popularity for therapeutic purposes. It has been reported that yoga has been recommended to nearly 14 million adult Americans by physicians or therapists. In fact, approximately 80% of yoga practitioners from America indicated that they practise yoga explicitly to improve their well-being [203]. Yoga is a blend of exercise and controlled breathing and relaxation practices that are often combined with lifestyle advice and a specific diet plan. An study of the prevention and rehabilitation of cardiac diseases [204,205] showed that yoga improves several components of MetS [206].

An interesting review that aimed to evaluate the efficacy of yoga as a treatment for patients with MetS based on a meta-analysis has been recently published [207]. The review included 794 participants from seven trials. Eligible yoga interventions were yoga intervention studies that included at least one physical

activity that included meditation and breath control. Studies including several model interventions, including yoga and other exercises, were not included. The results indicated that there was no improving effect of yoga on MetS, diastolic blood pressure, triglycerides, fasting plasma glucose or high-density lipoprotein cholesterol. Nevertheless, yoga was found to be more beneficial than the usual care such as diet for reducing waist circumference (standardized mean difference (SMD) = - 0.35; 95%, confidence interval (CI) = - 0.57 to - 0.13; $p < 0.01$) and systolic blood pressure (SMD = - 0.29; 95 % CI = - 0.51 to - 0.07; P = 0.01). Regarding metabolic requirements, yoga practice is considered a low-level physical movement. Yoga enhances the metabolic rate, cardiopulmonary capacity, and perfusion. Yoga can decrease the manifestations of MetS and can improve lipid profiles. Yoga also improves insulin actions and is therefore influential in reducing the risk of MetS. Yoga is protective against heart distress, atrial fibrillation and cardiovascular diseases [208–211].

The mechanism underlying yoga is believed to work through the autonomic sensory system via parasympathetic pathways to restore the relaxing effects of neurohormonal pathways. For instance, the renin angiotensin aldosterone complex is vital to controlling heart rate increases, myocardial-localized necrosis, pulse increases, congestive heart failure and arterial fibrillation [212–214]. The components of neurohormonal pathways are thought to affected by yoga. Yoga has a positive impact on reducing stress and maintaining heart rate variability because it has an impact on the components of autonomic pathways. Yoga practice advances wellbeing and decreases psychological complications.

Table 2.7: Summary of yoga intervention studies conducted till date

Reference study	Origin	Intervention, program length, frequency, duration,	Control group Intervention, program length, frequency, duration,	Sample size	Mean age	Criteria used
Cohen et al., 2008	USA	Yoga 10 weeks 3-hour introductory course, 2x/week(week1-5) 1x/week(weeks 6-10) 90 mins each	No intervention	N=26(Yoga n=14, Control n=12)	Yoga=52±9 yrs Control 52±8 yrs	NCEP
Harbans et.al., 2011	India	Yogic exercise 8 weeks 2x/day 45 minutes each	Herbal medicine	N=63(Yoga n=34, Control n=29)	Not reported	AHA
Kanaya et al., 2014	USA	Restorative yoga 48 weeks 2x/week(week1-12) 1x/week(weeks 13-24) 1x/month(weeks 25-48) 90 mins each	Stretching 48 weeks 2x/week(week1-12) 1x/week(weeks 13-24), 1x/month(weeks 25-48), 90 mins each	N=180(Yoga n=91, Control n=89)	Yoga=55±7 yrs Control 54±7 yrs	Not reported
Khatri et al., 2017	India	Yoga 3 month Additional usual care	usual care	N=101(Yoga n=55, Control n=46)	Yoga=54±9 yrs Control 54±11yrs	NCEP

Kim et al., 2013	South Korea	Hatha yoga 12 weeks 3x/week 60 mins each	usual care	N=41(Yoga n=20, Control n=21)	Yoga=48 ± 7yrs Control 50±8 yrs	NCEP
Manchanda et al., 2013	India	Yoga 1 week yoga instruction +12 months home practice (60 mins daily)	usual care	N=100(Yoga n=50, Control n=50)	Yoga=62±13 yrs Control 57±13 yrs	NCEP
Siu et al., 2015	China	Yoga 1 year 3x/week 60 mins each	No intervention	N=283(Yoga n=146, Control n=137)	Yoga=62±13 yrs Control 57±13yrs	NCEP

Source: [207]

Dietary Modification

NCEP:ATP III guidelines recommend a low intake of trans fats, saturated fats, cholesterol and simple sugars and an increased intake of vegetables, fruits, and whole grains. Very high carbohydrate diets may increase atherogenic dyslipidaemia. Dyslipidaemia may not be caused by increasing the intake of unsaturated fats. The clinical significance of atherogenic dyslipidaemia induced by diet is unresolved. Recent clinical trials on a small scale indicate that atherogenic dyslipidaemia is improved less by increasing the consumption of unsaturated fat compared with standard dietary recommendations [215,216].

Metabolic risk factors management

Apart from lifestyle modifications, numerous drug therapies have been recommended to patients to reduce metabolic risk factors. Setting therapy goals for metabolic risk factors during a patient's risk assessment is fundamental.

In the 2004 National Heart, Lung, and Blood Institute (NHLBI)/American Heart Association (AHA) conference proceedings regarding MetS [54], Framingham Heart Study researchers showed that their standard Framingham risk equation, which include blood pressure, cigarette smoking, HDL cholesterol, total cholesterol, and age, explains most of the CVD risk in patients with MetS. However, the addition of central/abdominal obesity, fasting glucose, and triglycerides to the Framingham risk equation provides a negligible increase or no increase in predicting CVD risk. Nevertheless, the addition of other parameters contributing to the risk of MetS (apolipoprotein B, CRP, small LDL

and fibrinogen) to the Framingham risk equation has not been tested extensively. However, the risk of developing T2D is dependent on the presence of abdominal obesity and impaired fasting glucose. People who have diabetes diagnosed by only oral glucose tolerance test (OGTT) are expected to develop diabetes and are diagnosed with high fasting plasma glucose in a relatively short time. Thus, OGTT is not commonly suggested for obese peoples who have MetS and may be suggested for voluntary testing based on clinical requirements [217].

Management of atherogenic dyslipidemia

Beyond lifestyle modifications, several drug alternatives may be considered in patients with atherogenic dyslipidaemia. The NCEP:ATP III emphasizes that LDL cholesterol is the primary target of lipid-lowering therapies. Statins, also called HMG-CoA reductase inhibitors, reduce all apolipoprotein B-containing lipoproteins. Statins can mostly achieve the NCEP:ATP III goals for non-HDL cholesterol and LDL cholesterol [217]. Numerous clinical investigations have established the benefits of statins [218]. Fibrates are a class of amphipathic carboxylic acids used to improve atherogenic dyslipidaemia and reduce CVD risk [218]. Statins with fibrates are a particularly attractive therapy. Nevertheless, both statins and fibrates may cause myopathy. When both drugs are used together, myopathy risk is increased [219]. Several reports have been published stating that the combination therapy of a statin and gemfibrozil leads to severe myopathy [219]. Evidence indicates that gemfibrozil hinders statin catabolism in the liver and raises blood statin levels, thus leading to myopathy. However, fenofibrate does not interact with statin catabolism and is safer to use in

combination therapy. Nicotinic acid, a water-soluble B-complex vitamin, has characteristics similar to fibrates. The combination of statins and nicotinic acid is promising. Nicotinic acid is efficient at raising HDL cholesterol levels, but its higher doses can increase plasma glucose levels [217].

Management of increase in blood pressure

A new category of elevated blood pressure called prehypertension(systolic BP = 120-139, diastolic BP = 80-89 mm Hg) has been introduced by The Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [220]. This categorization was to recognize the fact that increases in blood pressure increases CVD risks. The designation agrees with NCEP:ATP III criteria and adds a blood pressure range of ≥ 130 / ≥ 85 mm Hg to the list of MetS risk factors. Patients with hypertension (blood pressure ≥ 140 / ≥90 mm Hg) are recommended to receive drug intervention by the JNC. In diabetic patients, antihypertensive drugs should be prescribed at lower blood pressures (≥ 130 / ≥ 80 mm Hg). However, no antihypertensive agents have been considered desirable for patients with hypertension and MetS. Diuretics (water pills) and β-blockers (beta-adrenergic blocking agents) in high doses have been reported to deteriorate insulin resistance and atherogenic dyslipidaemia. Thiazide diuretic (a drug that increases urine flow), doses are relatively low in accordance with current therapeutic recommendations. Interestingly, β-blockers are no longer controversial in patients with T2D due to their well-known cardioprotective property in patients with CHD. Some investigations have shown that angiotensin receptor blockers and angiotensin-converting enzyme inhibitors

are good antihypertensive drugs as they have advantages over other therapeutics in diabetic patients. Currently, mainstream clinical trials indicate that antihypertensive drugs reduce most of the cardiovascular risk [217].

Management of insulin resistance and hyperglycemia

There are mounting queries regarding whether drugs that decrease insulin resistance delay the onset of T2D and reduce CVD risk in patients with MetS. For instance, the Diabetes Prevention Program disclosed that metformin therapy in prediabetic patients prevents or delays the development of diabetes. Data on use of the thiazolidinedione troglitazone (a medication used to treat T2D) suggested a comparable effect. However, this drug is commercially banned. Even though insulin resistance has been associated with an increase in CVD risk, neither any of the thiazolidinediones nor metformin has been shown to reduce CVD risk in prediabetic or diabetic patients with MetS. Therefore, there is inadequate information to endorse these drugs for anything other than their glucose-lowering action. Diabetic patients with MetS are at a high risk for developing CVD. Therefore, in the presence of both, the proper treatment of hypertension and dyslipidaemia is important. The choice of drug treatment beyond lifestyle interventions to attain glycaemic goals rests on decisions made in the clinical setting [217].

Management of prothrombotic state

A prothrombotic state in MetS patients is defined by increased PAI-1, fibrinogen, and additional coagulation factors. Nevertheless, coagulation factors are not measured routinely in clinical practice. Aspirin therapy has been

used to reduce thrombotic events in patients. The American Heart Association (AHA) recommends aspirin treatment for patients with a ≥ 10 % 10-year risk for coronary heart disease and MetS patients with a ≥10% 10-year risk for CHD as evaluated by Framingham risk scoring [217,221].

Management of proinflammatory state

A proinflammatory state is characterized by higher cytokine levels (such as interleukin-6 and tumour necrosis factor-α) as well as by an increase in acute-phase reactants (fibrinogen and C-reactive protein). Measuring C-reactive protein (CRP) has been proposed as a practical way to estimate the presence of an inflammatory state. CRP levels have been reported to be higher in MetS patients than those in normal patients. An elevated CRP level (≥ 3 mg/L) indicates the emergence of risk for developing CVD. The Centers for Disease Control and Prevention (CDC) and the AHA have issued a guiding principle for the measurement of CRP in clinical practice [222]. The aim of testing CRP levels in a patient with an intermediate risk is to determine whose risk category should be increased to high. The practical significance of increasing the category of risk would be to implement more stringent lifestyle therapies. The guidelines of the AHA / CDC emphasize that CRP testing belongs in an optional category based on clinical advice because the extent of its independent predictive power remains ambiguous [217,218].

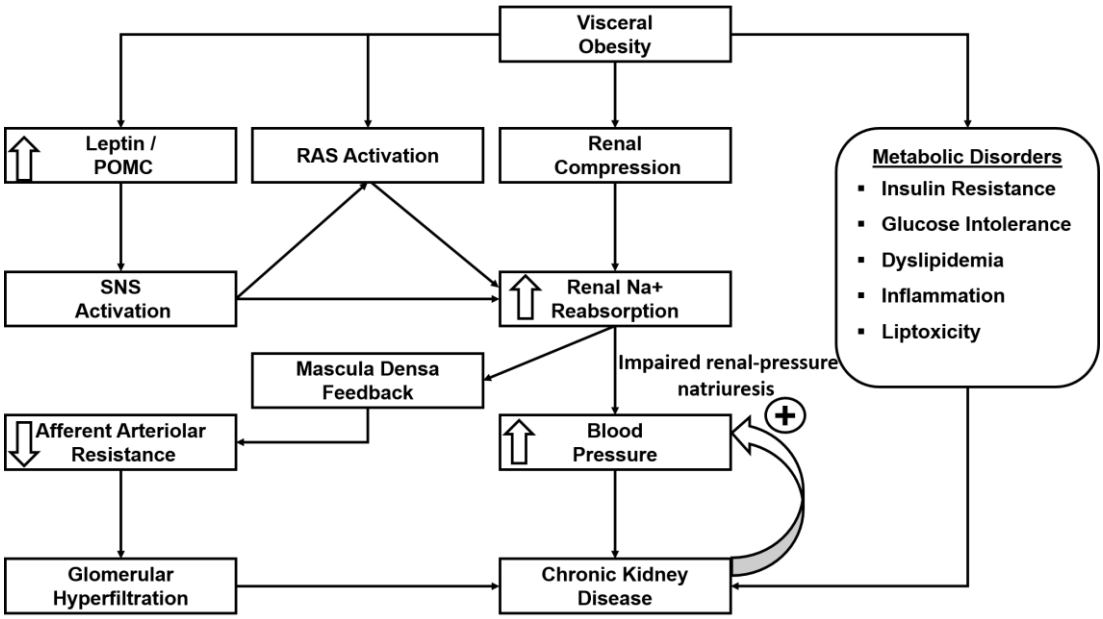

Figure 2.2: Summary of the mechanisms of obesity linked hypertension and renal injury.

(MR=mineralocorticoid receptor, RAAS=renin-angiotensin-aldosterone system, POMC=proopiomelanocortin and SNS=sympathetic nervous system [223])

2.1.2. Type 2 Diabetes

Diabetes is projected to be one of the top ten leading causes of death by 2030, as estimated by the WHO [224]. There are two types of diabetes: type 1 and type 2. Approximately 80% of deaths due to diabetes are due to T2D. T2D is also known as insulin-resistant diabetes mellitus and accounts for 90–95% of all diabetes cases. Over the past three decades, the number of subjects with T2D has doubled [225]. The signs and symptoms of T2D are not always as obvious, but it is often diagnosed during a routine check-up. Clustering of the associated medical conditions (central obesity, elevated fasting plasma glucose, elevated blood pressure, high serum triglycerides, and low HDL levels) is sometimes referred as prediabetes or MetS leading to diabetes and can further lead to cancer or cardiovascular disorders. One of the potential causes of MetS is obesity. The National Institutes of Health (NIH) has reported that MetS is

becoming more common due to increasing obesity rates, especially among adults. As the name MetS suggests, it is a breakdown of conserved biochemical processes involved in the body's normal functions. The biochemical processes involved in normal body functions are commonly called metabolic and immune pathways. Lifestyle, food habits and exercise affect our metabolic pathways and increase our immunity. Unhealthy lifestyle or eating habits and sedentary lifestyles cause obesity, which breaks down the conserved metabolic and immune response pathways [225]. These nutrient and pathogen sensing systems are conserved, but once there is a metabolic surplus from a sedentary lifestyle and unhealthy food habits, inflammation is triggered by the metabolic pathways, thus inducing MetS. It is worthwhile to hypothesize that focusing on the link between these two pathways might provide some early signs of MetS or prediabetes as these pathways are triggered in response to obesity. To date, no biomarkers or therapeutic drugs that focus on the link between inflammation and metabolic pathways have been developed for the early detection of MetS leading to T2D.

The availability of drugs that affect the underlying mechanisms may lead to a new therapeutic paradigm for the early detection and prevention of T2D and MetS. As reviewed by David E. Moller, the current therapeutic approaches are largely developed in the absence of defined molecular targets or even a solid understanding of the disease pathogenesis. Within the past few years, our understanding of the biochemical pathways related to new drug targets for T2D and MetS has been expanded. Within these pathways, there are number of targets that have not yet been discovered [226].

T2D and cancer are two of the leading causes of mortality in the world according to data reported by the WHO. Epidemiological studies have evidently demonstrated that there is a strong connection between particular types of cancers (such as colorectal and breast cancer) and T2D [227], even though the detailed mechanisms explaining this connection are not very clear. Indeed, T2D has been shown to increase the risk and mortality of breast, liver, colorectal and pancreatic cancers [228]. Thus, it is common to diagnose both diabetes and cancer in the same individual. As the number of people suffering from T2D is predicted to further increase in the upcoming years, it is expected that the number of diabetic cancer patients will also significantly increase.

2.1.2.1. Worsening of type 2 diabetes

Over past three decades, the number of individuals with T2D has doubled [229]. T2D is also known as insulin-resistant diabetes mellitus, is characterized by pancreatic β-cell dysfunction [230,231], and accounts for 90–95 % of all diabetes [225]. The confluence of the immune system and metabolism has recently emerged as a breakthrough target in T2D. Immune system activation by metabolic stress is providing new insights for understanding the pathogenesis and progression of T2D. Metabolic stress due to metabolic dysregulation leads to the pathological activation of the immune system. Thus, metabolic disorders such as T2D manifest and progress as an inflammatory disorder with severe consequences. B-cell hyperinsulinaemia due to insulin resistance occurs in the preclinical period of the disease. Relative insulin deficiency to compensate for insulin resistance progresses towards the clinical symptoms of T2D [231]. Recently, inflammatory pathways that are downstream of oxidative stress have been focused on as a unifying mechanism for the worsening of T2D. Many

studies indicate the link between metabolic alterations in T2D and inflammation [232].

Worsening of type 2 diabetes by doxorubicin induction

Epidemiologic evidence has suggested a strong connection between T2D and certain cancers, such as kidney, pancreas, liver, colon, and others. The risk and mortality of these cancers have been demonstrated to be increased by diabetes [228]. The connection between diabetes and certain cancers might partly result from the common risk factors of these two diseases, including ageing, obesity, diet, and physical inactivity [233]. A meta-analysis of 43 studies performed by Hardefeldt and co-workers found that type 2 diabetic patients had a significant increase in the risk of breast cancer when compared to non-diabetic controls [234]. The link between T2D and breast cancer has been extensively investigated. It was proposed that the link between these two disorders was attributed to activation of the insulin/IGF pathway via hyperinsulinaemia and dysregulation of sex hormones [235]. Diabetes and colorectal cancer studies in humans have demonstrated an association between T2D and colorectal cancer. There was a relative risk of 1.43 for colorectal cancer and a relative risk of 2.39 for fatal colorectal cancer in type 2 diabetic patients [236]. The findings of a meta-analysis of 24 cohort studies suggested that patients with T2D had a higher risk of colorectal cancer development [237]. Additionally, colorectal cancer mortality is also affected by T2D. Coughlin and colleagues reported increased mortality in diabetic patients with colorectal cancer who were over 30 years old [238]. The proposed potential mechanisms for the connection between colorectal cancer and T2D include a slower bowel transit time in type 2 diabetic patients with subsequent increased exposure to toxins, increased production of carcinogenic

bile acids, and hyperinsulinaemia [239]. Among these potential mechanisms, hyperinsulinaemia has been the most thoroughly explored, and it appears to be a major player in the T2D-mediated increase in colorectal cancer risk [240]. A high risk of pancreatic cancer in T2D patients has been extensively documented [241],[242]. A meta-analysis of 35 cohort studies found that the relative risk of pancreatic cancer was increased in T2D for both males and females and was negatively associated with the duration of diabetes. Patients with the highest risk of pancreatic cancer were found among the diabetic patients diagnosed within one year [241]. Hyperinsulinaemia in T2D may lead to pancreatic cancer. Pancreatic carcinogenesis was enhanced by insulin resistance via increased proliferation of islet cells. On the other hand, T2D was thought to be a result of pancreatic cancer. However, it is not clear how pancreatic cancer can lead to the development of insulin resistance [241]. Taken together, T2D has been linked to different types of cancer. Hyperinsulinaemia is proposed to play a fundamental role in explaining the connection and potential mechanism for the link between T2D and cancer. The high levels of insulin associated with T2D lead to increased production of insulin-like growth factor 1 (IGF-1), which plays a major role in cell growth, proliferation, and differentiation. The induction of bioavailable IGF-1 by insulin might result from the inhibition of the two transporters of IGF-1: insulin-like growth factor binding protein (IGFBP) and IGF-1 receptor (IGF-1R); moreover, the insulin receptor (IR) has been found to be upregulated in cancer cells [228]. Mutations in tumour suppressor genes (such as p53 and p63) cause tumour growth and proliferation through a defect in the inhibition of IGF-1R [244]. In addition to the direct effects of hyperinsulinaemia on cancer cells via IR and IGF-1R, insulin might play an indirect role in the growth

and development of cancer cells. Insulin stimulates aromatase activity and inhibits the production of sex hormone-binding globulin (SHBG), which increases the bioavailable oestrogen level. Collectively, the increases in IGF-1 and oestrogen levels are hypothesized to contribute to the development of cancer [228].

Doxorubicin (DOX) is an anti-neoplastic drug that is one of the most effective treatments for various types of cancer [245]. Doxorubicin-induced cardiotoxicity in diabetic hearts is a recent clinical challenge. It exerts side effects on the heart by inducing myofibrillar loss and vacuolar degeneration in cardiomyocytes [245]. In one study, DOX effects on the function of cardiac, skeletal and smooth muscle tissues were monitored for 5 days in rats. A time-dependent progressive decrease was reported both in cardiac and skeletal muscle function during the 5 days following DOX treatment. Decreases in the maximal twitch force and the rate of force development were reported in skeletal muscle [246]. Doxorubicin increases serum triglyceride and blood glucose levels by inhibiting adipogenesis, which is required for lipid absorption. This inhibition of adipogenesis occurs through the downregulation of peroxisome proliferator-activated receptor γ (PPARγ). PPARγ inhibits blood glucose and lipid clearance and is a major component of lipid metabolic pathways. Inhibition of this molecule by doxorubicin causes hyperglycaemia, hyperlipidaemia, inflammation and insulin resistance, mimicking T2D [247]. In skeletal muscle, increases in apoptotic DNA fragmentation, TUNEL-positive nuclei and Bax expression after exposure to doxorubicin have been reported. However, autophagic markers, including the LC3 II-to-LC3 I ratio, Atg12-5 complex, Atg5 and beclin-1, show no change after doxorubicin treatment. Additionally,

histological analyses revealed that doxorubicin-treated muscle has increased centre-nucleated myofibres [248]. Doxorubicin effects on diabetic skeletal tissue have not been studied. Doxorubicin leads to increased mitochondrial ROS, resulting in muscle cell death [87,249]. Increased ROS levels are also a common feature in obesity-induced diabetes. Therefore, it may be hypothesized that doxorubicin in the skeletal tissue will cause more ROS accumulation. In recent studies, it has been indicated that inflammatory signalling pathways can also be activated by metabolic stresses. Additionally, increased glucose metabolism leads to mitochondrial production of ROS. High levels of ROS are produced in obesity, which in turn enhance the activation of inflammatory pathways [250–252]. Overlap of this immune inflammatory pathway and metabolic pathways has been reported many times. Therefore, the worsening effects of doxorubicin can be monitored in metabolic pathways triggered by this inflammatory pathway. Therefore, it is evident that there is a link between inflammatory and metabolic pathways that might be affected before any other signalling pathways are disrupted.

2.2. Metabolic diseases as inflammatory disease

The combination of metabolism and immune systems has recently emerged as a breakthrough target for treating metabolic diseases such as T2D. Immune system activation by metabolic stress has provided new insights for understanding the pathogenesis and progression of T2D. Metabolic stress causes pathologic activation of the immune system that leads to metabolic disorders and progresses as an inflammatory disorder with severe consequences. T2D is now considered a metabolic disorder characterized by insulin resistance and pancreatic β-cell dysfunction [230,231]. IR in insulin-sensitive

tissues and a relative decrease in insulin secretion by β-cells of the pancreas are the major features of T2D. Hyperinsulinaemia due to IR occurs in the preclinical period of the disease [231]. To date, four mechanisms have been reported to contribute to the pathogenesis and progression of T2D and are mentioned below [232].

i) *Increased polyol pathway flux:* In T2D, cells are unable to use glucose, and unused glucose thus enters the polyol pathway where aldose reductase reduces it to sorbitol. This reaction oxidizes nicotinamide adenine dinucleotide phosphate (NADPH) to NADP+. Then, hexokinase oxidizes sorbitol so that it can enter the glycolysis pathway. In T2D, blood glucose levels exceed the glycolysis pathway limit, and the mass balance continues to favour sorbitol production.

ii) *Increased advanced glycation end product (AGE):* Glycation is the process by which glucose or fructose molecules are bonded covalently to a protein, carbohydrate or lipid molecule and occurs without the involvement of an enzyme. The glycation process leads to cell damage. The formation of advanced glycation end products is increased in diabetes because of hyperglycaemia. Increased accumulation of glycated plasma proteins has a significant role in the pathogenesis of various diseases [253].

iii) *Increased hexosamine pathway flux:* The hexosamine biosynthesis pathway (HBP) is a small branch of glycolysis. In this pathway, fructose 6-phosphate is converted to glucosamine 6-phosphate, which is catalysed by glutamine fructose-6-phosphate amidotransferase, a rate-limiting enzyme. One study suggested that

increased hexosamine pathway flux leads to NF-κB-dependent promoter activation [254].

iv) *Activation of protein kinase C (PKC) isomers*: The protein kinase C (PKC) family has been reported to contain worthy candidates that are activated upon fat oversupply. They are called lipid-dependent kinases. In skeletal tissues, an association between PKC activation and insulin has been demonstrated. PKC translocation occurs in defective insulin-stimulated glucose metabolism [254].

Inflammatory pathways that are downstream of oxidative stress have been focused on as unifying mechanisms for these four pathways. Many studies indicate that there is a link between metabolic alterations in T2D and inflammation. A list of inflammatory molecules and their particular role in T2D has been compiled in Table 2.8.

Table 2.8: Role of various inflammatory molecules in Metabolic diseases

Catagory	Module	Metabolic and inflammatory roles
Proinflammatory cytokines and signalling molecules	TNF-α IL-6 CRP IL-1 IL_1β	-Increased levels related to IR and T2D -Reduces insulin sesitivityby influencing the phosphorylation of insulin receptor -Major proinflammatory cytokine that induces inflammation and IR leading to T2D -Elevated serum CRP associated with the incidence of T2D -Associated with obesity and IR -Affects insulin signalling directly through the induction of SOCS-3 -Leads to IR via the inhibition of insulin induced Akt phosphorylation in adipocytes -

Transcription factors	NFkβ JNK IKKβ	-Increase the expression of genes encoding cytokines, chemokines, transcription factors and various receptors involved in IR and pathogenesis of T2D -Promotes IR through phosphorylation of serine residues in IRS-1 -Leads to IR through transcriptional activation of NFkβ.
Adipokines/cytokines	Leptin Adiponectin Resistin Adipsin Visfatin MCP-1	-High leptin levels, reflecting leptin resistance predict increased risk of T2D. -Low levels of adiponectin correlate with T2D and is downregulated by TNF-α. -Promotes IR and decreases insulin stimulated glucose transporters in adipose tissue. - Role in maintaining β cell function -Lower levels of adipsin found in T2D patients -Visfatin binds to the insulin receptor at a site distinct from that of insulin and causes hypoglycaemia by reducing glucose release from liver cells and stimulating glucose utilization in adipocytes and monocytes. -MCP-1 expression in adipose tissue contributes to the macrophage infiltration to the tissue -causes IR and T2D
Toll like receptor	TLR2 and TLR4	-Play critical role in pathogenesis of IR and T2D
Adhesion molecules	E-selectin/P-selectin ICAM-1/VCAM-1	-Leads to the leukocyte recruitment in the local tissue and promotes inflammation, IR and T2D -Alters endothelaia and sub-endothelial structure leading to reduced vascular permeability
Nuclear recetors	PPAR and VDR	-Mutations in these genes causes IR and T2D -Regulated expression of insulin receptor

Source: [254,255]

Experimental studies demonstrate that adipose tissue acts as a site of inflammation. Increases in adiposity have been linked with the upregulation of proinflammatory genes such as TNF-α [255]. NF-kB and c-Jun NH2-terminal kinase (JNK) have been reported to cause hypoxia stress activation of toll-like receptor (TLR) via excess free fatty acid (FFA) intake [256]. Due to a sustained accumulation of lipids, proinflammatory macrophages cluster around adipocytes called foam cells and result in the switching of macrophages from an anti-inflammatory “M2” phenotype (alternatively activated) to a proinflammatory “M1” phenotype (classically activated). When the equilibrium between M1 and M2 phenotypes is disturbed, inflammatory molecules are secreted, leading to metabolic disorders [257,258]. It has also been demonstrated that inflammatory responses in macrophages are induced by adipocyte-derived FFAs via TLR or the NOD-like receptor family [259,260]. In addition, nutrient overload leads to the increased infiltration of macrophages into metabolic tissues that promotes an proinflammatory environment characterized by increases in TNF-α, IL-1β, and inducible nitric oxide synthase (iNOS) levels. Further, the accumulation of these proinflammatory macrophages in metabolic organs, such as the liver, adipose tissue and muscle, directly suppresses the action of insulin by phosphorylating IRS1 at the serine position [261].

Proper regulation of metabolic and immune systems is the most important requirement for healthy living and survival. This interaction can be treated as a central homeostatic mechanism in the animal kingdom whose proper maintenance can prevent a cluster of metabolic disorders, especially obesity, T2D, MetS, cardiovascular disorder and cancer. During the past two decades,

it has been demonstrated several times that inflammation is a key feature of obesity and T2D [262,263]. Evidence also indicates that there is a link between metabolism and immunity. Inflammation caused by obesity activates metabolic and inflammatory pathways and leads to diabetes. Metabolic and immune pathways are interdependent as many hormones, cytokines, regulatory proteins, bioactive lipids and transcription factors are common in both metabolic and immune roles [262]. To fight against infection, the normal inflammatory response depends on the metabolic pathway, particularly on the mobilization of stored lipid [264]. The basic inflammatory response suppresses anabolic pathways and supports catabolic pathways, such as the highly conserved insulin signalling pathway [262]. Long-term consequences of prolonged inflammation are often found in metabolic diseases, and most of the mediators are involved in obesity and diabetes. During obesity, a continuous nutrition supply is present, which leads to an overactive metabolic state [265]. It has been reported that there are overlapping pathways that regulate both metabolic and immune functions through common key regulatory molecules and signalling systems. This overlap allows nutrients to trigger to pathogen-sensing systems such as TLRs, which have been reported to cause metabolically or nutritionally induced inflammatory responses. The potential benefit of inflammatory activation due to metabolic triggers is to block major anabolic signalling pathways such as the insulin/insulin growth factor pathway. These anabolic pathways need to be blocked to divert energy sources away from synthetic pathways.

2.2.1. Overlap of Inflammatory and metabolic pathways

In recent studies, it has been shown that inflammatory signalling pathways can also be activated by metabolic stress. Additionally, increased glucose metabolism leads to the production of ROS. High levels of ROS are produced in obesity, which in turn enhances the activation of inflammatory pathways [266,267]. Several serine/threonine kinases are activated by inflammatory or stressful stimuli, which leads to the inhibition of insulin signalling proteins, such as JNK, NF-κB and PKC-θ [268]. NF-κB and JNK kinase activation in obesity indicates the overlap of inflammation and metabolic pathways. The JNK group of serine/threonine kinases has three members, namely, JNK-1, -2, and -3, which belong to the MAPK family and regulate multiple cell activities in development and cellular functions. JNK has been investigated as a central metabolic regulator that plays a major role in insulin resistance in obesity [269,270]. In obesity, JNK activity is increased in the liver, muscle, and fat tissues [271]. Experiments have shown that the modulation of hepatic JNK1 in adult animals had effects on glucose metabolism via the release of cytokines [272]. PKC and IκB kinase are the other two inflammatory kinases that play a significant role in insulin action, particularly in response to lipid metabolites. Increases in diacylglycerol (DAG) and fatty ACC (indicators of the intracellular fatty acid level) are correlated with increases in the Ser307 phosphorylation of IRS-1 [263] and the activation of NF-κB (a transcription factor for multiple inflammatory mediators, including TNF-α and IL-6) [262].

2.3. Targeting key players in metabolic and inflammatory pathways

An abnormal adipokine profile may contribute to MetS and thus other MetS-related diseases. Although numerous articles have reviewed the functions of adipokines, the biological sources of these adipokines are still vague. Adipokines are commonly referred to as cytokines (e.g., IL-6, TNF-α, and visfatin), which are secreted by macrophages, or adipose-derived hormones (e.g., leptin, adiponectin, and resistin), which are secreted by adipose tissue. Different combinations of cells in adipose tissue may give rise to distinct adipokine profiles, which may represent the progression of diseases from obesity to MetS or prediabetes.

2.3.1. Proinflammatory adipokines

2.3.1.1. Leptin

Leptin is a cytokine-like protein with a molecular mass of 16 kDa and is expressed by adipocytes. Under normal physiological conditions, leptin functions to increase energy expenditure, reduce appetite, increase sympathetic activity, improve insulin sensitivity and facilitate glucose utilization [273,274]. The concentration of leptin in plasma ranges from 5 to 15 ng/mL in lean subjects, whereas it has been reported to reach up to 50 ng/mL in the plasma of obese individuals [275]. Leptin levels vary proportionally with the adipose mass, and it is mainly produced by adipocytes. Leptin is also secreted by cardiomyocytes and vascular smooth muscle cells. The functional receptor of leptin is present in the hypothalamus; it functions to reduce appetite and increase energy expenditure. The leptin receptor is also present in other organs, including the kidneys, heart, pancreas, and liver. The receptors are also

present in the brain vasculature, myometrium and endothelium and smooth muscle cells of the heart [276]. Increased plasma leptin levels have been associated with obesity, hypertension, T2D, and MetS. Dysregulation of adipokines, such as leptin and adiponectin, has been reported to play an important role in the development of MetS [256].

Link between metabolic and inflammatory pathway

The role of leptin as a biomarker for MetS has been demonstrated in a wide range of populations. Irrespective of the demographic, exacerbated leptin levels have been associated with MetS. Upregulated leptin levels have also been linked to insulin resistance, obesity, congestive heart failure, and myocardial infarction [276]. It has been reported that leptin is the most delicate marker for predicting MetS and risk of cardiovascular disease in school children (n=321, age 6 to 12 years) [277]. Lee et al. demonstrated increased leptin serum levels in postmenopausal women (n=153) with MetS. They reported a positive correlation between leptin and obesity and leptin and the number of risk factors present in MetS [278]. A study conducted in a Lebanese population of nondiabetic males (n=53, mean age ± SD= 59.3 ± 7 years) demonstrated that increased leptin levels were associated with MetS. The authors also showed a strong correlation of leptin with waist size. Interestingly, they reported a weak correlation of leptin with lipid profiles that disappeared after BMI adjustment [279]. Similarly, in a Korean population (n=3272; men: 1915, women: 1357) aged 30 to 84 years old, elevated leptin levels were associated with MetS independent of BMI. The study reported that levels of leptin in the serum increased as the number of components of MetS increased, irrespective of the obesity status of the individuals [280]. In contrast, Martins et al., reported a positive association

between leptin and hyperinsulinaemia, obesity, and insulin resistance. They showed that there was a weak association between leptin and other risk factors of MetS [281]. Leptin has been reported to have proinflammatory properties and actions comparable to acute phase reactants. It upregulates the production of inflammatory cytokines, including IL-6, TNF-α, and IL-12 via p38 and mitogen-activated protein kinase (MAPK) in lipopolysaccharide (LPS)-stimulated Kupffer cells [282] and in obese mice [283]. On the other hand, in adipose tissue, IL-1β and TNF-α increase the expression of leptin mRNA in adipose tissue, suggesting that a feedback loop between leptin and inflammatory cytokines promotes inflammation [284]. Faggioni et. al reported that the leptin levels in adipose tissue and serum increase after the induction of inflammatory stimuli [285]. Leptin in the circulation affects the HPA axis during stress [286]. In conclusion, increased leptin levels due to overeating trigger a proinflammatory mechanism to prevent excessive stress at the cellular level. Fat cells are overburdened due to an excessive intake of calories, and they grow in size and number. The stress results in inflammation and unhealthy fat storage within muscles, blood vessels, and internal organs. In addition, the increase in insulin levels due to calorie overload exacerbates leptin levels and is potentiated by increased cortisol levels. Excessive caloric intake overburdens the capability of fat cells to increase in number and grow in size; the subsequent stress response results in inflammation in the cells and the storage of unhealthy fat within internal organs, such as muscles and blood vessels. The increase in insulin due to caloric overload aggravates increases in leptin levels potentiated by increased cortisol levels.

2.3.1.2. Resistin

Resistin is an adipose-derived cysteine-rich peptide hormone encoded by the RETN gene in humans [287]. Epithelial cells and immune cells are the sources of resistin in primates, dogs, and pigs. However, in rats, adipose tissue is the main source of resistin. The molecular mass of resistin is nearly 12.5 kDa. Resistin, an adipose-derived hormone, has been reported to be involved in obesity and T2D [288]. In normal physiological conditions, resistin concentrations in human serum range from 7 to 22 ng·mL^{-1}. Resistin shares structural homology with adiponectin. Resistin is mainly secreted by adipose tissue and adipose tissue macrophages [289].

Link between metabolic and inflammatory pathway

Resistin has been involved in metabolic, autoimmune and inflammatory diseases. Resistin is an important biomarker and therapeutic target for heart and other diseases [290]. Serum resistin levels have been shown to be inversely associated with serum HDL cholesterol concentrations in a general Japanese population (n = 2078) [291]. Furuhashi et al. showed that circulating levels of resistin are not correlated with blood pressure. They reported that the resistin levels were similar between controls and subjects with essential hypertension (n = 1090), irrespective of the presence or absence of insulin resistance [292]. In contrast, a study of 71 human female subjects with MetS (mean age 31.59 ± 4.88 years) and 99 healthy human female subjects without MetS (mean age 31.75 ± 6.34 years) suggested a positive correlation between circulating resistin levels and systolic and diastolic blood pressure [293]. It has been demonstrated that serum resistin levels are significantly higher in MetS subjects compared to

those in subjects without MetS (13.53 ± 4.11 ng/ml vs. 7.42 ± 2.31 ng/ml). Serum resistin levels have been significantly correlated with BMI [294], whereas some studies have shown that there is no correlation between serum resistin levels and BMI [295,296]. The circulating resistin levels were associated with serum triglyceride concentrations and BMI [297]. Studies have reported that resistin increases the level of LDL or bad cholesterol, which increases the risk of heart disease [298]. Resistin has been found to accelerate the accumulation of LDL in arteries and the liver in humans [299]. Resistin levels in plasma have a significant correlation with the components of MetS. Resistin has a positive correlation with triglycerides, waist circumference, and systolic blood pressure and has a negative correlation with HDL [300]. Another study discovered a positive correlation between resistin levels and MetS (waist circumference: r = 0.07; triglycerides: r = 0.07 and HDL: r = -0.09), with P-values less than 0.05. Moreover, increased resistin levels in the inflamed joints of rheumatoid arthritis patients have been correlated with other inflammatory markers, suggesting potent proinflammatory and regulatory role of resistin [301]. In mice, resistin is mostly secreted by adipocytes [302]. In humans, it was found to be highly expressed in immune cells including, monocytes and macrophages [303]. Numerous studies indicate that proinflammatory cytokines, including IL-6, IL-1 and TNF-α, may increase the expression of resistin in human peripheral blood mononuclear cells [304]. Studies in humans demonstrated that circulating resistin levels were positively associated with leukocytes [305], C-reactive protein (CRP) [306] and IL-6 [307].

2.3.1.3. Visfatin

Visfatin is a cytokine with a molecular mass of 52 kDa that is mainly expressed in visceral adipose tissue compared with subcutaneous adipose tissue [308]. Visfatin is an adipocyte-derived hormone and is also known as nicotinamide phosphoribosyltransferase [309]. Visfatin is found in the bone marrow, muscle, liver, heart, kidney, lung, and cartilage and a small amount can be detected in the placenta and testis [310]. To date, visfatin circulatory levels in humans have been divergent. For instance, visfatin plasma concentrations were reported to be elevated in obese subjects (0.037 ± 0.008 μg/ml), compared with controls (0.001 ± 0.000 μg/ml, $P < 0.001$) [311]. The concentration of circulating visfatin, which is 21.5 ± 8.3 ng/ml in non-diabetic subjects versus 42.0 ± 19.9 ng/ml in diabetic subjects, has also been reported [312]. Visfatin is produced in visceral adipose tissue and has been proposed as the missing link between intra-abdominal obesity and diabetes [313]. However, visfatin plays a similar role to that of insulin, and the plasma concentration of visfatin is much lower than that of insulin under physiological conditions, which raises doubts about the physiological importance of the systemic insulin-sensitizing effects of visfatin [313]. However, the dramatic elevation of visfatin levels in the visceral adipose tissue of obese mice suggests that it has a significant role in the pathophysiology of obesity. Human obesity-related diabetes and the accompanying metabolic disorders have been explicitly linked to increased visceral adipose tissue mass. Visfatin is also not regulated by fasting and feeding. Hence, visfatin production is proposed to be a compensatory response to tissue-specific insulin resistance [313,314]. Nevertheless, the paracrine insulin-mimetic effects of visfatin in intra-abdominal adipose tissue and the expansion

of the intra-abdominal fat depot may be more biologically relevant than the endocrine effects of visfatin on improving global insulin sensitivity [313,314].

Link between metabolic and inflammatory pathway

An elevated circulating visfatin plasma level in subjects with T2D has been reported several times. For instance, Chen et. al., conducted a study of people from Taiwan (n = 120, mean age = 65.3) with T2D. The study reported that visfatin levels were increased in subjects with T2D regardless of BMI. The visfatin levels were negatively correlated with inflammatory markers and the lipid profile [315]. Another study, based on Chinese patients (n = 166, mean age = 56.2 for males and 56.9 for females) with T2D, suggested that serum visfatin levels may be linked to visceral obesity in men. This study also suggested that the visfatin gene might account for the variation in the lipid parameters and glucose levels of Chinese subjects [316]. Additionally, visfatin has been correlated with obesity. For example, visfatin concentrations (16.4 ± 13.4 ng/ml vs 7.7 ± 5.2 ng/ml, P = 0.006) in plasma were found to be elevated in obese subjects (n = 36, mean age= 46.5 years, mean BMI = 28.4 kg/m^2) compared with non-obese subjects (n = 12, mean age = 48.4 years, mean BMI = 23.7 kg/m^2) [317]. A study conducted using subjects from Greece (n = 56, mean age = 50 years) demonstrated that plasma visfatin concentrations were elevated in obese subjects with MetS compared with individuals without MetS [318]. This study also reported that plasma visfatin levels were positively correlated with waist circumference ($r = 0.31$, $p < 0.05$), glucose levels ($r = 0.33$, $p < 0.05$) and triglycerides ($r = 0.59$, $p < 0.01$) but negatively correlated with high density lipoprotein levels ($r = -0.38$, $p < 0.05$) [319]. Visfatin has been reported to be overexpressed in numerous inflammatory diseases, including atherosclerosis,

osteoarthritis, and rheumatoid arthritis, and visfatin may be involved in an obesity-associated low-grade inflammation state and MetS [320,321].

2.3.1.4. Chemokine (C-C motif) ligand 2

Chemokine (C-C motif) ligand 2(CCL2), a monomeric polypeptide, has a molecular mass of 13 kDa. It is attached to the plasma membrane of endothelial cells through proteoglycans. CCL2 is mainly secreted by immune cells such as monocytes, dendritic cells, and macrophages [322]. In normal physiological conditions, CCL2 plasma concentrations are reportedly 200 pg/mL [323]. CCL2 is also known as monocyte chemotactic protein 1 (MCP-1). CCL2 is secreted by cells, such as malignant tumour cells, endothelial cells, fibroblasts, lymphocytes, and smooth muscle cells [324], to recruit different immune cells, such as T lymphocytes, mast cells and monocytes, to sites of inflammation [325].

Link between metabolic and inflammatory pathway

CCL-2 has been reported to worsen the condition of MetS by accumulating macrophages in the tissues and inducing insulin resistance [326]. Additionally, the presence of CCL-2 indicates the possibility of the presence of other diseases such as arthritis, neoplasia, and hypersensitivity [327]. CCL-2 is linked to several diseases that are related to inflammation, such as diseases that are caused by the infiltration of monocytes including rheumatoid arthritis psoriasis and atherosclerosis [328]. Additionally the upregulation of serum CCL-2 in an individual may contribute to myocardial infarction [329]. Several lines of evidence indicate that patients with MetS have a higher level of CCL-2. The higher level of serum CCL-2 has been associated with the accumulation of fat. CCL-2

exhibits a specific action of attracting different white blood cell types. For instance, CCL-2 attracts monocytes and basophils when it binds to its receptors, but CCL-2 does not attract neutrophils or eosinophils. However, an N-terminal deletion of CCL-2 reverses this situation, whereby CCL-2 only attracts neutrophils and eosinophils but not monocytes [330].

2.3.1.5. Chemerin

Chemerin is a chemoattractant protein with a molecular mass of 14 kDa, and chemerin acts as a ligand for CMKLR1 (a G protein-coupled receptor) [331]. Chemerin is secreted in an inactive form as prochemerin and then activated by inflammatory and coagulation serine proteases that cleave its C-terminus [332]. In humans, the mRNA of chemerin in mostly expressed in white adipose tissue, lung, and liver. However, the chemerin receptor, CMKLR1, is mainly expressed in adipose tissue and immune cells [333]. Chemerin is considered an adipokine because it plays a role in adipocyte differentiation and glucose uptake. In humans, chemerin concentrations in the serum and plasma under normal physiological conditions have been reported to be 3.0 and 4.4 Nm, respectively [334]. Healthy control subjects have been shown to have significantly lower serum levels compared with MetS subjects (median: 192.5 vs. 266.0 μg/l; $P < 0.01$) [334].

Link between metabolic and inflammatory pathway

Chemerin has been considered to be an independent marker of MetS because it plays a significant role in the pathogenesis of MetS [334]. In an animal study, significant associations were demonstrated between chemerin and MetS for T2D and obesity [333]. Chemerin acts as a mediator between obesity and

vascular inflammation [335]. Landgraf et. al., studied associations between the chemerin serum levels and metabolic and cardiovascular parameters in 105 obese and 69 lean children and discovered that the chemerin concentrations were significantly higher in the obese children compared with the lean children [336]. Chemerin has been reported to exhibit both pro- and anti-inflammatory properties. The pro-inflammatory and anti-inflammatory effects of chemerin have been shown by Cash et.al [337]. Chemerin exerts both pro- and anti-inflammatory effects through chemokine-like receptor 1. Animal and cell-based studies demonstrated that chemokine-like receptor 1 and chemerin have both pro- and anti-inflammatory roles in immune system pathways. A dual function for chemerin has been proposed due to the different roles of the chemerin isomers during different phases of inflammation [338].

2.3.1.6. Plasminogen activator inhibitor-1

Plasminogen activator inhibitor-1 (PAI-1) is also known as serpin E1 or endothelial plasminogen activator inhibitor. In humans, this protein is encoded by the SERPIN 1 gene. PAI-1 is a serine protease inhibitor and functions as the main inhibitor of urokinase (uPA) and plasminogen activator (tPA). uPA and tPA are activators of fibrinolysis [339]. An increased circulating level of PAI-1 has been reported to be associated with atherosclerosis and thrombosis [340]. PAI-1 is mainly produced by the cells present in the endothelium of the blood vessel. PAI-1 has also been reported to be secreted by other types of tissue such as adipose tissue, intra-abdominal adipocytes, platelets and the vascular endothelium. Tests that measure the PAI-1 level are indicated for unexplained delayed disorders of bleeding, which are characteristically associated with surgery or trauma [341]. The PAI-1 reference range in circulation is 2-15 AU/mL.

However, increased activity of PAI-1 is often detected in elderly individuals [340]. The normal plasma concentration of PAI-1 is 5-40 ng/mL [340].

Link between metabolic and inflammatory pathway

The primary function of PAI-1 is to prevent fibrinolysis and to support fibrin formation during inflammation [342,343]. An elevated level of PAI-1 in the circulation has been observed in many conditions such as bacterial and other infections [344]. The PAI-1 level is also elevated in several pathological conditions such as obesity, cancer, and MetS [342]. Furthermore, PAI-1 plays a significant role in inflammatory progression. In humans, an elevated level of PAI-1 indicates that there is internal damage and that fibrin is being formed to replace dead cells. However, the greater increase in PAI-1 level leads to a prothrombotic state and endothelial dysfunction, which becomes a hypercoagulable state and consequently causes MetS. Additionally, increases in free fatty acid levels reportedly lead to PAI-1 elevation. Increased circulating PAI-1 levels in acute lung injury may indicate a high risk of mortality [345,346]. Nevertheless, a congenital deficiency of PAI-1 leads to bleeding/haemorrhagic diathesis. Angiotensin II (a protein that promotes aldosterone secretion and raises blood pressure) increases the synthesis of PAI-1 that causes atherosclerosis [347]. Several researchers have also suggested an interaction between PAI-1 expression and ORM-1, a protein that is also known as Alpha-1-acid glycoprotein 1, during the acute phase of inflammation that plays a role in immunosuppression [348].

It is well known that increased concentrations of PAI-1 in the blood are associated with a preference towards pulmonary embolism and venous

thrombosis. Such a linkage is predicted based on inhibition by PAI-1. Additionally, increased concentrations of PAI-1 in circulation are associated with MetS, obesity, and T2D [349]. An influential article reported that young male survivors of myocardial infarction (MI) displayed augmented concentrations of PAI-1 in the blood compared with age-matched normal subjects. The patients were characterized one year after the index infarction when the patient's haemodynamic status was not visibly compromised. The authors proposed that the patients manifested elevated levels of PAI-1 in circulation before the MI occurrence [350]. It has also been reported that the patients with T2D or obesity, compared with lean individuals, show increased concentrations of PAI-1 in the arterial wall and blood [351]. Researchers have also speculated that the augmentation of PAI-1 that is distinctive of T2D and IR contributes to the higher incidence of MI and coronary artery disease [352]. Metabolic imbalances including hypertriglyceridemia and hyperglycaemia are due to a relative/absolute deficiency in insulin. These metabolic aberrations contribute to the raised PAI-1 concentration in vivo. Elevated levels of glucose have been shown to elevate the PAI-1 level in both smooth vascular cells and endothelial cells in vitro [353]. High triglycerides and their elements (fatty acids) also increase the expression of PAI-1 in human liver cancer cell lines [354]. Furthermore, the combination of high concentrations of both triglycerides and insulin exerts a synergistic effect on PAI-1 in human liver cancer cell lines [355]. The results of mechanistic studies have demonstrated that insulin reduces the degradation rate of PAI-1 mRNA [356]. In conclusion, the results of in vitro studies have established that the combination of T2D and IR and the metabolic derangements and hormonal

abnormalities associated with T2D and IR have direct effects on PAI-1 expression.

2.3.1.7. Interleukin-6

IL-6 is a pro-inflammatory cytokine. It is a glycosylated protein, weighing 21 – 28 kDa and encoded by the IL6 gene located on the seventh chromosome. IL-6 is secreted by a large variety of cells, such as macrophages, monocytes and even several tumour cells [357] and adipocytes [358]. IL-6 is involved in different important processes for reacting to stimuli, for example, differentiation of monocytes, survival of cells, apoptosis, and proliferation [359]. Due to its property of promoting inflammation, it is suggested that the concentration of IL-6 would increase, contributing to the pro-inflammatory state of MetS and central obesity [360].

Link between metabolic and inflammatory pathway

IL-6 is a pleiotropic cytokine that plays a major role in various metabolic processes [361]. IL-6 has been considered as an important player for the progression of inflammation from innate to acquired immunity. During acute inflammation, IL-6 replaces neutrophils by T cells and monocytes after one to two days of the tissue damage. It prevents increased tissue damage caused by the accumulation of neutrophil-secreted proteases and ROS at the inflamed location [362–364]. Endothelial cells and other vascular elements are activated by microbial products releasing several cytokines like TNF-α and IL-6, attracting neutrophils at the site of infection. Proteolytic processing of IL-6R from neutrophils later leads to IL-6 trans-signaling in the nearby tissue cells. IL-6 trans-signaling further switches the recruitment of neutrophils to monocytes by

reducing neutrophil-attracting chemokines (IL-8, fractalkine) and increasing monocyte-attracting chemokines (CCL-2, Chemokine (C-X-C motif) ligand 6 and 8) [365,366]. Also, by IL-6 trans-signaling, cell adhesion molecules for examples, intercellular Adhesion Molecule 1, vascular cell adhesion protein 1, and E-selectin on endothelial cells, and L-selectin on lymphocytes are upregulated. Hence, leukocyte transmigration is enhanced. Apart from attracting monocytes, monocyte differentiation can be skewed towards macrophages by IL-6 trans-signaling through increasing macrophage colony-stimulating factor receptor expression [367]. IL-6 can also induce apoptosis in neutrophils, suggesting that IL-6 resolves the acute neutrophil infiltration [363,368]. IL-6 inhibits transforming growth factor beta (TGFβ)-mediated differentiation of naive cluster of differentiation 4 (CD4+) T cells into T regulatory cells. Hence, autoimmunity and tissue injury are prevented. On the contrary, when TGFβ and IL-6 combines, the formation of T helper 17 cells is stimulated [369]. It has been reported that during exercises, muscle cells produces a large amount of IL-6 [370]. In contrast, adipocytes in obese individuals also produce IL-6, and its activity has been correlated with the adipocyte volume [371]. Obesity is considered as a state of chronic, low-grade inflammation [372]. Glucose intolerance and insulin resistance were developed in IL-6−/− mice as compared to wild-type mice. Moreover, signs of liver inflammation were found in IL-6−/− mice [373]. Whereas IL-6 gene deleted in hepatocytes were also found to have decreased insulin sensitivity and glucose tolerance [374]. Interestingly, TNF-α blockade inhibited this inflammation, reflecting that the balance between IL-6 and TNF-α signaling is essential in the liver [374]. In humans, a link between impaired metabolic homeostasis and the blockade of IL-6 signaling has been

suggested [375]. The increase in body weight of about 4 kg (7 %) with hypertriglyceridemia and hypercholesterinemia are found in individuals receiving IL-6R neutralizing tocilizumab [375,376].

2.3.1.8. Interleukin -8

Interleukin-8 (IL-8) is one of the proinflammatory cytokine weighing about 8-10 kDa with atherogenic properties. It has multiple features including intimal thickening, recruitment of T lymphocytes and neutrophils into the subendothelial space, migration of vascular smooth muscle cells and favoring adhesion of monocyte to endothelium [377,378]. In humans, macrophage-derived foam cells contain IL-8 in high amounts [379]. Under normal physiological conditions, IL-8 level in serum has been reported to be12.9 ± 13.93 pg/ml [380].

Link between metabolic and inflammatory pathway

IL-8 has been reported to be secreted in vitro by human adipocytes [381]. In a study conducted on 75 subjects with normal glucose tolerance, it was established that plasma IL-8 levels were increased in obese subjects with normal glucose tolerance (n = 40) compared to lean subjects (n = 35) with normal glucose tolerance. Increased IL-8 levels have also been related to increases in body mass index, percent body fat waist-to-hip ratio, and TNF-α [382]. IL-8 levels have found to be increased in both type 1 and type 2 diabetic patients [383]. Similarly, a study on 100 subjects (obese: n = 50, BMI > or = 25 kg/m^2 and non-obese: n = 50, BMI < 25 kg/m^2) investigated the circulating levels of IL-8 in the serum. The study reported that in obese subjects (BMI > 30 kg/m^2) the level of IL-8 was higher compared with nonobese controls (BMI < 25 kg/m^2). It was also demonstrated that IL-8 is related to obesity-related parameters

including waist circumference, BMI, CRP, HDL-cholesterol and IL-6 levels. These findings suggested that the circulating IL-8 may be a potential candidate that links obesity with metabolic complications such as MetS and diabetes [384]. However, the relationship between chemokines and obesity has not been fully established at the moment. In the pathogenesis of atherosclerosis IL-8 has been reported to be involved in recruiting the neutrophils and T-lymphocytes into the sub-endothelial space and monocyte adhesion molecules to endothelium [385]. IL-8 induces the expression of vascular endothelial growth factor in vascular endothelial cells and functions as an autocrine factor for endothelial cell growth and angiogenesis [385].

2.3.1.9. Tumor necrosis factor -α

Tumor necrosis factor α (TNF-α) is a proinflammatory 25 kDa cell signaling protein. It is mainly produced by activated macrophages, while it can be produced by other immune cells including natural killer cells, lymphocytes, mast cells, neutrophils, eosinophils, and neurons [386]. The physiological serum level of TNF-α in healthy controls have been reported 11.2 ± 7.3 pg/ml [380]. TNF-α is mainly involved in the regulation of immune cells. Exacerbation in the TNF-α production has been related to numerous human diseases including cancer [387], depression [388], inflammatory bowel diseases [389] and T2D [390].

Link between metabolic and inflammatory pathway

TNF-α is a proinflammatory cytokine mainly secreted by visceral adipose tissue. Visceral adipose tissue expansion is a common characteristic of MetS [391]. MetS are often characterized by dysregulation of metabolism and hence, the adipocyte secretes proinflammatory adipokines at higher levels including

TNF-α and IL-6. Therefore, abdominal or central obesity often leads to MetS and may be a risk factor for elevated circulatory TNF-α levels [392]. Additionally, increased TNF-α levels has been associated with insulin resistance through its abnormal activation of the protein kinase C and mechanistic target of rapamycin (mTOR) signaling pathways [392]. In a study based on MetS subjects (n = 80 subjects) aged between 40 and 60 years, elevated levels of proinflammatory cytokines including TNF-α and IL-6 were associated with hypertriglyceridemia and insulin resistance. They found that TNF-α, leptin and IL-6 levels in MetS patients were higher compared to those in the control group, suggesting that these cytokines are directly correlated with MetS [393]. It was hypothesized that early detection of the inflammatory status of the patient including TNF-α and IL-6, might be useful for early diagnosis and early intervention for MetS and its comorbidities [393]. In another study, TNF-α levels were found to be higher in MetS patients with coronary artery disease (n = 37), than the controls (n = 23) [394]. Indulekha et al also reported that increased TNF-α levels were significantly correlated with MetS [395]. It has also been demonstrated that elevated levels of the soluble TNF-α receptor (sTNF-α-R) is associated with increased TNF-α activity, in patients with MetS and hypertension. The reason stated was the widespread systemic effects of TNF-α contributing to the various disease processes related to MetS. TNF-α is a cytokine associated with coronary atheroma and is secreted from endothelial, smooth muscle cells as well as macrophages and adipose cells. It also enhances monocyte recruitment into developing atherosclerotic lesions and links obesity with atherosclerosis. Association of LDL accumulation in rat arteries with TNF-alpha expression suggests a role for inflammation in early-stage atherosclerosis [396]. Its

contribution to the various characteristics of MetS suggest that TNF-α may be a significant contributor to the development and progression of its associated disease processes.

2.3.2. Anti-inflammatory adipokines

2.3.2.1. Adiponectin

Adiponectin is the most abundant adipokine that circulates at relatively high concentrations in the serum. In the plasma of healthy volunteers, the adiponectin has been reported to be present in the range from 1.9 to 17.0 mg/ml. However,in obese people, plasma concentrations of adiponectin were significantly lower than those compared to non-obese people [397]. It is an anti-inflammatory collagen-like protein, solely synthesized in the white adipose tissue. It is secreted during adipocyte differentiation [398]. Both in humans and animals, adiponectin has been demonstrated to modulate lipid and glucose metabolism in the insulin sensitive tissues. In the diet-induced murine models of obesity, the circulatory level of adiponectin has been reported to be decreased [399]. In humans, the diet-induced obesity also reduces the circulatory adiponectin level [397]. Low levels of adiponectin have also been demonstrated to be involved in the development of IR in mouse models of obesity and also in mouse models of lipoatrophy [399]. In humans, adiponectin plasma levels are lower in insulin-resistant states together with T2D [400]. Adiponectin levels has been shown to be increased after induction of the insulin-sensitizing compounds thiazolidinedione (TZD) in humans [401,402]. It has also been suggested that adiponectin has anti-atherogenic properties because, in diabetic subjects with coronary artery disease, the adiponectin level in plasma is relatively lower than in diabetic patients without coronary artery disease [403]. In

human aortic endothelial cells, adiponectin decreases the surface expression of vascular adhesion molecules that modulates inflammatory responses in endothelial cells [404]. Adiponectin inhibits the proliferation of vascular smooth muscle cells [405]. It is also now known as an important marker of the MetS [406].

Link between metabolic and inflammatory pathway

Adiponectin has many roles, including insulin sensitization, anti-atherogenesis, vasodilatation and lipid oxidation enhancement. Since, adiponectin is related to all the component of MetS, it is related to MetS. Researches indicate that adiponectin suppresses nearly all processes that are involved in atherosclerotic vascular change including adhesion of monocytes to endothelial cells by inhibiting TNF-α, adhesion molecule's expression in vascular endothelial cells, foam cells formation by inhibiting oxidized LDL and inhibiting vascular smooth cell migration and proliferations [407]. Adiponectin has insulin-sensitizing effects, its high levels exert a protective effect against T2D [408], and its low level acts as an independent risk factor for the development of T2D [409.]. Subjects with hypertension or with obesity have low adiponectin level, but its level increases after losing the weight [410].

A study based on Japanese adults (men: n = 479, mean ± SD age = 53 ± 10 years; women: n = 182, mean ± SD age = 56 ± 10 years) demonstrated that adiponectin levels were negatively correlated with visceral fat, waist circumference, fasting plasma glucose, serum triglycerides, systolic and diastolic blood pressure and fasting plasma insulin levels, in both males and females. However, adiponectin was shown to be positively correlated with HDL [408]. Interestingly, they showed that the mean number of MetS components

increased with the decrease in plasma adiponectin levels. They also demonstrated that men had lower adiponectin levels than women [408]. Gannage et al discovered that adiponectin is inversely correlated with MetS which is independent of BMI, consistent with other studies [411,412]. Santaneimi et al studied a Finnish population (n=1041) and showed that the decrease in adiponectin levels is correlated with an increase in the number of components of MetS in both male and female, independent of BMI [410]. However, researchers believe that high molecular weight adiponectin is more active form as compared to low molecular weight adiponectin. Additionally, it has been suggested that high molecular weight adiponectin is the most reliable biomarker for MetS diagnosis [413]. Hara et al. showed that the ratio of high molecular weight adiponectin to plasma adiponectin was a better indicator of insulin resistance and MetS [414]. Several pieces of evidence support the notion that adiponectin has a strong anti-inflammatory function. It is reported that adiponectin strongly suppresses production of TNF-α in macrophages which is a potent proinflammatory cytokine [415]. Cultured macrophages treated with adiponectin have shown that the phagocytic activity and lipopolysaccharide-induced production of TNF-α in macrophages were inhibited [416]. Therefore, it is suggested that adiponectin act as an important negative regulator inflammatory system that may be involved in dismissing inflammatory responses by its inhibitory functions. The mechanisms of adiponectin's actions are not known and are controversial. However, evidence indicates a dominant role of AMP-activated protein kinase (AMPK), also called regulatory molecule of the anti-inflammatory microenvironment. AMPK exerts its effect in regulating extracellular-signal-regulated kinases including Akt, ERK p38, and cAMP.

Adiponectin also exerts its effect on NFκB which is regulatory molecule of proinflammatory pathway [417].

2.3.2.2. Interleukin-10

Interleukin-10 (IL-10) is mainly an anti-inflammatory cytokine that plays a role in the modulation of systemic inflammation. It is secreted by monocytes or M2 (anti-inflammatory) macrophages [392]. It is a pleiotropic cytokine and is produced by T helper cells 2, B cells, monocytes and macrophages that can inhibit a broad range of immune parameters [392]. Apart from immune cells, IL-10 is also discovered to be secreted from human adipocytes, as well as the stromal vascular fraction and tissue matrix of human fat depots [418]. In vivo, IL-10 demonstrates its anti-inflammatory effects through inhibition of proinflammatory cytokines and chemokine production by macrophages and lymphocytes, for example, T regulatory cells suppress the proinflammatory immune cells through the production of IL-10 [419]. Due to its ability in down-regulating the production of proinflammatory cytokines, it is suggested that IL-10 level would increase in the proinflammatory state, attempting to inhibit further production of proinflammatory cytokines via feedback loop [420]. For obese patients with MetS, which is a chronic inflammatory state, IL-10 would exert its anti-inflammatory function as in response to the increase in proinflammatory cytokines and acute-phase proteins that provide the proinflammatory status in obesity and MetS.

Link between metabolic and inflammatory pathway

IL-10 has been correlated with MetS and its risk factors. A study conducted on obese children demonstrated that IL-10 levels were elevated in MetS, even after adjusting for BMI [421]. Calcaterra et al. suggested that the elevated levels

of IL-10 are due to the development of MetS in children [421]. A study also reported that IL-10 level was reported to be elevated in obese women (n = 50) compared to nonobese women (n = 50). Interestingly IL-10 levels were lower in both nonobese and obese women with MetS [422]. Studies have also indicated that IL-10 levels significantly decreases in a subject with MetS in both sexes [423,424]. For demonstrating the specific role of IL-10 in insulin signaling, a cross-sectional study of adults (n = 599, mean age = 85 years) was conducted. The study showed that the decreasing level of IL-10 was associated with IR and T2D. Also, the study demonstrated that IL-10 levels was inversely correlated with levels of high triglycerides, high blood glucose, high total cholesterol (LDL), and hemoglobin A1c, but was positively correlated with low levels of HDL [425]. 70 patients with MetS and 30 age-matched controls were selected in a study conducted by Chen et al. They concluded that old male with MetS has a lower concentration of serum IL-10 but higher level of hs-CRP in serum. Some studies also demonstrated that IL-10 levels are significantly correlated with other cytokines including TNF-α and IL-6. Another study assessed MetS components in drug naive middle-aged men (n = 117). They also concluded that IL-10 is positively correlated with adiponectin, in the MetS subjects. It was suggested that IL-10 might be involved in the inflammatory pathways of MetS linked with adiponectin [426]. The results advise that if both and adiponectin and IL-10 are low, the risk of MetS is likely larger. Hence, to increase the sensitivity and specificity, the use of several biomarkers in a panel is recommended. An interesting study reported that IL-10 might moderate inflammatory reactions by reducing oxidative stress by inhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. This method was reported to be associated with

impaired insulin signaling. Additionally, the insulin signaling pathway may be dysregulated by an upsurge of the proinflammatory cytokines such as IL-6 and TNF-α [392,427].

2.3.3. Insulin

Insulin is a peptide hormone that is initially synthesized as proinsulin in pancreatic beta cells, and it is encoded by a gene located on the short arm of chromosome 11 [428]. Proinsulin further undergoes cleavage to remove a connecting peptide to form a smaller insulin molecule with 51 amino acid residues. Insulin is essential for the regulation of blood glucose levels as it can stimulate liver cells and muscle cells to take up more glucose, as well as convert glucose into glycogen for storage. Insulin performs its action by attaching to the insulin receptor, which consists of two alpha subunits and two beta subunits linked by disulfide bonding. Then, the autophosphorylation and tyrosine phosphorylation of insulin receptor substrates (IRS-1 and IRS-2) is triggered. Further phosphorylation and dephosphorylation allow insulin to achieve its final cellular effects, such as activating an enzyme for the translocation of glucose transport proteins to facilitate glucose uptake by skeletal muscle. The cellular actions of insulin lower blood glucose levels, and normal insulin control failure will result in metabolic disorders such as T2D. Two different mechanisms of failed insulin control result in two different types of diabetes mellitus. Type 1 diabetes mellitus is caused by the inability to produce sufficient amounts of insulin, and one of its most common causes is the autoimmune destruction of beta cells in the pancreas [429]. T2D is often associated with the inability of cells to respond properly to insulin, and this pathological state is known as insulin resistance [430]

Insulin resistance has been associated with several risk factors, such as advanced age, obesity and chronic sleep loss [431,432]. Insulin resistance stimulates the production of insulin in an attempt to compensate for the situation and leads to hyperinsulinaemia. Several lines of evidence have associated obesity with insulin resistance that leads to metabolic disorders [433,434]. It has also been demonstrated that the accumulation of adipose tissue stimulates the secretion of various cytokines and hormones that are involved in the induction of insulin resistance [400]. Inflammatory cytokines, such as IL-6, IL-8, and TNF-α, were discovered to play essential roles in regulating glucose metabolism, and the levels of these cytokines were found to be increased in MetS [435].

Insulin resistance has been investigated using various techniques in individuals. The glucose clamp technique was considered the best method for measuring insulin resistance in vivo [436]. This method is expensive and requires many subjects; therefore, it is not suitable for large-scale studies. Later, inexpensive methods that require only insulin levels and fasting glucose levels were proposed, including the homeostasis model assessment index, fasting glucose/insulin ratio, and quantitative insulin sensitivity check index. Among all the proposed methods, homeostatic model assessment (HOMA) index was found to be more reliable for studying insulin resistance in obese children and adolescents (boys: n = 27; girls: n = 30 girls; mean age: 12.04 ± 2.90 years; mean BMI: 29.57 ± 5.53) (Keskin et al., 2005). The authors concluded that the HOMA index is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index methods. They propose a HOMA cut-off point of 3.16 for the diagnosis of insulin resistance [437]. HOMA-insulin resistance scores increase with decreasing insulin sensitivity, and the cut-off

score for insulin resistance is set at 2.5 in adults. Nevertheless, no standardized cut-off scores can be established as the score varies among ages and ethnicities. For instance, the cut-off score for insulin resistance in a Hispanic population was found to be 3.80 [438]. Whereas in Caucasians, the cut-off score for insulin resistance was 2.71 [439]. Additionally, the cut-off score for a Japanese population was reported to be 2.5 [440]. The HOMA index is a non-invasive method for testing insulin resistance, and it requires only simple laboratory testing [441].

Chapter 3

Adipokines demonstrate the interacting influence of central obesity with other cardiometabolic risk factors of metabolic syndrome

3.1. Introduction

Metabolic syndrome (MetS) is a complex disorder that is defined by the clustering of cardiometabolic risk factors, including obesity, hypertriglyceridemia, reduced high-density lipoprotein (HDL) cholesterol, hypertension, and insulin resistance that together, culminate in an increased risk of type 2 diabetes mellitus and cardiovascular disease [1]. Obesity, in particular, central obesity plays an important role in the development of MetS [154] and is associated with an increased risk of CVD, type 2 diabetes and certain cancers[442].While several organizations have proposed criteria for Mets, debate has been ongoing regarding the decision on whether or not the central obesity component should be regarded as a prerequisite for the diagnosis of MetS. The prognostic importance of central obesity has been recognized by the International Diabetes Federation which includes central obesity as the *sine qua non* of diagnosing MetS [6]. Central obesity has been associated with an increased risk of cardiovascular diseases in subjects with or without MetS [34]. On the other hand, non-obese individuals with ≥ 3 MetS risk factors have been shown to have an equal or slightly higher risk of cardiovascular mortality and

renal dysfunction than obese subjects with ≥ 3 metabolic factors [443,444]. Furthermore, a number of studies have shown that while central obesity is important, it is not an essential component of predicting type 2 diabetes in Asian populations [445,446]. Nonetheless, these findings are difficult to interpret because they did not specify the two or more MetS risk factors other than central obesity in the selection of their subjects with MetS.

Cardiometabolic disease risk factors are derived from a combination of insulin resistance and abdominal obesity resulting from surplus intra-abdominal adiposity. Intra-abdominal adiposity interacts with other MetS risk factors to unfavorably influence overall cardiometabolic risk. The Quebec Health Survey analyzed the relationships between indices of obesity, blood pressure and hyperinsulinemia and found that variations in waist circumference explained the associations between obesity, hypertension and insulin resistance (8). A study conducted in Japanese Americans also revealed that visceral obesity was associated with hypertension independently of fasting plasma insulin [447]. Moreover, intra-abdominal adiposity has been allied with adverse changes in the lipid profile in older subjects before and after adjustment for overall adiposity [448]. Few cross-sectional studies in Hong Kong and Taiwan have proposed that central obesity is an independent determinant of insulin resistance and other metabolic risk factors. They have shown that the cluster of MetS risk factors results from multiple adiposity linked factors [449–451].

Obesity occurs in association with the enlargement of white adipose tissue and is often emphasised in the pathogenesis of MetS [6,452,453]. Specifically, excess

intra-abdominal obesity has the potential to influence cardiometabolic risk directly, through alternations in the secretion of adipokines [454]. Adipose tissue is primarily composed of a stromal vascular fraction and adipocytes. The stromal vascular fraction consists of preadipocytes, fibroblasts, endothelial cells and immune cells, including B cells, T cells, and macrophages [455] cells play critical roles in various stages of obesity. In a healthy state, adipocytes and M2 macrophages secrete adiponectin and interleukin-10 (IL-10), which assist in anti-inflammatory regulation and tissue repair [456,457]. On the other hand, during the preliminary stage of obesity, the positive energy balance increases the size of adipocytes. These enlarged adipocytes secrete pro-inflammatory adipokines, such as leptin [458], interleukin-6 (IL-6) [459], tumour necrosis factor-α (TNF-α) [22], chemokine (C-C motif) ligand 2 (CCL2) [460], and chemerin, which enhance M1 macrophage chemotaxis. In the advanced stage of obesity, the obese adipose tissues activate $CD8^+$ T cells, which recruit monocytes into the adipose tissues and stimulate the pro-inflammatory M1 macrophages in the adipose tissues. Subsequently, large numbers of migrated M1 macrophages suppress the anti-inflammatory effects of M2 macrophages, promote systemic inflammation and subsequently contribute to MetS [461,462].

Increase in perivascular and visceral obesity disrupts the equilibrium between pro-inflammatory and anti-inflammatory adipokines. Subsequently, triacylglycerol overloaded adipocytes produce increasing amounts of proinflammatory adipokines with numerous undesirable cardiovascular consequences [463]. For example, a study reported that adiponectin (anti-inflammatory adipokine) level decreases even lower if obesity is combined with

type 2 diabetes mellitus, optionally with atherosclerosis [400,464]. In contrast, increases in chemerin (pro-inflammatory adipokine) level have been positively correlated with body mass index, serum low-density lipoprotein cholesterol, blood pressure and triglycerides; and negatively with serum high-density lipoprotein-cholesterol [465]. Chemerin has been linked to progression of atherosclerosis and stable chronic coronary artery disease [466]. Therefore, the importance of controlling obesity-induced inflammation in MetS patients to prevent cardiovascular risk has been suggested [467]. A study reported that subjects with elevated levels of more than two pro-inflammatory adipokines had a higher MetS prevalence compared with subjects with elevated levels of less than two pro-inflammatory adipokines. Additionally, subjects with reduced levels of anti-inflammatory adipokines showed a higher MetS prevalence compared with subjects with elevated levels of anti-inflammatory adipokines. In another study, the prevalence of MetS was 1.49 times higher for subjects with low adiponectin, an anti-inflammatory adipokine and high retinol binding protein 4, a pro-inflammatory adipokine profiles compared to subjects with low retinol binding protein 4 and high adiponectin profiles [31]. Thus, a single adipokine may not induce MetS but the interaction between pro-inflammatory and anti-inflammatory adipokines contributes to systemic metabolic abnormalities.

Abdominal obesity has a potentially unifying pathogenetic role that is strongly associated with inflammation and oxidative stress leading to MetS [468]. Biomarkers for these clinical and pathophysiological changes have a strong potential to improve early patient identification and predict MetS associated morbidity and mortality. We hypothesised that central obesity plays a unique

role in mediating circulatory levels of adipokines through its interaction with the clustering of the other 4 MetS cardiometabolic risk factors, including hypertriglyceridemia, reduced HDL cholesterol, hypertension, and high fasting glucose. Therefore, the present study was specifically designed to examine the influence of central obesity on circulating levels of adipokines through its interaction with the clustering of cardiometabolic risk factors of MetS.

3.2. Materials and Methods

MetS risk factors data and serum sample of Hong Kong Chinese adults of both sexes (N = 83, mean ± SD age = 56 ± 9.1) were retrieved from a total of 1492 archived blood samples of participants screened for MetS parameters defined by NCEP-ATP III [2]. In this study, inclusion of subjects was based on 4 groups: 1) subjects with none of the NCEP-ATP III-defined MetS cardiometabolic risk factors, 2) subjects with only central obesity, 3) subjects without central obesity and with the other 4 MetS cardiometabolic risk factors, and 4) subjects with all five cardiometabolic risk factors of MetS. We adopted a 2 x 2 factorial design with central obesity and/or 4 other MetS cardiometabolic risk factors (high systolic and diastolic blood pressure, elevated fasting blood glucose, high triglycerides, and low HDL). All subjects were screened for MetS according to the diagnostic guideline of the NCEP-ATP III criteria [469]. Individuals diagnosed with MetS have more than two of following characteristics: (1) central obesity (waist circumference exceeds 90 or 80 cm for Asian male and female, respectively), (2) hypertension (systolic pressure equals or exceed 130 mmHg, or diastolic pressure equals or exceeds 85 mmHg), (3) elevated blood glucose (fasting glucose level equals or exceeds 5.5 mmol/L [100 mg/dL]), (4) elevated plasma triglycerides (level equals or exceeds 1.70 mmol/L [150 mg/dL]), and

(5) low level of HDL-C (level equals or is less than 1.0 mmol/L [40 mg/dL] for male and 1.3 mmol/L [50 mg/dL] for female) are regarded as MetS positive. Participants with severe or acute cardiovascular diseases, post-stroke, neuromusculoskeletal illness, dementia or mental disorders, acute medical illness, symptomatic heart or lung diseases, osteoarthritis or pulmonary illness, severe rheumatoid arthritis, smoker and participants who were immobile, or under treatment for metabolic abnormalities were excluded in the study. Subject participation was voluntary and written informed consent was obtained before this study. Human research ethics approval was provided by the Human Subjects Ethics Subcommittee of the Hong Kong Polytechnic University (HSEARS20150205001). A brief chart explaining the methodology has been presented in Figure 3.1.

3.2.1. Selection of subjects and group assignment

In this study, “4RF” was used to identify the subjects with the cluster of all 4 MetS cardiometabolic risk factors (i.e., elevated blood pressure, increased fasting glucose, increased blood triglycerides, and reduced HDL-cholesterol). Subjects without the cluster of all 4 MetS cardiometabolic risk factors were identified as "N4RF”. In addition, central obesity was specifically investigated as an independent factor. Subjects with central obesity were identified as "O", whereas subjects without central obesity were indicated as "NO”. All subjects were divided into 4 groups including subjects with none of the MetS risk factors (N4RF_NO; n=20), subjects without the cluster of 4 MetS risk factors but with central obesity (N4RF_O; n=35), subjects with the cluster of 4 MetS risk factors but without central obesity (4RF_NO; n=9), and subjects with the cluster of 4 MetS risk factors and central obesity (4RF_O; n=19).

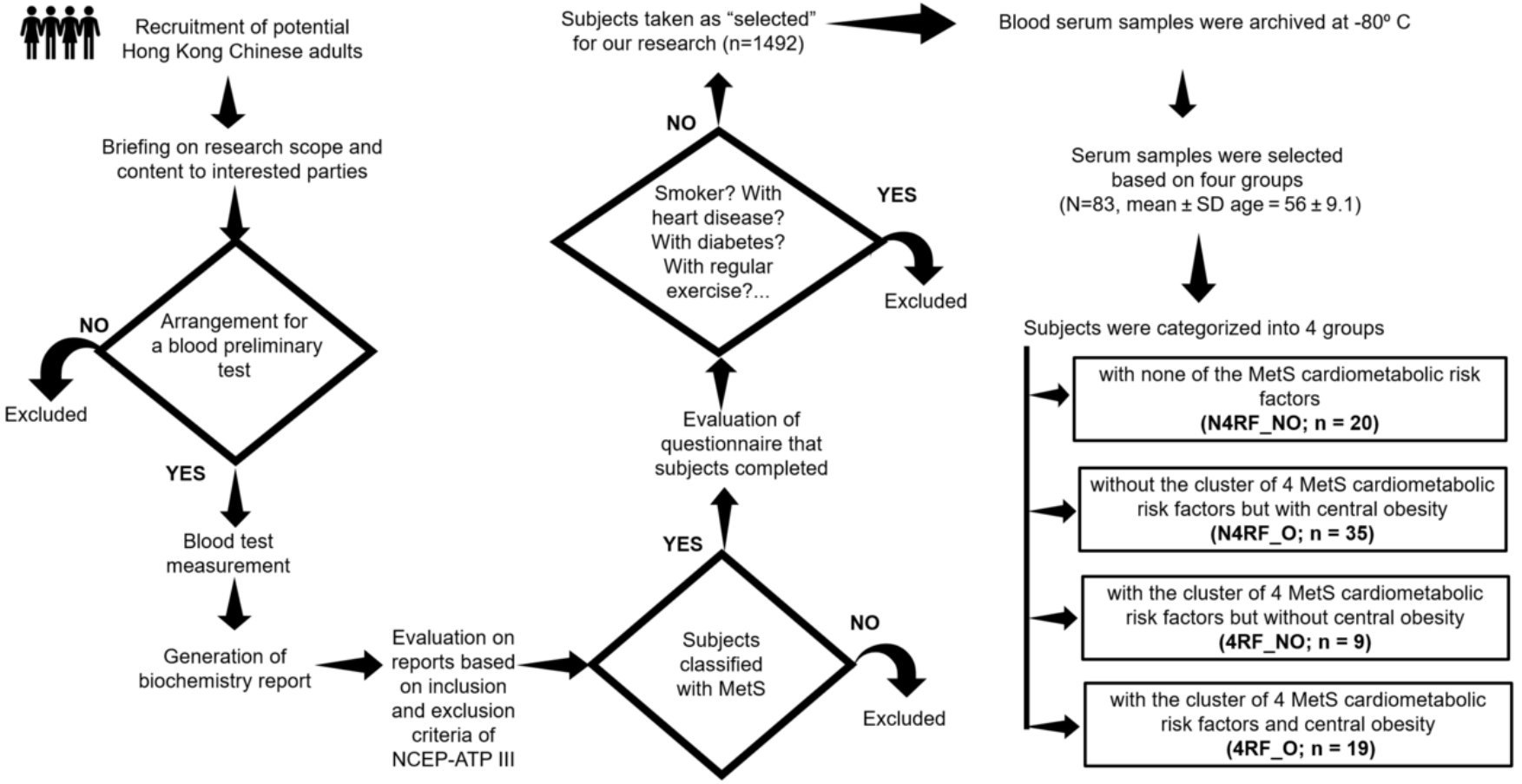

Figure 3.1: Brief flowchart of the methodology involved in the study design

3.2.2. Measurements of cardiometabolic risk factors of MetS

All MetS diagnostic parameters were measured by trained research personnel. For central obesity, the waist circumference was measured using inelastic measuring tape on the bare skin region between the lowest rib and superior border of the iliac crest. Systolic and diastolic blood pressure were measured using an electronic blood pressure monitor (Accutorr Plus, Datascope) over the brachial artery region on the right arm after 5 minutes of rest, and the arm was supported at the level of the heart with the use of an appropriately sized cuff. Fasting venous blood samples were collected after at least 10 hours of fasting by certified phlebotomists. Fasting blood glucose, blood triglycerides, and blood HDL-cholesterol were determined using an automatic clinical chemistry analyser (Architect CI8200, Abbott Diagnostics) in an accredited medical laboratory.

3.2.3. Measurements of adipokines and insulin

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to perform the biochemical measurements of adipokines and insulin in the blood samples according to the manufacturer's instructions (Visfatin kit was from BioVision; chemerin, PAI-1, resistin, CCL-2, IL-6, IL-8, IL-10 and TNF-α kits were from R&D; leptin, adiponectin and insulin kits were from Thermo Fisher Scientific). The coefficient of variability (CV) for the ELISA kits were shown as follows: visfatin (intra-assay: 4.4 - 8 %; inter-assay: 8.2 %), chemerin (intra-assay: 3.9 %; inter-assay: 7.3 %), PAI-1 (intra-assay: 6.8 %; inter-assay: 7 %), resistin (intra-assay: 4.7 %; inter-assay: 8.4 %), CCL-2 (intra-assay: 5 %; inter-assay: 5.1 %), IL-6 (intra-assay: 2.6 %; inter-assay: 4.5 %), IL-8 (intra-assay: 4.7 - 6.7 %; inter-assay: 5.8 - 7.7 %), IL-10 (intra-assay: 3.7 %; inter-

assay: 6.9 %), TNF-α (intra-assay: 4.9 - 7.8 %; inter-assay: 4.7 - 5.8 %), leptin (intra-assay: 3.9%; inter-assay: 5.3%), adiponectin (intra-assay: 3.8%; inter-assay: 5.5%), and insulin (intra-assay: 4.8 - 6%; inter-assay: 8.1 - 9%). All measurements were performed in duplicates or triplicates. Seven controls (one blank, two at lower concentrations, two at medium concentrations, and two at higher concentrations) were also quantified in duplicates to check for the reproducibility of measurements and confirm acceptable reproducibility.

3.2.4. Statistical analysis

Data are expressed as the mean ± standard deviation (SD). The generalized estimating equation (GEE) was adopted to analyse the non-normal distribution of the data regarding the main effect of central obesity, the main effect of the cluster of all 4 MetS risk factors, and the interaction effect between central obesity and the cluster of all 4 MetS risk factors. The Kruskal-Wallis test followed by post hoc tests with Dunn-Bonferroni correction were used to analyse multiple group-wise comparisons. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 22 for Windows. Statistical significance was accepted at $P < 0.05$.

3.3. Results and Discussion

Participant's baseline characteristics comparison

No significant differences were observed in sex and age among all the four groups. Baseline comparisons of all the five MetS risk factors between groups were also calculated. The summary table of the participant characteristics is presented in Table 3.1.

Circulatory levels of TNF-α and leptin exacerbated, and adiponectin decreased in people with central obesity and the cluster of other 4 MetS risk factors

Interaction effects between central obesity and the cluster of the other 4 MetS cardiometabolic risk factors were found for TNF-α (Wald chi square = 5.47, P = 0.019; Figure 3.2 A), leptin (Wald chi square = 4.92, P = 0.027; Figure 3.2 B) and adiponectin (Wald chi square = 9.17, P = 0.002; Figure 3.22 C).

Table 3.1: Baseline characteristics of gender, age and metabolic risk factors in the 4 groups

1) subjects with none of the cardiometabolic risk factors (N4RF_NO; n = 20), 2) subjects with only central obesity without the other 4 MetS cardiometabolic risk factors (N4RF_O; n = 35), 3) subjects without central obesity but with the other 4 MetS cardiometabolic risk factors (4RF_NO; n = 9), and 4) subjects with all five MetS cardiometabolic risk factors (4RF_O; n = 19). The data are expressed as the mean ± standard deviation. Statistical significance was accepted at $P < 0.05$.

	Group 1 (N4RF_NO) N=20	**Group 2 (N4RF_O) N=35**	**Group 3 (4RF_NO) N=9**	**Group 4 (4RF_O) N=19**		**P value**
Gender	17 Females 3 Males	19 Females 6 Males	5 Females 4 Males	21 Females 7 Males		0.402
Age (Years)	62 ± 6	58 ± 11	65 ± 5	65 ± 11		0.135
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group Comparisons**	
Diastolic blood pressure (mmHg)	71.5 ± 7	71.5 ± 6	82.4 ± 9	83.9 ± 12.5		
Systolic blood pressure (mmHg)	123 ± 7.7	122 ± 6.3	170.2 ± 11.2	159 ± 17.2		
Fasting glucose (mmol/L)	4.9 ± 0.4	5.1 ± 0.3	6.3 ± 0.8	6.8 ± 1.3	1-3 1-4 2-3 2-4	<0.05 <0.05 <0.05 <0.05
Blood triglycerides (mmol/L)	0.9 ± 0.3	1.1 ± 0.3	2.1 ± 0.4	2.5 ± 1.0		
Blood high density lipoprotein-C (mmol/L)	1.8 ± 0.4	1.7 ± 0.3	1.0 ± 0.2	1.0 ± 0.2		
Waist circumference (cm)	72.9 ± 7	86.6 ± 5	80.6 ± 4.5	92.6 ± 10.2	1-2 1-4 2-3 2-4	<0.05 <0.05 <0.05 <0.05

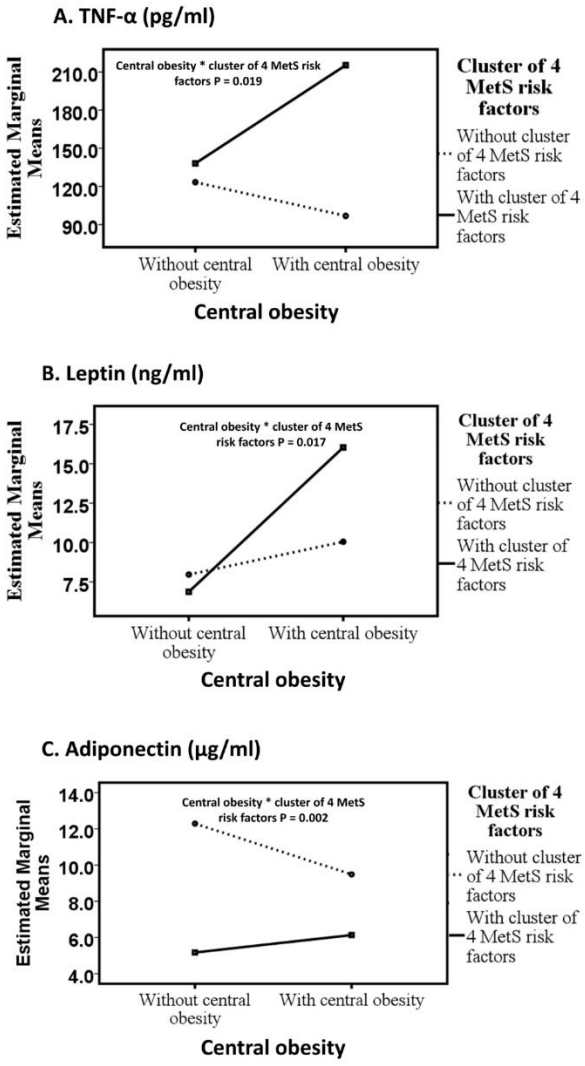

Figure 3.2: The interaction of central obesity with the cluster of the other 4 MetS risk factors on adipokines

The line graphs represent the directions of interaction effect of central obesity and the cluster of other 4 MetS risk factors (high fasting blood glucose, high triglycerides, low HDL and high systolic and diastolic blood pressure) on adipokines including TNF- α (A), leptin (B) and adiponectin (C) in Hong Kong Chinese women categorized into four groups: 1) subjects with none of the cardiometabolic risk factors (N4RF_NO; n = 20), 2) subjects with only central obesity without the other 4 MetS cardiometabolic risk factors (N4RF_O; n = 35), 3) subjects without central obesity but with the other 4 MetS cardiometabolic risk factors (4RF_NO; n = 9), and 4) subjects with all five MetS cardiometabolic risk factors (4RF_O; n = 19). The data are expressed in estimated marginal means. Statistical significance was accepted at $P < 0.05$.

Furthermore, the four-group comparisons indicated that the 4RF_O group had a 74 % higher TNF-α level (mean difference = 92 pg/ml, P = 0.048), 101.3 % higher leptin level (mean difference = 8 ng/ml, P < 0.001), and 50 % lower adiponectin level (mean difference = - 6.2 µg/ml, P < 0.001) than the N4RF_NO group (i.e., difference between central obese subjects with 4RF and non-central obese subjects without 4RF). The 4RF_O group showed a 122 % higher TNF-α level (mean difference = 118.4 pg/ml, P < 0.001) and 35.3 % lower adiponectin level (mean difference = - 3.4 µg/ml, P = 0.006) compared with the N4RF_O group (i.e., difference between the presence and the absence of the cluster of 4 cardiometabolic risk factors in central obese subjects). The 4RF_O had a 133 % higher leptin level (mean difference = 9.2 ng/ml, P = 0.023) compared with the 4RF_NO group (i.e., the difference between central obesity and non-central obesity in the 4RF subjects). The 4RF_NO group had a 57 % lower adiponectin level (mean difference = - 7.1 µg/ml, P < 0.001) than the N4RF_NO group (i.e., the difference between the presence and the absence of the cluster of 4 cardiometabolic risk factors in non-central obese subjects) (Figure 3.3 A, 3 B and 3.4 A).

Circulatory levels of insulin, chemerin, IL-6 and PAI-1 exacerbated in people with only central obesity

The main effects of central obesity were observed on insulin (Wald chi square = 17.4, P < 0.001, Figure 3.5), chemerin (Wald chi square = 4.7, P = 0.031, Figure 3.3 C), IL-6 (Wald chi square = 7.6, P = 0.001, Figure 3 E), and PAI1 (Wald chi square = 5.9, P = 0.016, Figure 3.3 F). We found increases (mean difference ± standard error) in the insulin (78.5 ± 18.8 pg/ml), chemerin (117.2

± 54.2 ng/ml), PAI-1 (604.7 ± 250.1 pg/ml) and IL-6 (7.7 ± 2.8 pg/ml) levels in obese subjects compared with non-obese subjects (Figure 3.6 A, B, C and D).

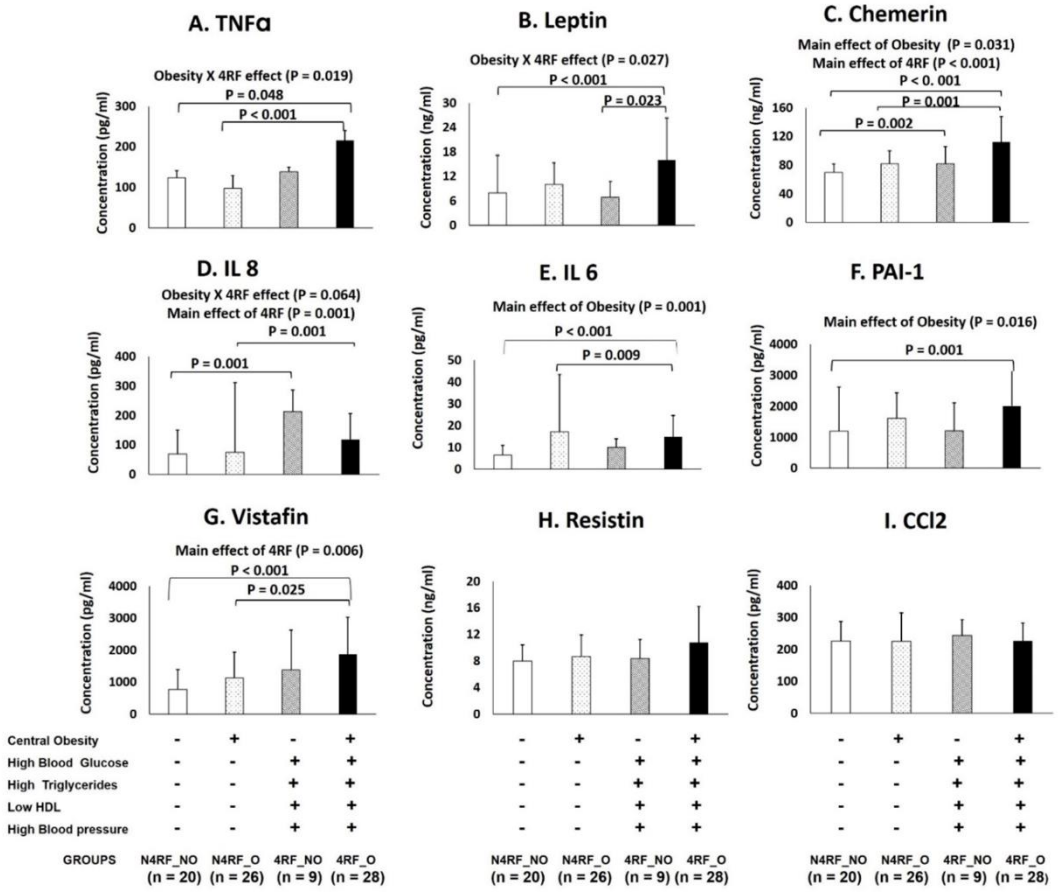

Figure 3.3: Pro-inflammatory adipokines

The bar graphs (A-I) represent the serum concentrations of pro-inflammatory adipokines including TNF- α (A), leptin (B), chemerin (C), IL-8 (D), IL-6 (E), PAI-1 (F), visfatin (G), resistin (H), and CCL2 (I) in the following 4 groups: 1) subjects with none of the cardiometabolic risk factors (N4RF_NO; n = 20), 2) subjects with only central obesity without the other 4 MetS cardiometabolic risk factors (N4RF_O; n = 35), 3) subjects without central obesity but with the other 4 MetS cardiometabolic risk factors (4RF_NO; n = 9), and 4) subjects with all five MetS cardiometabolic risk factors (4RF_O; n = 19). The five MetS cardiometabolic risk factors include central obesity, high fasting blood glucose, high triglycerides, low HDL and high systolic and diastolic blood pressure. The data are expressed as the mean ± standard deviation. Statistical significance was accepted at $P < 0.05$.

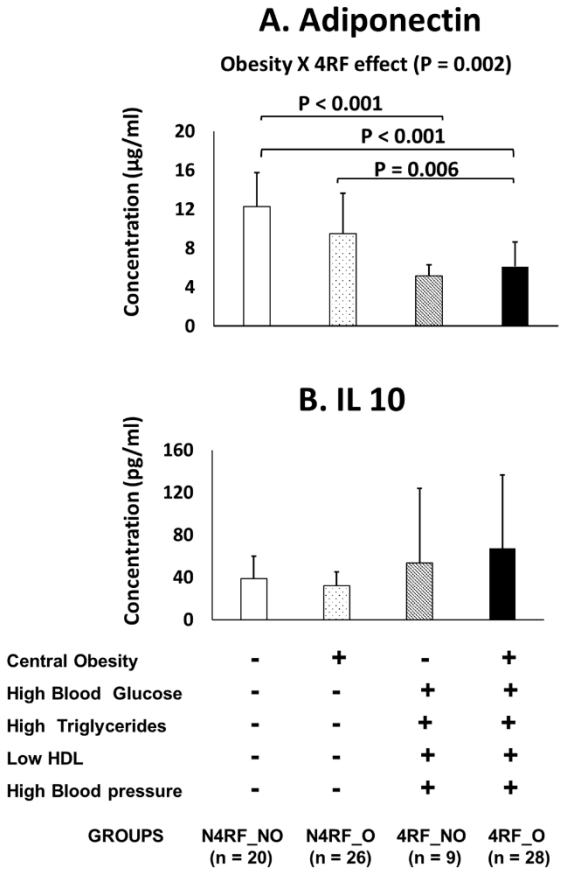

Figure 3.4: Anti-inflammatory adipokines

The bar graphs (A and B) represent the serum concentrations of anti-inflammatory adipokines including adiponectin (A) and IL-10 (B) in the following 4 groups: 1) subjects with none of the cardiometabolic risk factors (N4RF_NO; n = 20), 2) subjects with only central obesity without the other 4 MetS cardiometabolic risk factors (N4RF_O; n = 35), 3) subjects without central obesity with the other 4 MetS cardiometabolic risk factors (4RF_NO; n = 9), and 4) subjects with all five MetS cardiometabolic risk factors (4RF_O; n = 19). The five MetS cardiometabolic risk factors include central obesity, high fasting blood glucose, high triglycerides, low HDL and high systolic and diastolic blood pressure. The data are expressed as the mean ± standard deviation. Statistical significance was accepted at $P < 0.05$.

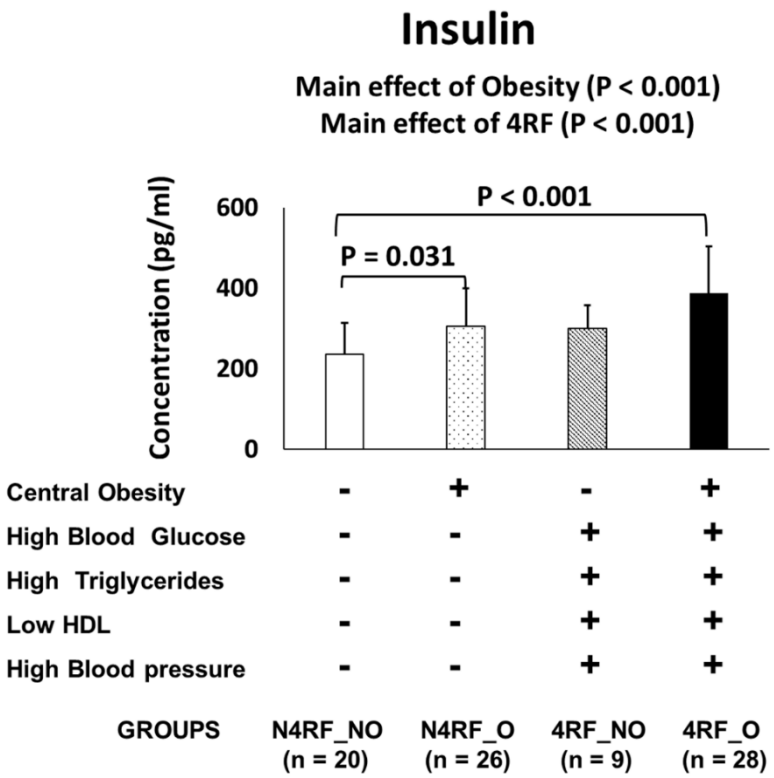

Figure 3.5: Insulin concenteration in the groups

The bar graph represents the serum concentration of insulin in the following 4 groups: 1) subjects with none of the cardiometabolic risk factors (N4RF_NO; n = 20), 2) subjects with only central obesity without the other 4 MetS cardiometabolic risk factors (N4RF_O; n = 35), 3) subjects without central obesity but with the other 4 MetS cardiometabolic risk factors (4RF_NO; n = 9), and 4) subjects with all five MetS cardiometabolic risk factors (4RF_O; n = 19). The five MetS cardiometabolic risk factors include central obesity, high fasting blood glucose, high triglycerides, low HDL and high systolic and diastolic blood pressure. The data are expressed as the mean ± standard deviation. Statistical significance was accepted at $P < 0.05$.

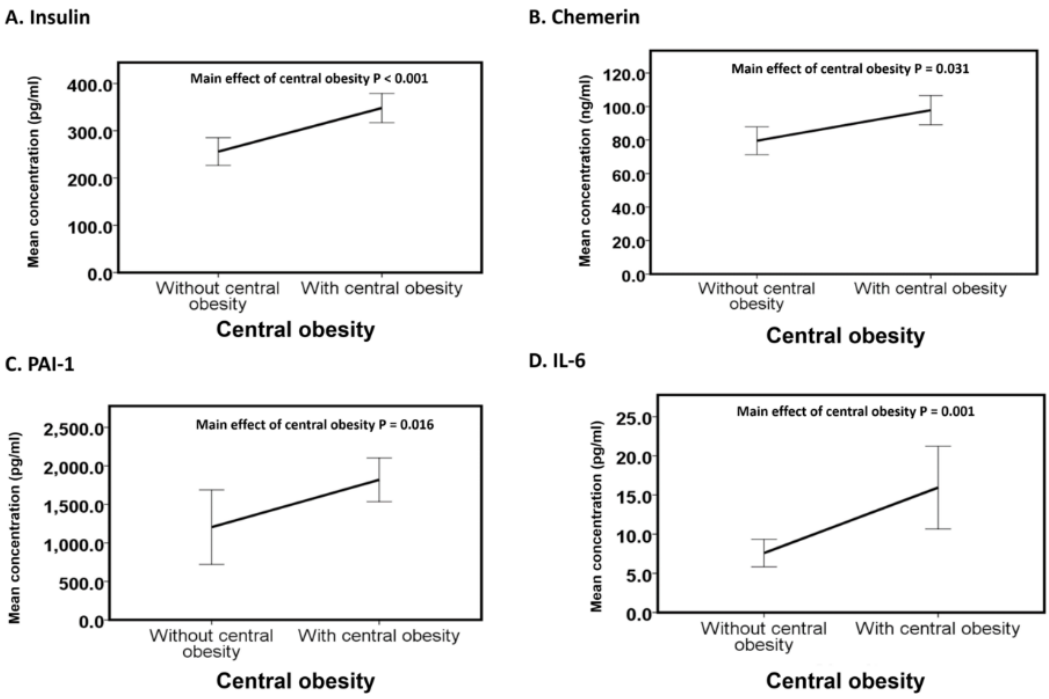

Figure 3.6: Main effect of obesity on adipokines

The line graphs (A-D) represent the means of insulin, chemerin, IL-6, and PAI1 of subjects without central obesity (n = 29) versus subjects with central obesity (n = 54) irrespective of the presence or absence of the cluster of 4 MetS risk factors (high fasting blood glucose, high triglycerides, low HDL and high systolic and diastolic blood pressure. The data are expressed as the mean ± 1 standard deviation. Statistical significance was accepted at $P < 0.05$.

Circulatory levels of insulin, chemerin, IL-8 and Visfatin exacerbated in people with only the cluster of other 4 MetS risk factors

The main effects of the cluster of 4 MetS cardiometabolic risk factors were observed on insulin (Wald chi square = 15.1, $P < 0.001$, Figure 3.4), chemerin (Wald chi square = 32.3, $P < 0.001$ Figure 2C), IL-8 (Wald chi square = 10.4, $P = 0.001$, Figure 3.2D), and visfatin (Wald chi square = 7.6, $P = 0.006$, Figure 2G). We found increases (mean difference ± standard error) in the insulin (73 ± 18.8 pg/ml), chemerin (30.8 ± 54.2 ng/ml), IL-8 (91.3 ± 28.3 pg/ml) and visfatin

(677.7 ± 245.6 pg/ml) levels in subjects with the cluster of all 4 MetS cardiometabolic risk factors compared with subjects without the cluster of 4 MetS cardiometabolic risk factors (Figure 3.7 A, B, C & D).

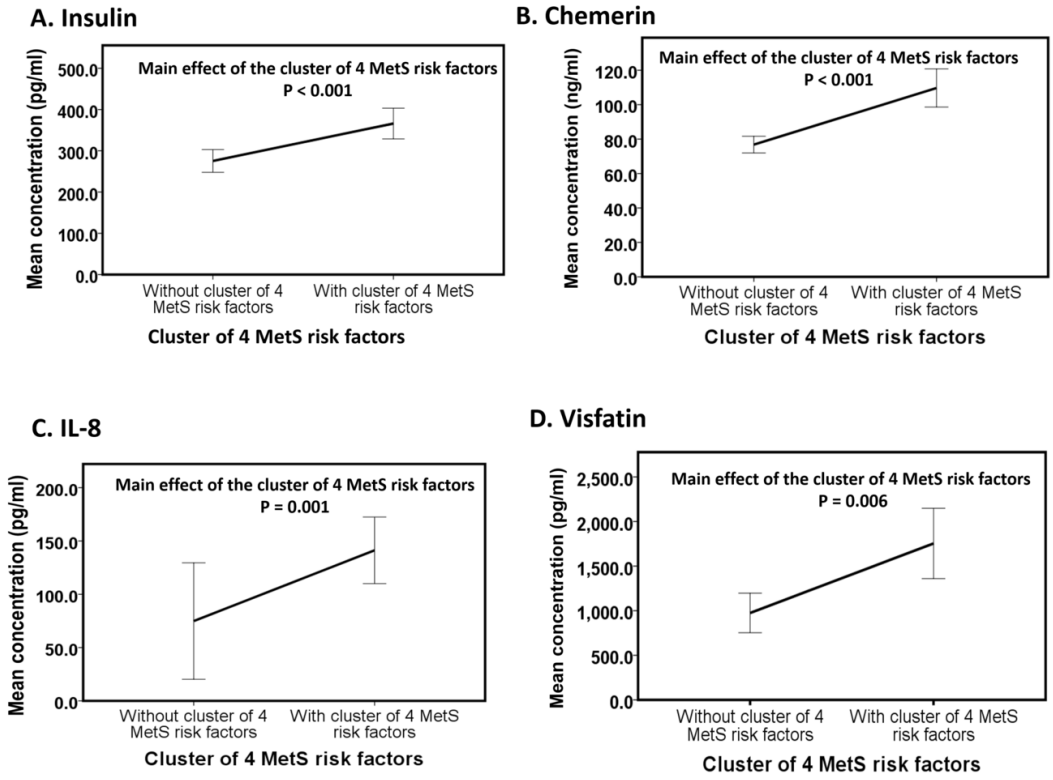

Figure 3.7: Main effect of the cluster of 4 MetS risk factors on adipokines

The line graphs (A-D) represent the means of insulin, chemerin, IL-8, and visfatin of subjects without the cluster of 4 MetS risk factors (high fasting blood glucose, high triglycerides, low HDL and high systolic and diastolic blood pressure) (n = 46) versus subjects with the cluster of 4 MetS risk factors (n = 37) irrespective of the presence or absence of central obesity. The data are expressed as the mean ± 1 standard deviation. Statistical significance was accepted at $P < 0.05$.

3.4. Discussion and Conclusion

Metabolic and cardiovascular complications are major obesity-associated burdens, and thus recognising obese individuals at threat for MetS is a foremost healthcare priority. The enduring worldwide obesity epidemic is a significant

threat to the population and healthcare systems owing to the related morbidity and high costs [470]. The present study was designed to examine the interaction of central obesity and the clustering of the other 4 MetS risk factors (including high blood pressure, high triglycerides, high fasting blood glucose and reduced HDL cholesterol) on circulatory proinflammatory and anti-inflammatory adipokines. Our results indicated that the interaction effects between central obesity and the cluster of other 4 MetS cardiometabolic risk factors existed for TNF-α, adiponectin and leptin. TNF-α increased, and adiponectin decreased in centrally obese subjects that exhibited a clustering of 4 risk factors compared to central obese subjects without this clustering. Leptin was increased in centrally obese subjects, with the clustering of 4 MetS risk factors compared with subjects with no 4 MetS risk factors. For subjects with the cluster of 4 risk factors, leptin was increased in centrally obese subjects when compared to non-obese subjects.

Exacerbated circulatory proinflammatory (TNF-α and leptin), and decreased anti-inflammatory (adiponectin) adipokine levels in people with central obesity and the clustering of other 4 MetS risk factors might be associated with the risk of type 2 diabetes

TNF-α is a cell signalling protein that is responsible for metabolic imbalances such as insulin resistance and altered lipid and carbohydrate metabolism [471,472]. For subjects with central obesity, the clustering of 4 risk factors was associated with an increase in TNF-α concentration. Although the exact molecular mechanisms that link obesity to other MetS risk factors remains unclear, TNF-α has been demonstrated to be associated with MetS risk factors [473,474]. TNF-

α has been reported to be increased in proportion to the number of the MetS components [473,475]. Increases in circulating TNF-α has also been suggested to be related to insulin resistance and augmented systemic inflammation [476,477]. Apart from insulin resistance, obesity-associated hypertension led to an increase in TNF-α in a French Canadian cohort [475]. Similarly, subjects with severe hyperinsulinaemia, hypertriglyceridemia, high body mass index, and low HDL-cholesterol were reported to have high circulating TNF-α levels [473,478]. Increases in circulating TNF-α are associated with peripheral insulin resistance, increased plasma glucose levels and insulin levels before the onset of type 2 diabetes, whereas the increased fasting glucose level induced by obesity can be prevented by TNF-α deficiency [473].

In addition, our data revealed the interaction effect between central obesity and the clustering of the other 4 MetS risk factors on adiponectin and leptin. We observed relatively lower circulating adiponectin in centrally obese subjects with the clustering of 4 MetS risk factors as compared to the subjects with only central obesity, with the clustering of 4 MetS risk factors or without any MetS risk factors. Consistent with our study, a low circulating adiponectin level has been previously shown to correlate with an increase in MetS risk factors [479]. Similarly, hypoadiponectinemia has been suggested to place MetS patients at a higher risk of developing type 2 diabetes [480]. Adiponectin activates 5' adenosine monophosphate-activated protein kinase (AMPK) [481], which regulates lipogenesis and cholesterol synthesis [482]. Cross-sectional studies have demonstrated that hypoadiponectinemia might be an independent risk factor for hypertension [483,484]. The renin-angiotensin system (RAS) plays an

important role in the regulation of blood pressure and cardiovascular function. This system is activated in MetS and leads to arterial wall inflammation, elevation of the angiotensin II level, and oxidative stress [485,486]. Suppressing RAS by elevating adiponectin has been proposed as an effective strategy for treating MetS [487].

In addition to adiponectin, we also observed relatively higher leptin in centrally obese subjects with the cluster of 4 MetS risk factors as compared to the subjects with only central obesity, with the clustering of 4 MetS risk factors or without any MetS risk factors. Consistently, high circulating leptin level have been observed in people with MetS risk factors compared with people without MetS risk factors [488]. The exacerbation in the leptin level in obese patients with the cluster of 4 risk factors indicates that leptin may be involved in the severity of MetS and may lead to type 2 diabetes [489,490] associated with central obesity. The inclined pattern of serum leptin has been reported in MetS. Specifically, associations between leptin and other risk factors of MetS ($r \geq \pm 0.1$) and body mass index ($r \geq \pm 0.5$) for both sexes have been established [491]. In contrast, serum leptin has been reported to be increased in proportion to the number of MetS risk factors, regardless of the obesity status of the subjects [492]. The controversial results are explainable by the complexities of the definition of MetS; for instance, an identical number of MetS risk factors might not indicate that the subjects would share precisely the same cardiometabolic risk factor profiles and characteristics.

The interaction between the components of the clinical phenotype (e.g., central obesity, insulin resistance, hypertension, and dyslipidaemia) of MetS might contribute to the development of a pro-inflammatory state [493]. Previous studies have suggested that the anti-inflammatory: pro-inflammatory adipokine equilibrium is important for preventing MetS, especially in central obese subjects [31]. For instance, a low adiponectin: leptin ratio indicates the severity of MetS in patients with central obesity compared with people without MetS [467]. Additionally, the adiponectin: leptin ratio has been shown to correlate with variations in systolic blood pressure, insulin sensitivity, total cholesterol and low-density lipoprotein in MetS patients with obesity [467]. Consistently, our results also indicate the interaction effect between central obesity and the clustering of 4 MetS risk factors on the adiponectin: leptin ratio ($P = 0.016$) (data not shown). Physiologically, a disproportion in the expression of anti-/pro-inflammatory adipokines results in the increased size of adipocytes. These hypertrophied adipocytes result in a shift towards the dominance of the pro-inflammatory adipokines [494]. The imbalance in the production of pro-inflammatory and anti-inflammatory biomolecules precedes increased immune cell infiltration and the induction of a macrophage phenotype switch in visceral adipose tissue [495]. We propose that the interaction between central obesity and the clustering of all 4 MetS risk factors would favour a dominance of pro-inflammatory adipokines that might be involved in increasing the severity of MetS.

Elevated circulating levels of insulin, chemerin, PAI-1 and IL-6 might represent the first-line adipokines that initiate subsequent inflammatory cascades in people with only central obesity

The main effect of central obesity was observed in insulin, PAI-1, chemerin and IL-6. Interestingly, all 4 biomarkers were shown to be augmented during the differentiation of preadipocytes to adipocytes. Hyperinsulinaemia enhances the differentiation of preadipocytes to adipocytes, which is a major contributor to the increased PAI-1 in obese subjects [496]. Chemerin is a chemoattractant recruiting macrophages to adipose tissue. Furthermore, adipocytes serve as both secretary cells of chemerin and target for autocrine chemerin signaling, regulation of adipogenesis and adipocyte differentiation [497]. Similarly, several studies have reported that serum IL-6 is elevated in obese patients [3] and that IL-6 is highly expressed in preadipocytes compared with the mature adipocytes of obese mice [498]. Our results show that increases in adipokines (PAI-1, IL-6, and chemerin), which favour the differentiation of preadipocytes attributed to the effect of central obesity, might represent the first-line adipokines that initiate subsequent inflammatory cascades in people with only central obesity.

Elevated circulating levels of insulin, chemerin, IL-8 and visfatin in people with only the clustering of 4 MetS risk factors might result from the migration and activation of macrophages

On the other hand, insulin, chemerin, IL-8 and visfatin demonstrated the main effect of the clustering of 4 MetS risk factors. Insulin resistance has been reported as a combination of macrophages accumulation that secretes proinflammatory adipocytokines and altered outcome of insulin target cells

[499]. Notably, in this context, the primary sources of the above adipokines are macrophages, implying that migration and activation of macrophages may be the key events that mediate the development of MetS. The migrated macrophages secrete chemotactic cytokines such as IL-8 that further induce macrophage migration to adipose tissues. Visfatin is produced in visceral adipose tissues and has been proposed as the missing link between intra-central obesity and diabetes [313]. Visfatin is secreted by neutrophils and is regulated by pro-inflammatory factors (such as TNF-α and IL-6) in monocytes [500,501]. Likewise, chemerin is a chemokine that recruits macrophages to adipose tissue and play an important role in pro-inflammatory processes such as chemotaxis modulation and dendritic cell and macrophage activation [502]. Researchers have verified that the serum levels of chemerin are higher in MetS patients compared with controls and are correlated with the levels of the MetS components [503]. The adipokines mentioned above have been reported to increase the severity of MetS; however, the adipokine expression levels in the MetS patients with all 4 MetS risk factors have not been reported. Therefore, our results suggest that changes in the insulin, IL-8, visfatin and chemerin levels may indicate the severity of MetS with the clustering of 4 MetS risk factors. These adipokines recruit pro-inflammatory macrophages and, hence, promote the transition from a mild metabolic dysfunction phenotype to a full metabolic dysfunction phenotype [504].

In this study, our findings are based screening insulin and 11 adipokines to examine the interaction of central obesity and the clustering of the other 4 MetS risk factors on circulatory proinflammatory and anti-inflammatory adipokines.

Therefore, a study profiling all adipokines for these clinical and pathophysiological changes to improve early patient identification is suggested. Although the adopted sample size has resulted in a statistical power of 80% or above for most of our significant outcome measures, the constraint of sample size might also be a limitation in the present study. In conclusion, our results revealed the interaction of central obesity and the clustering of the other MetS cardiometabolic risk factors for exacerbating TNF-α and leptin; and reducing adiponectin in the circulation. This combined effect of central obesity with all the 4 MetS risk factors increases the proinflammatory status of adipokines. These altered adipokine patterns (low circulating adiponectin and high TNF-α and leptin) could provide useful information on the additional risk of metabolic derangements. Our results indicate that MetS disorders might become severe in the presence of central obesity. These results also confirm the IDF definition of MetS by placing central obesity as the compulsory risk factor for MetS diagnosis. Further research is needed to fully understand exact roles of adipokines in the progression of MetS and the subsequent chronic diseases such as type 2 diabetes and cardiovascular diseases.

Chapter 4

Complications of the interaction of central obesity and hypertension on circulatory adipokines in adult women

4.1. Introduction

Obesity and hypertension are commonly associated with chronic disorders, including metabolic syndrome, renal disease, stroke, and cardiovascular diseases [186,191,193,505]. According to the obesity paradox, lean individuals with hypertension have a smaller chance of survival than obese people [39,40]. On the other hand, obese hypertensive individuals have a higher mortality rate and an increased risk of cardiovascular diseases compared with non-obese hypertensive subjects [506,507]. Indeed, the inter-relationship between obesity and hypertension is unclear. Very few researchers have tried to examine the subtle differences between the sequelae observed in lean individuals with hypertension versus those in obese counterparts. Obese individuals have been reported to develop more cardiovascular structural abnormalities, whereas obese people with hypertension might have an increased risk of renal insufficiency [186,191,193,505,508]. Nevertheless, when obese individuals become hypertensive, they show a higher mortality rate and an increased risk of ischaemic heart disease compared with non-obese hypertensive subjects [506,507]. Therefore, researchers hypothesized that the higher death rate in obese hypertensive patients compared with only obese people might be the outcome of a combination of several overlapping factors, including the regulation of

appetite and satiety, endothelial function, energy expenditure, haemostasis, insulin sensitivity, blood pressure, adipogenesis, fat distribution and insulin secretion in pancreatic β-cells, that have been attributed to obesity and hypertension [509].

Most of the studies in the past few years have focused on the differences between obese hypertensive subjects and lean normotensive subjects or obese normotensive subjects [509–511]. For example, it has been shown that it is more difficult to control blood pressure and end-organ damage in obese individuals with hypertension compared with their non-obese counterparts [512]. Several mechanisms, including neurohumoral pathways, the function of adipose tissue derivatives (adipokines and cytokines), metabolic functions and the modulation of pressor/depressor mechanisms, have been proposed [513]. Simulation of the renin-angiotensin system (RAS) has been introduced as one of the essential mechanisms in obesity-related hypertension [189,514]. In obesity, exacerbation of the RAS components of adipocytes leads to systemic RAS consequences [191], including dysregulation of renal vasomotor activity, uncontrolled tissue growth in the kidneys, and disruption of optimal salt and water homeostasis [515]. Diet-induced obesity upregulates RAS in adipose tissue that increases reactive oxygen species, elevates lipogenesis, impairs insulin signaling, and promotes inflammation in the circulation [189,191,514]. Thus, the activation of RAS in the circulation represents a link between inflammation during obesity and hypertension [191].

Intriguingly, adipokines have been shown to be associated with RAS. Several lines of evidence indicate that the adipose tissue is a large endocrine organ, which secretes biologically active substances called adipokines, such as adiponectin, plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor-α (TNF-α), and leptin [516]. A RAS blockade by an angiotensin-converting enzyme inhibitor and angiotensin II receptor blocker has been demonstrated to increase plasma adiponectin and to improve differentiation and expression of adiponectin in human pre-adipocytes in subjects with metabolic syndrome [517]. Leptin has been shown to upregulate RAS and pro-inflammatory cytokines, as well as to mediate angiotensin II-elicited hypertension in rats fed a high-fat diet for 3 weeks [28]. Furthermore, an increase in PAI-1, which is a pro-inflammatory adipokine involved in the coagulation system, is associated with RAS and cardiovascular risk factors, such as hypertension, obesity, insulin resistance and diabetes [29,30]. TNF-α, which is a well-known pro-inflammatory cytokine with a wide range of biological effects, has also been exhibited to be involved in functional crosstalk with angiotensin II that causes adverse left ventricular remodeling and hypertrophy in hypertension [518].

There are sex-specific differences in the prevalence of hypertension and obesity. The association of obesity with prehypertension and hypertension are stronger in young women than in men [519]. Similarly, in adult women, waist-to-hip ratios are associated with cardiovascular risk factors and hypertension more than obese men [520]. A study examining 813 Hong Kong people aged 51 ± 12 years (393 men and 420 women) also confirmed that elevated blood pressure is related to increased waist circumference, especially in women [521]. Therefore,

this study was designed to differentiate the effects of central obesity and hypertension by examining the profile of adipokines that have focused separately on obesity and hypertension (adiponectin, PAI-1, leptin, and TNF-α) in middle-aged Hong Kong Chinese women. We hypothesized that the interaction between central obesity and hypertension would exacerbate the adipokine equilibrium by upregulating the pro-inflammatory adipokines and downregulating the anti-inflammatory adipokine.

4.2. Materials and Methods

4.2.1. Study design

This is a cross-sectional study in which blood samples from a total of 387 women (mean age ± SD = 58 ± 11 years) were selected from a pool of 1,492 Hong Kong Chinese adults who were previously screened for metabolic syndrome using the United States National Cholesterol Education Program (NCEP) Expert Panel Adult Treatment Panel (ATP) III guidelines [2].

Subjects with central obesity (i.e., a waist circumference ≥80 cm based on NCEP ATP III criteria [2]), with hypertension (i.e., a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg based on the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [13]) and with central obesity and hypertension were selected for this study.

In this study, the subjects with a high fasting blood glucose of ≥ 5.5 mmol/L, high plasma triglycerides ≥ 1.70 mmol/L and low high-density lipoprotein (HDL) cholesterol (HDL-C ≤ 1.3 mmol/L were excluded. This study design allowed us to supply an unambiguous interpretation solely based on the individual and

interaction effects of central obesity and hypertension, not confounded by other metabolic syndrome risk factors, including hyperglycemia, hypertriglyceridemia, and reduced HDL cholesterol. Participants with neuromusculoskeletal illness, post-stroke, severe or acute cardiovascular diseases, dementia or mental disorders, symptomatic heart or lung diseases, acute medical illness, osteoarthritis or pulmonary illness, smoker, severe rheumatoid arthritis, and participants who were under treatment for metabolic abnormalities or were immobile were excluded in this study [452]. Subject participation was voluntary and written informed consent was obtained before this study.

4.2.2. Subject selection

Subjects were divided into four groups: 1. Non-central obese subjects with normal blood pressure (NO_NBP; n = 105), 2. Non-central obese subjects with hypertension (NO_HBP; n = 102), 3. Central obese subjects with normal blood pressure (O_NBP; n = 74), and 4. Central obese subjects with hypertension (O_HBP; n = 106). All subjects participated voluntarily, and their written informed consent was obtained before this study began. Human research ethics approval was given by the Human Subjects Ethics Subcommittee of Hong Kong Polytechnic University (HSEARS20150205001). All methods were performed in accordance with the relevant guidelines and regulations.

4.2.3. Measurements of cardiometabolic risk factors of metabolic syndrome

All the metabolic syndrome diagnostic parameters were measured by trained research personnel. Electronic blood pressure monitor (Accutorr Plus, Datascope) was used to measure systolic, and diastolic blood pressure over the brachial artery region on the right arm supported at heart level after 5 minutes of rest with the use of appropriate sized cuffs. The inelastic measuring tape was used on the open skin region between the lowest rib and superior border of the iliac crest to measure waist circumference. Venous blood samples were collected by certified phlebotomists from subjects after at least 10 hours of fast. An automatic clinical chemistry analyzer (Architect CI8200, Abbott Diagnostics) in an accredited medical laboratory was used to determine fasting blood glucose, blood HDL-cholesterol, and blood triglycerides.

4.2.4. Measurements of adipokines and insulin

Commercially available ELISA kits were used to perform biochemical measurements for adipokines and insulin in the harvested serum samples according to the manufacturer's instructions. Adiponectin, PAI-1, leptin and TNF-α. ELISA kits were purchased from R&D, and insulin kits were purchased from Thermo Fisher Scientific. The coefficient of variability (CV) for the ELISA kits were: adiponectin (intra-assay: 3.5 %; inter-assay: 7 %), PAI-1 (intra-assay: 6.8 %; inter-assay: 7 %), leptin (intra-assay: 3 %; inter-assay: 4.4 %) and TNF-α (intra-assay: 4.9-7.8 %; inter-assay: 4.7-5.8 %).

4.2.5. Statistical analysis

Data are expressed as the mean ± standard deviation. Generalized estimating equation (GEE) was implemented to analyze the non-normal distributed data

concerning the interaction effect between central obesity and hypertension, the main effect of central obesity, and the main effect of hypertension on adipokines. Kruskal-Wallis test followed by post hoc test with Dunn-Bonferroni correction were used to investigate multiple group-wise comparisons. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 22 for Windows. Data are expressed as a mean ± standard deviation. Statistical significance was accepted at $P < 0.05$.

4.3. Results

Circulatory level of adiponectin dropped and TNF-α exacerbated, in people with obesity and hypertension

Interaction effects of central obesity and hypertension were found on adiponectin (Wald chi square = 10.3, P = 0.001) (Figure 4.1) and TNF-α (Wald chi square = 10.1, P = 0.002) (Figure 4.2A). Compared to the non-obese and normotensive subjects, the main effects of central obesity and hypertension were significantly positive (mean difference = 2.9 µg/ml, and 2.3 µg/ml, P < 0.001) on adiponectin, but the combined effect (central obesity + hypertension) appeared to be equal to the individual effect of central obesity only (mean difference = 2.9, P < 0.001) on adiponectin. Compared to the non-obese and normotensive subjects, the individual effects of central obesity and hypertension were both significantly negative (mean difference = -11.01 pg/ml and -7.50 pg/ml, P < 0.001, respectively) on TNF-α, but the combined effect (central obesity + hypertension) seemed to be more negative (mean difference = -12.67 pg/ml, P < 0.001) than the individual effects of central obesity or hypertension on TNF-α.

Furthermore, the four group comparisons indicated that NO_NBP had 23.8 % (P = 0.002) higher adiponectin level and 98.7 % (P < 0.001) lower TNF-α level than NO_HBP (i.e., the difference between normotensive and hypertensive in non-obese subjects). NO_NBP had 28.7 % higher (P < 0.001) adiponectin level and 146 % lower (P < 0.001) TNF-α level than O_NBP (i.e., the difference between non-obese and central obese in normotensive subjects). NO_NBP had 28.7 % higher (P < 0.001) adiponectin level and 168 % lower (P < 0.001) TNF-α level than O_HBP (i.e., the difference between non-obese normotensive

and central obese hypertensive subjects). NO_HBP had a 34.4 % lower (P < 0.001) TNF-α level as compared to O_HBP (i.e., the difference between non-obese and central obese in hypertensive subjects) (Figure 4.1 and 4.2A).

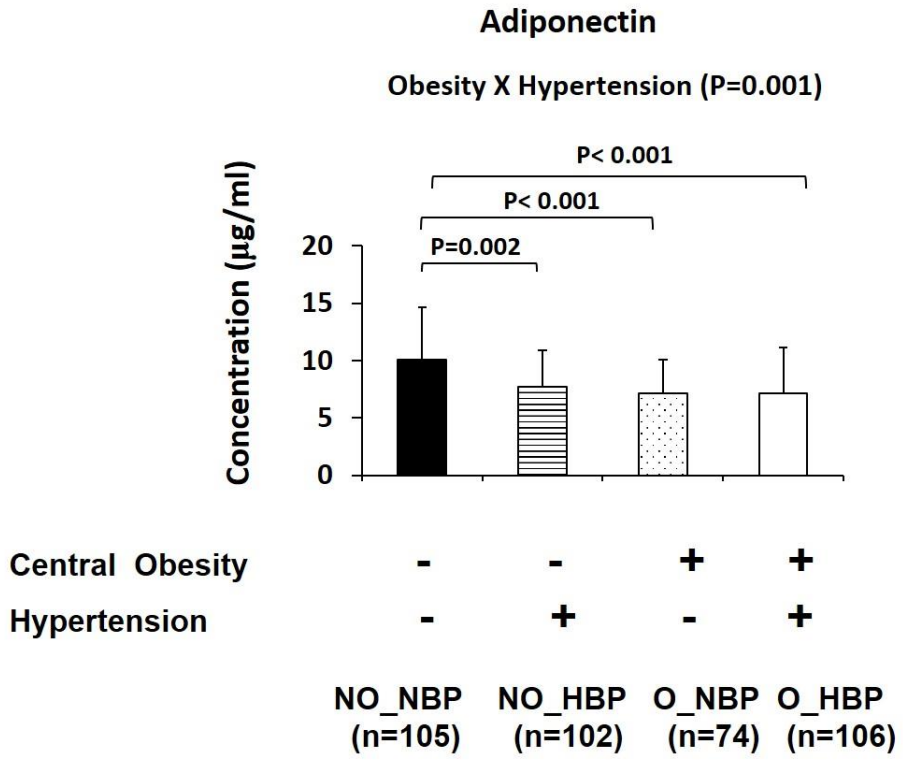

Figure 4.1: Anti-inflammatory adipokine profile for demonstrating distinctive influence of central obesity and hypertension

Bar graph represents circulatory abundance of anti-inflammatory adipokine, adiponectin, in Hong Kong Chinese women categorized into four groups namely: 1) Non-central obese subjects with normal blood pressure (NO_NBP; n = 105), 2) Non-central obese subjects with hypertension (NO_HBP; n = 102), 3) Central obese subjects with normal blood pressure (O_NBP; n = 74), and 4) Central obese subjects with hypertension (O_HBP; n = 106). Subjects with hypertension were defined with systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg, and subjects with central obesity were defined with waist circumference ≥ 80 cm. The data are expressed as mean ± standard deviation. Statistical significance was accepted at P < 0.05.

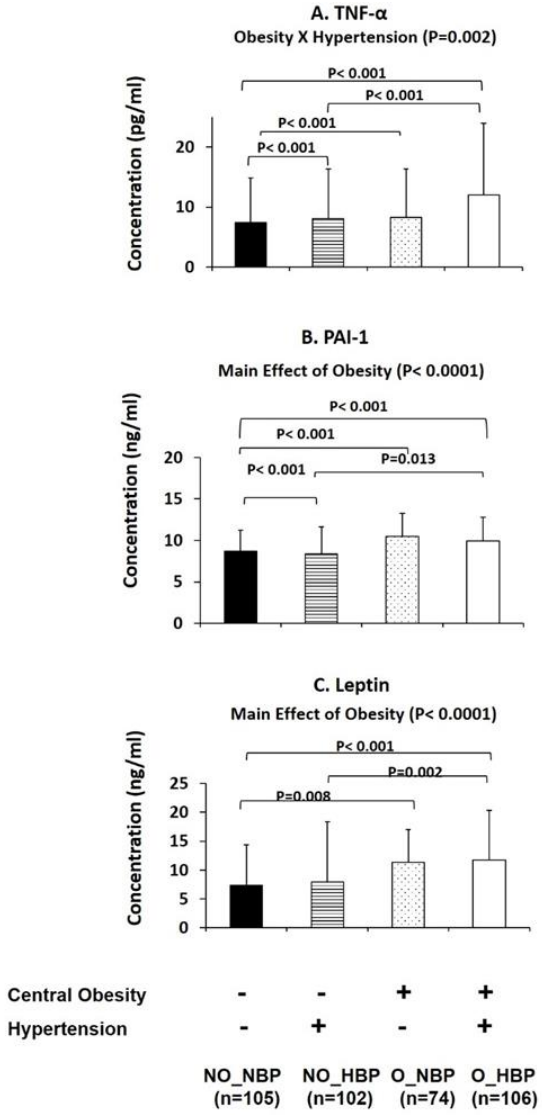

Figure 4.2: Proinflammatory adipokines profile for demonstrating distinctive influence of central obesity and hypertension

Bar graphs represent circulatory abundances of proinflammatory adipokines including PAI-1 (A), TNF-α (B), and leptin (C) in Hong Kong Chinese women categorized into four groups namely: 1) Non-central obese subjects with normal blood pressure (NO_NBP; n = 105), 2) Non-central obese subjects with hypertension (NO_HBP; n = 102), 3) Central obese subjects with normal blood pressure (O_NBP; n = 74), and 4) Central obese subjects with hypertension (O_HBP; n = 106). Subjects with hypertension were defined with systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg, and subjects with central obesity were defined with waist circumference ≥ 80 cm. The data are expressed as mean ± standard deviation. Statistical significance was accepted at $P < 0.05$.

4.3.2. Circulatory level of leptin and PAI-1 exacerbated in people with only central obesity

Main effects of central obesity were observed on PAI-1 (Wald chi-square = 31.7, $P < 0.001$; Figure 4.2B), and leptin (Wald chi-square = 21.1, $P < 0.001$; Figure 4.2C). The means for PAI-1 and leptin were lowered in non-central obese subjects (8.52 ± 0.18 ng/ml and 7.61 ± 0.44 ng/ml, respectively) when compared with central obese subjects (10.19 ± 0.23 ng/ml and 11.53 ± 0.73 ng/ml, respectively) (Figure 4.2B & 2C).

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4.4. Discussion

Obesity and hypertension are commonly associated with chronic disorders, including metabolic syndrome, renal disease, stroke, and cardiovascular diseases [186,191,193,505]. Adipokines have been proposed as the link between obesity and hypertension and are also linked with the pathogenesis of metabolic diseases. There are sex-specific differences in the prevalence of obesity-related hypertension. Obesity-related hypertension is more common in adult women than men [520,521]. Therefore, we attempted to determine the adipokine profile (adiponectin, PAI-1, leptin, and TNF-α) in Hong Kong Chinese middle-aged and older women to differentiate the interaction effects of central obesity and hypertension. Our findings demonstrated that there is a reduction in anti-inflammatory adipokines (adiponectin) and an upsurge of the pro-inflammatory adipokine TNF-α because of the interaction between central obesity and hypertension. These findings are specific to adult women and support the concept that central obesity-related hypertension is disadvantageous because it leads to shifting towards more pro-inflammatory state compared with central obesity or hypertension alone.

Our results showed that central obesity and hypertension interacted to affect the circulatory abundance of adiponectin and TNF-α. The decrease in circulatory adiponectin in subjects with hypertension only (23 %; 7.7 ± 3.0 versus 10.0 ± 4.53 μg/mL) was relatively smaller than obese hypertensive subjects and subjects who were only obese (29 %, 7.1 ± 3.1 and 7.1 ± 4.0 versus 10.0 ± 4.53 μg/mL, respectively) compared with non-obese normotensive subjects. Similar to a study reported that individuals with

hypertension had significantly lower circulatory adiponectin compared with the body mass index-matched normotensive healthy individuals (9.1 ± 4.5 versus 13.7 ± 5.2 μg/mL) [522]. Furthermore, we found an interaction effect between central obesity and hypertension on the circulatory TNF-α level. The increase in the circulatory TNF-α level in hypertensive-only subjects (145 %, 18.4 ± 8.3 versus 7.5 ± 7.3 pg/mL) and in central obese-only subjects (100 %, 15.0 ± 8.0 versus 7.5 ± 7.3 pg/mL) was relatively smaller than obese hypertensive subjects (168 %; 20.12 ± 12.0 versus 7.5 ± 7.3 pg/mL) compared with non-obese normotensive subjects. Obese hypertensive people, as well as adipokines, are associated with renal complications or cardiovascular diseases by mediating endothelial dysfunction, oxidative stress, inflammation and changes in immune response [523]. Therefore, diminution of adiponectin (an anti-inflammatory adipokine) and exacerbation of TNF-α (a proinflammatory adipokine) in obese hypertensive subjects might be associated with severe metabolic disorders such as adverse cardiovascular or renal diseases.

Elevated circulatory TNF-α and decreased adiponectin levels in people with obesity and hypertension might be associated with the increased risk of developing cardiometabolic diseases.

The mechanisms through which central obesity interacts with hypertension are mostly unknown. Some mechanisms underlying the pathogenesis of obesity-related hypertension were proposed based on studies in humans and animals, and these mechanisms include neurohumoral pathways and adipose-tissue derivatives (adipokines and cytokines) in both functional and metabolic

mechanisms. The circulating adiponectin concentration has been shown to be negatively associated with pro-inflammatory cytokines, such as IL-6 and TNF-α [524,525]. Adiponectin shows anti-inflammatory action through multiple mechanisms by inducing changes in phenotype and function of macrophages [526]. The increase in adiponectin has also been shown to inhibit the expression of adhesion molecules and to suppress the adherence of TNF-α-stimulated endothelial cells to monocytes [527]. Interestingly, adiponectin has been shown to decrease the agonist-stimulated production of TNF-α in cultured macrophages, which was accompanied by reduced nuclear factor-κB (NFκB) activation [528]. NFκB is a regulatory molecule that regulates pro-inflammatory cytokines such as TNF-α [529]. TNF-α is a key pro-inflammatory cytokine associated with the pathology of obesity-linked vascular and metabolic disorders. Cross-talk between angiotensin II and pro-inflammatory adipokines (TNF-α) participates in self-amplifying and sustaining positive feedback loops, which results in the progression of hypertension and cardiac remodeling [518]. Mice deficient in the TNF-α gene were found to attenuate the angiotensin-II-induced hypertensive response [530]. Inhibition of TNF-α was also shown to decrease oxidative stress, NF-κB activation and mitogen-activated protein kinases (MAPK) phosphorylation [513]. Collectively, TNF-α induced hypertension and adverse cardiac remodeling via angiotensin II, which was associated with changes in the MAPK / TGF-β / NF-κB pathway [513]. Therefore, according to our results, the interaction between central obesity and hypertension might lead to a reduction in anti-inflammatory adipokines, adiponectin, and further increases in pro-inflammatory adipokines and TNF-α compared with subjects with central obesity or hypertension alone. Our results might provide clues for explaining

the high mortality rate documented in central obese individuals with hypertension. Additional research is needed to further determine the exact relationship between our observed interaction and the modulation of adipokines in cardiovascular health. In overweight and obese subjects, the cardiovascular risk is not significantly increased unless hypertension is present [531].

Elevated circulatory TNF-α and decreased adiponectin levels in people with obesity and hypertension might be associated with the increased risk of developing renal dysfunction and chronic kidney disease.

Activation of the sympathetic nervous system is considered to be an important mechanism among all mechanisms underlying the pathogenesis of obesity-related hypertension [513]. In obese individuals, the arterial pressure controlling the function of natriuresis and diuresis shifts towards a higher level of blood pressure. It is proposed that renal tubular reabsorption is increased in the early stages of obesity. This reabsorption results in primary sodium retention that causes the expansion of extracellular fluid volume and the resettlement of the kidney-fluid apparatus to a hypertensive level, which mimics the developmental model of hypertension [513]. Obesity and hypertension are strongly correlated, and both are major risk factors for renal dysfunction and chronic kidney disease. Studies have shown that only specific adipokines are associated with the development of renal dysfunction and chronic kidney disease [532,533]. Adiponectin has been explored as an independent predictor of moderate chronic kidney disease and is inversely associated with renal dysfunction and chronic kidney disease [532]. The mechanism is still unknown in humans, but the

mechanism has been investigated in mice with hypoadiponectinemia and albuminuria (a pathological condition where the protein albumin is abnormally present in the urine) and demonstrated the link between hypoadiponectinemia and kidney dysfunction. Podocytes (specialized cells) are present in the Bowman's capsule in the kidneys. Podocytes wrap around glomerulus capillaries and form multiple interdigitating foot processes [534–536]. Adiponectin treatment in mice with albuminuria improved the glomerular podocyte foot processes by activating 5' adenosine monophosphate-activated protein kinase (AMPK). This activated AMPK downregulated nicotinamide adenine dinucleotide phosphate oxidase production in the podocytes [537]. Apart from adiponectin, TNF-α has also been associated with the development of renal dysfunction and chronic kidney disease. It is mainly produced by macrophages infiltrating adipose tissue in obesity, but can also be produced in the renal cells of the kidney. In the renal cells, TNF-α synthesis can be stimulated by activation of RAS system [538]. In rats with renal failure, neutralization of TNF-α decreased NFκB activity that was associated with an improvement of nitric oxide released by reduction in renal transforming growth factor beta 1 and endothelin-1 production. Therefore, TNF-α has also been proposed as one of the key adipokines in the progression of renal injury and chronic kidney diseases [533].

Elevated circulating levels of leptin and PAI-1 level in people with central obesity might indicate the risk of hypertension.

Our analyses showed significant main effects of central obesity on circulating leptin and PAI-1. Consistent with our results, leptin has been shown to be

remarkably upregulated with increased fat mass [539]. The circulatory leptin is transported across the blood-brain barrier to a hypothalamic region that controls the transmission of appetite neuropeptides to the peripheral tissues [45]. The main function of leptin is to stimulate sympathetic activity, upregulate thermogenesis, upregulate energy expenditure, and decrease food consumption [540,541]. These effects of leptin are known to be mediated by two main pathways, one driven by a high level of leptin called a positive regulatory action, and the other by an agouti-related peptide, which is an antagonistic ligand activity that stimulates the sympathetic nervous system by activating the hypothalamus-pituitary-adrenal axis [540,541]. Interestingly, in anesthetized animals, systemic administration of leptin has been shown to exert no effect on the heart rate or increase in arterial pressure. Therefore, long-term exposure to hyperleptinemia has been suggested for full expression of the expected renal sympathoexcitation pressor effect [542]. Nevertheless, leptin has been demonstrated to upregulate RAS and pro-inflammatory cytokines and mediate angiotensin II-elicited hypertension after feeding rats a high-fat diet for 3 weeks [28]. Therefore, it is plausible that obesity exerts its effect on leptin and that leptin subsequently participates in increasing blood pressure by slowly increasing sympathetic nervous system activity. In addition to leptin, our data also indicated that obesity exerts its effect on PAI-1. Consistent with our findings, increased PAI-1 levels in plasma have been observed among non-diabetic abdominal obese subjects [543,544]. Our result also indicated that there was no significant change in PAI-1 level in hypertensive subjects. In line with this result, RAS blockade has been shown to slow the progression of processes related to vascular disorders without affecting the PAI-1 level [545]. These results can

probably be explained by the involvement of additional pathways, such as decreased fibrinolysis and thrombosis, as well as the interaction between RAS and hemostasis. Therefore, it is possible that obesity exerts its effect on PAI-1 and that PAI-1 subsequently participates in increasing blood pressure by associating with plasma renin activity and insulin resistance [546].

In conclusion, our data suggests that the interaction between central obesity and hypertension might lead to the reduction in adiponectin that probably exacerbates the TNF-α production and increases the proinflammatory status of adipokines in circulation. These altered adipokine patterns (low circulating adiponectin and high TNF-α) indicates that effect of obesity might become severe in the presence of hypertension and vice versa. However, the results of this study are specific to adult women, and similar investigation specific to men remains to be explored. Additional research that clarifies the mechanisms concerning adipose tissue derivatives, including adipokines and cytokines, may help to understand and prevent severe outcomes from obesity-related hypertension.

Chapter 5

Yoga training modulates circulatory adipokines in adults with high-normal blood pressure and metabolic syndrome

5.1. Introduction

Metabolic syndrome (MetS) is a clinically significant predictor of all-cause and cardiovascular mortality. MetS represents a cluster of metabolic abnormalities including central obesity, high blood pressure, dyslipidaemia, hypertriglyceridaemia, and hyperglycaemia. Central obesity and insulin resistance are considered important underlying contributors to MetS [547]. Furthermore, some researchers believe that hypertension might be another chief contributor to MetS as hypertension increases the risk for obesity and insulin resistance [7,8]. Approximately 65-75 % of hypertensive individuals are obese, and 50 % of hypertensive individuals are insulin resistant [548]. Notably, it has been shown that the prevalence of elevated blood pressure or hypertension among people with MetS could be as high as 85 % [8]. Thus, it has been proposed that blood pressure control might be an important strategy in reducing the risk of MetS in healthy individuals.

Lifestyle modification has been suggested to be the keystone for successful management of MetS [549,550]. Yoga is a blend of exercise, controlled breathing and relaxation practice, often combined with lifestyle advice and specific diet plan. Hatha yoga, a commonly practised yoga stream, consists of asana

(control of postures) and pranayama (manipulation of respiration). Yoga appears to have an anti-hypertensive effect as well as a positive impact on self-rated quality of life. Improvements of MetS risk factors in middle-aged and older adults have been demonstrated to be associated with yoga intervention [2]. One year of yoga training was shown to exert beneficial effects on reducing abdominal obesity and tended to decrease blood pressure in MetS people [2]. Moreover, a short-term intensive yoga programme (90 min/day for 15 consecutive days) has also been demonstrated to cause favourable changes in body mass index, waist and hip circumference, total cholesterol, postural stability, and hand grip strength [42]. Indeed, yoga exercise has been shown to decrease stress, reduce depression and anxiety, and increase perceived self-efficacy in healthy individuals. The reduction of stress has been proposed as one of the potential underlying mechanisms explaining the benefits of yoga exercise [551]. Intriguingly, a cross-sectional study comparing the stress hormones between novice and expert yoga practitioners demonstrated that leptin was lowered and the ratio of adiponectin-to-leptin was doubled in experts when compared to novice yoga practitioners [551].

An epidemiological link between adiposity and hypertension has been illustrated. Adipose tissue is a heterogeneous organ comprised of subcutaneous and visceral adipose tissue. The incidence of hypertension has been reported to be strongly associated with increased visceral adiposity [26]. Compared to subcutaneous adipose tissue, visceral fat is more sensitive to lipolysis and secretes higher amounts of inflammatory cytokines such as PAI-1 [552], visfatin [308], and chemerin [333]. In addition to visceral adipokines,

subcutaneous adipokines have been correlated with hypertension. Subcutaneous adipose depot secretions of leptin and adiponectin might be useful in the prediction of hypertension [27]. The progressive interaction between pro-inflammatory and anti-inflammatory adipokines is commonly thought to play a significant role in the developmental process of systemic metabolic abnormalities. Therefore, the understanding of the equilibrium and balance of adipokines (i.e., pro- vs. anti-inflammatory adipokines) in response to lifestyle components such as physical exercise is critically needed for the new development of regimens to combat MetS and other metabolic disorders.

Yoga reduces MetS risk factors [206] as well as inflammatory adipokines [553,554]. Our previous study indicated that participants with MetS showed a significant decrease in waist circumference and a decreasing trend in blood pressure (p = 0.067) with moderate effect size after 1 year of yoga intervention [2]. Therefore, we hypothesized that yoga training would induce a favourable modulation of the adipokine profile by reducing the circulatory abundance of pro-inflammatory adipokines and increasing anti-inflammatory adipokines in adults with MetS and high-normal blood pressure.

5.2. Materials and Methods

5.2.1. Study design, settings, and subject recruitment

This study was a follow-up to our previous randomized controlled trial in which Chinese participants aged between 30-80 years who were diagnosed with MetS according to the diagnostic guidelines of the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III) criteria underwent a 1-year yoga intervention program in Hong Kong. Individuals with MetS were defined as having 3 or more of the following characteristics: 1) central obesity (waist circumference ≥ 90 cm for Asian males or ≥ 80 cm for Asian females); 2) elevated blood glucose (fasting glucose level ≥ 5.5 mmol/L); 3) hypertension (systolic pressure ≥ 130 mmHg or diastolic pressure ≥ 85 mmHg); 4) elevated plasma triglycerides (triglyceride level ≥ 1.7 mmol/L); and 5) a low level of high-density lipoprotein-cholesterol (HDL-C; level ≤ 1.03 mmol/L for males or ≤ 1.3 mmol/L for females). Participants were randomly selected by a computer program.

Participants having symptomatic heart or lung disease, pulmonary illness, severe rheumatoid arthritis or osteoarthritis, dementia or mental disorder, previous stroke, severe cardiovascular illness, major orthopaedic problems in the lower back, neuromusculoskeletal illness, pelvis or lower extremities were excluded. Participants who were on drug therapy treating metabolic abnormalities, regular tobacco users, wheelchair users, immobile, with physical conditions not appropriate for yoga exercise were excluded. Additionally, participants who exercised at moderate-to-vigorous intensity at least 30 minutes per session regularly (3 or more days a week) were also excluded. All

the experimental procedures received human research ethics approval from The Hong Kong Polytechnic University (ethics approval number: HSEARS20090820001 and HSEARS20160810001) [2].

In the current study, we specifically selected 97 blood samples of participants (control n = 45, yoga n = 52) who had MetS specifically with high-normal blood pressure (systolic pressure ≥ 130 mmHg or diastolic pressure ≥ 85 mmHg) from the pool of 182 archived blood samples from our previous study. The samples from control and yoga intervention group were selected from two-time points: Pre (baseline measurement at the beginning of the study) and Post (the measurement upon accomplishment of the 1 year experimental period). Attrition rate in the study was 4.4% as some of the participants quitted due to personal reasons and some never showed up in the program.

5.2.2. Yoga Intervention

Subjects in control group were not given any intervention but were contacted monthly to monitor their health status. Subjects in yoga group attended 3 yoga sessions weekly for 1 year. Each session was 60-min long consisted of 10-min of warm-up, 40-min of Hatha yoga practice, and 10-min of breathing exercise and relaxation cool-down [2]. Yoga classes were conducted in a small group (~10 subjects) by certified yoga instructors. All subjects were requested to follow to their usual daily dietary intake and physical activities throughout one year of the experimental period. Subjects received supermarket coupon once they have completed the study as an incentive. Subjects in yoga group with below 70 % class attendance were excluded [2].

5.2.3. Determination of MetS risk factors

Subjects were assessed for the MetS diagnostic parameters including blood pressure, waist circumference, fasting glucose, triglycerides, and HDL-C conducted by trained research personnel. Blood pressure measurement was determined after 5-min rest on the right arm in sitting position. Systolic and diastolic blood pressure were obtained over the brachial artery region with the arm supported at heart level using appropriate sized cuff by an electronic blood pressure monitor (Accutorr Plus, Datascope). Waist circumference was measured on bare skin, midway between the lowest rib and the superior border of the iliac crest using an inelastic measuring tape. Venous blood samples were harvested by certified phlebotomists after an overnight fast. Blood glucose, triglycerides, and HDL-cholesterol concentrations were measured by an accredited medical laboratory by commercial test kit methods using an automatic clinical chemistry analyzer (Architect CI8200, Abbott Diagnostics)[2]. Systolic blood pressure, diastolic blood pressure, fasting plasma glucose, waist circumference, HDL-cholesterol, and triglycerides were examined at baseline and the end of the 1-year experimental period. Furthermore, the detailed explanations about the methods used for assessment of MetS risk factors are described in our previous publication [2].

5.2.4. Measurements of adipokines

Commercially available ELISA kits (Visfatin from BioVision; PAI-1 and chemerin from R&D, leptin, and adiponectin from Thermo Fisher Scientific) were used to examine the concentrations of adipokines in the serum samples according to the manufacturer's instructions. The coefficient of variability (CV) for the kit assays were shown as follows: visfatin (intra-assay: 4.4 – 8 %, inter-assay: 8.2

%), PAI-1 (intra-assay: 6.8 %, inter-assay: 7 %), chemerin (intra-assay: 3.9 %, inter-assay: 7.3 %), leptin (intra-assay: 3.9 %, inter-assay: 5.3 %) and adiponectin (intra-assay: 3.8 %, inter-assay: 5.5 %). Measurements were performed in duplicates or triplicates by a single observer to minimize the observer variation.

5.2.5. Data analyses

Data are expressed as the means ± standard deviation. The generalized estimating equation (*GEE*) was adopted to examine the interaction effect between 1 year of time and the intervention, the main effect of time, and the main effect of intervention on adipokines and MetS risk factors. GEE was also adopted to examine the interaction effect between 1 year of time and the yoga intervention on MetS risk factors after taking age as covariate. Normality of the data was verified by the Shapiro-Wilk test. The Mann-Whitney U-test was performed to examine the baseline differences in MetS risk factors, age, sex and change (post-pre) in the MetS risk factors and changes in adipokines between control and yoga groups. The chi-square test was performed to examine the baseline differences in the categorical data. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 22 for Windows. Statistical significance was indicated by $p < 0.05$. Power analyses were performed, and the statistical power was found to be ≥ 80% for most of the outcome measure of the study with significant results including adiponectin, leptin, chemerin, visfatin, diastolic and systolic blood pressure.

5.3. Results

MetS risk factors

No significant differences were observed in the MetS risk factors, habitual physical 209 activity level (as assessed by international physical activity questionnaire or IPAQ) and adipokines at the baseline assessment between control and yoga groups (Table 5.1).

Table 5.1: Baseline characteristics of metabolic syndrome risk factors and adipokines in control and yoga groups

	Control Group (n= 45)	**Yoga Group (n = 52)**	**P-value**
Gender	17 M, 28 F	17 M, 35 F	0.60
Age (years)	56.5 ± 8.6	58.5 ± 9.5	0.26
Diastolic blood pressure (mmHg)	85.8 ± 8.8	84.5 ± 7.8	0.35
Systolic blood pressure (mmHg)	141.7 ± 13.8	140.3 ± 13.5	0.35
Waist circumference (cm)	89.8 ± 7.1	90.4 ± 9.2	0.78
Fasting glucose (mmol/L)	100.2 ± 15.9	100.6 ± 9.9	0.23
Blood triglycerides (mmol/L)	186.2 ± 90.5	166.6 ± 76.9	0.33
Blood high density lipoprotein-C (mmol/L)	48.6 ± 12.0	46.8 ± 10.5	0.52
Adiponectin (ng/mL)	6484 ± 1803	6409 ± 1753	0.88
Plasminogen activator inhibitor-1 (ng/mL)	6.4 ± 2.6	6.0 ± 2.6	0.28
Visfatin (ng/mL)	3.5 ± 2.9	3.5 ± 2.0	0.18
Chemerin (ng/mL)	221.4 ± 70.7	230.1 ± 90.4	0.60
Leptin (ng/mL)	22.2 ± 9.8	25.4 ± 11.5	0.18

Our analysis revealed that the mean of the change (i.e., post-pre) in waist circumference was significantly lower in the yoga group as compared to the control group (-3.6 vs. -1.4, P=0.029) (Figure 5.1).

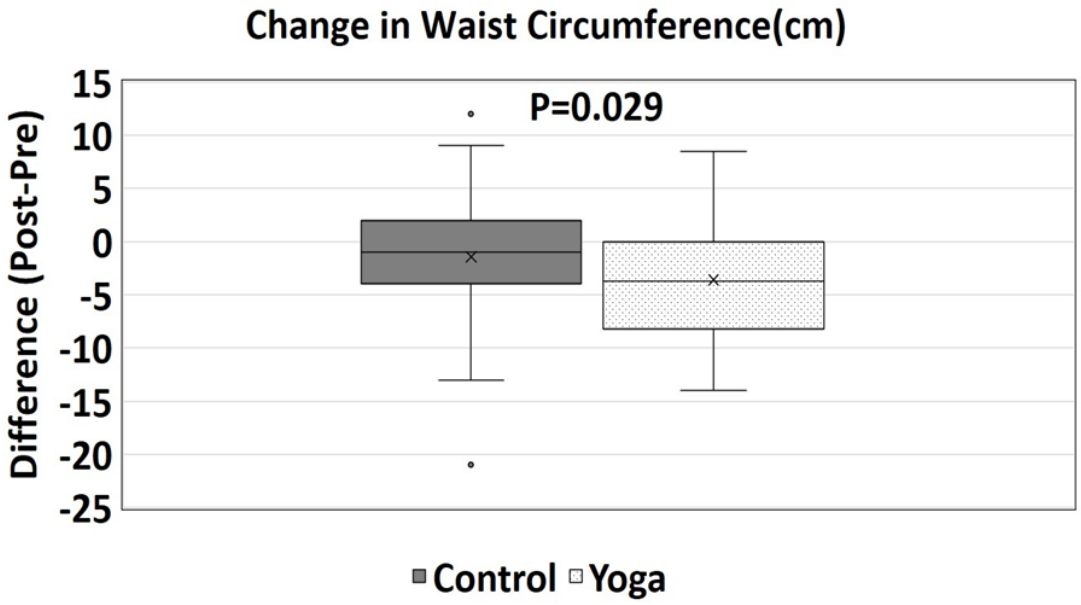

Figure 5.1: Change in waist circumference in control vs. yoga group

Box plot represents the change in waist circumference after yoga training in subjects with MetS and high-normal blood pressure. Statistical significance was accepted at $P < 0.05$. "Difference" refers to change during the 1-year experimental period (i.e., post-pre).

The mean value of waist circumference was reduced by 4 % in the yoga group and 2 % in control group (Figure 5.2 F). Further analysis revealed that there was no significant interaction effect between time and intervention on diastolic blood pressure, systolic blood pressure, high density lipoprotein, triglycerides, fasting glucose, and waist circumference in our examined MetS subjects with high-normal blood pressure (Figure 5.2). Of note, we also found no significant interaction effect between time and intervention on MetS risk factors in MetS participants with high-normal blood pressure after adjusting for age (mean age ± SD = 57.63 ± 9.03 years) as a covariate. The main effect of time was observed on systolic blood pressure (Wald chi square = 12.4, $P < 0.001$), diastolic blood pressure (Wald chi square = 6.3, $P = 0.012$), and waist circumference (Wald chi square = 4.4, $P = 0.037$) (Figure 5.2A, B and F).

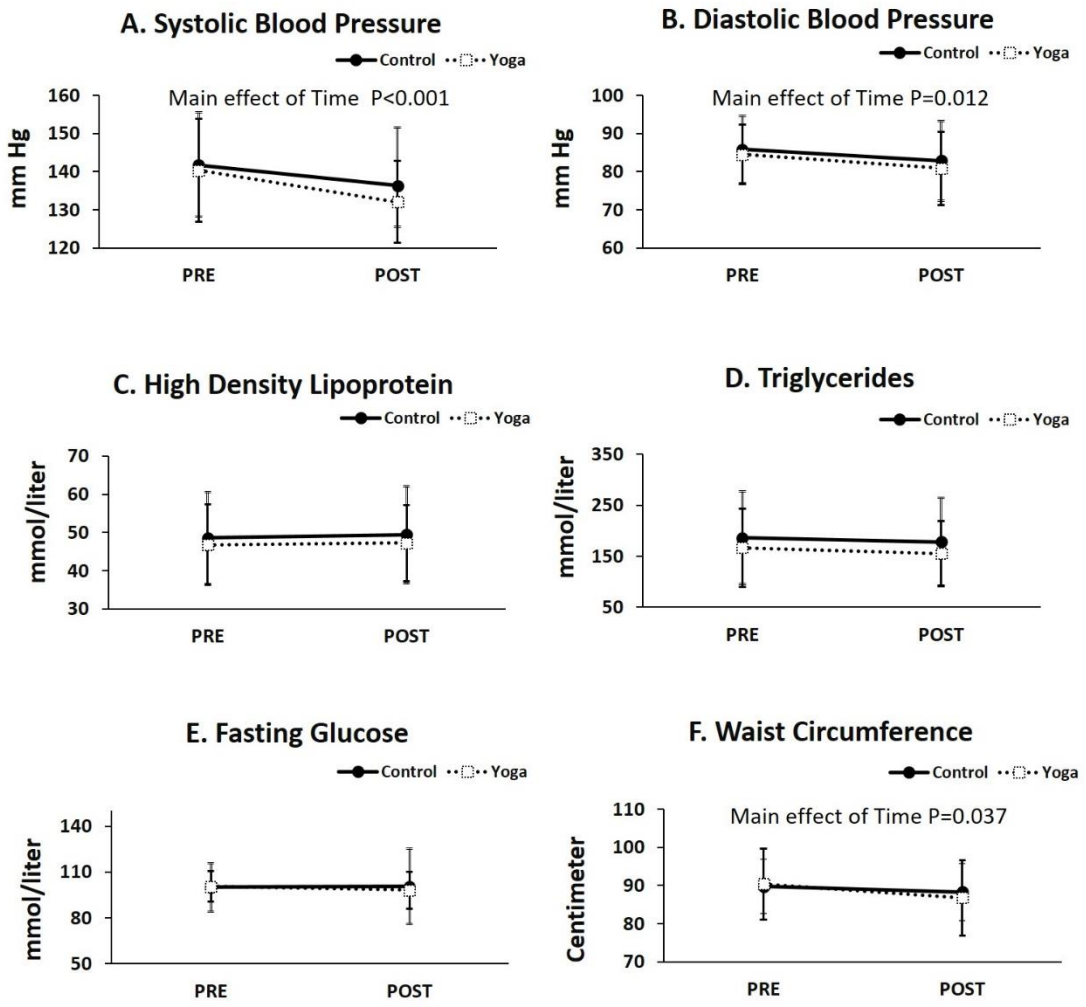

Figure 5.2: Effect of 1-year yoga on metabolic syndrome risk factors

Line graphs represent measurement of the changes in (A) Systolic Blood Pressure in mm of Hg (B) Diastolic Blood Pressure in mm of Hg (C) High density lipoprotein in mmol/liter (D) Tiglycerides in mmol/liter (E) Waist circumference (F) Fasting Glucose of MetS subjects with high-normal blood pressure before (Pre) and after (Post) the 1-year experimental period in control (n = 46) and yoga group (n = 53). Statistical significance was accepted at $P < 0.05$. Data are expressed as mean ± standard deviation.

Anti and proinflammatory adipokines

Our results revealed significant difference in the mean value of change (i.e., post-pre) in adiponectin (-1.0 vs. 1.3, P < 0.001), PAI-1 (0.9 vs. 0.4, P = 0.03), chemerin (46.3 vs. -32.8, P < 0.001) and leptin (1.9 vs. -6.7, P < 0.001) in control vs. yoga group (Figure 5.3A, B, C and D).

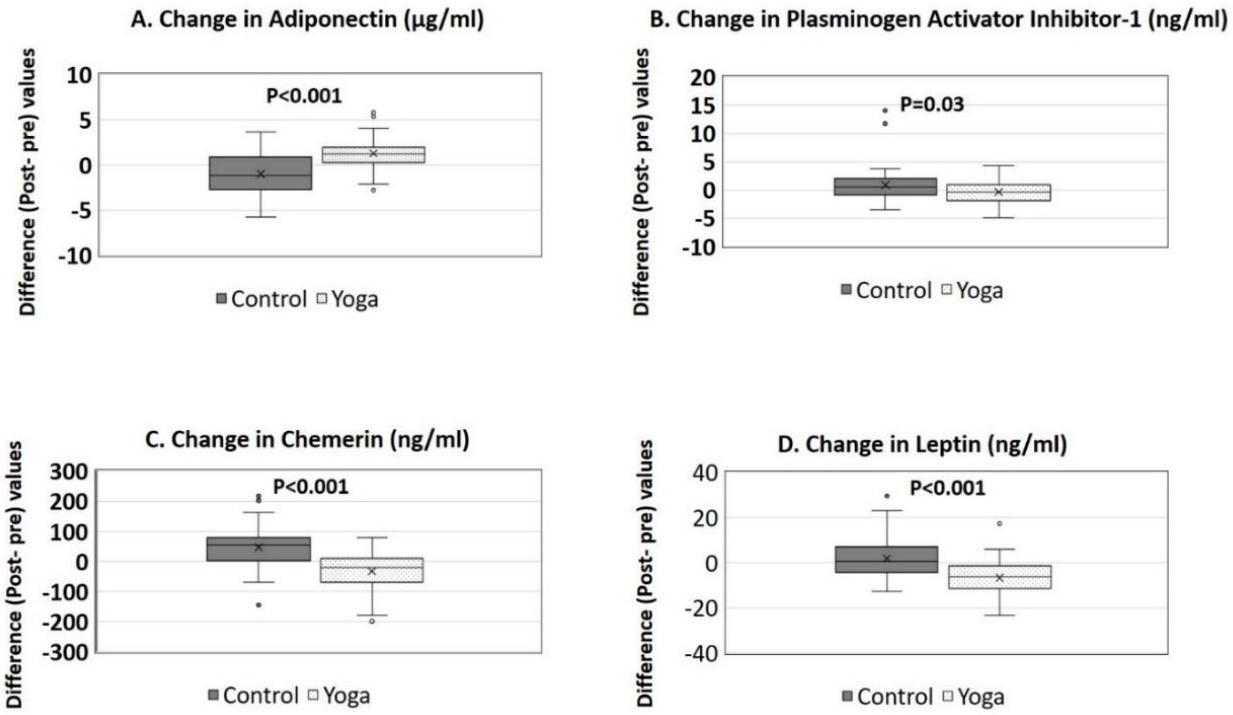

Figure 5.3: Change in adipokines in control vs. yoga group

Box plots represents the changes in adipokines including adiponectin (A), plasminogen activator inhibitor-1 (B), chemerin (C), and leptin (D) after yoga training in subjects with MetS and high-normal blood pressure. Statistical significance was accepted at P < 0.05. “Difference” refers to change during the 1-year experimental period (i.e., post-pre).

The mean value of adiponectin level in yoga group was increased by 20.1 % as compared to a decrease of 15.5 % in control group (Figure 5.4A). On the other hand, the mean values of leptin, PAI-1 and chemerin were significantly decreased (26.5 %, 6.5 % and 14.3 % respectively) in yoga group as compared to increases (9 %, 13.5 % and 21 % respectively) in the control group (Figure 5.4B, C and D). Our further analysis revealed the significant interaction effects between time and intervention on adiponectin (Wald chi square = 16.2, $P < 0.001$), leptin (Wald chi square = 9.4, $P = 0.002$), chemerin (Wald chi square = 11.6, $P = 0.001$), and (Figure 5.4A, B and D). The main effect of yoga intervention was found on PAI-1 (Wald chi square = 5.9, $P = 0.015$) (Figure 5.4 C). However, the main effect of time was observed on visfatin (Wald chi square = 11.78, $P = 0.001$) (Figure 5.4 E).

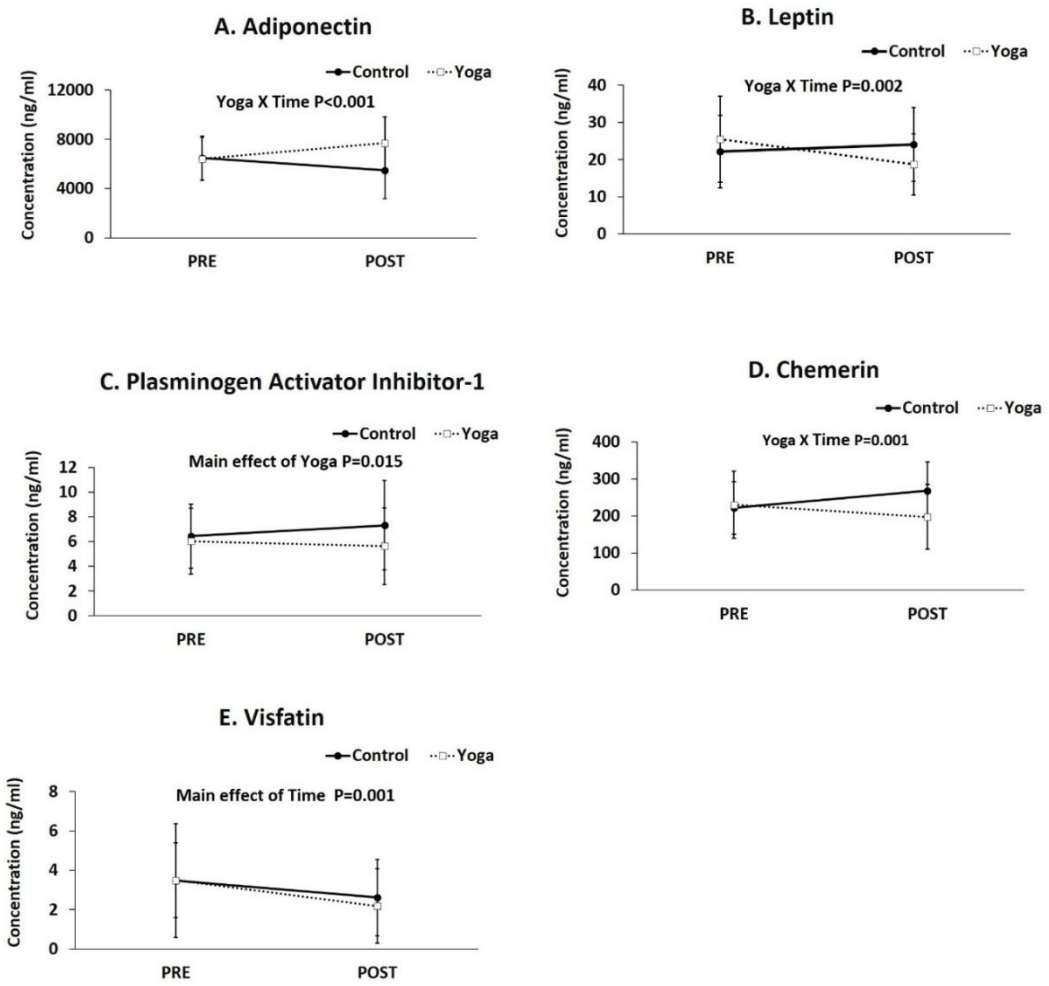

Figure 5.4: Effect of 1-year yoga on anti and proinflammatory adipokines profile

Line graphs represent serum concentration (ng/mL) of adiponectin (A), leptin (B), plasminogen activator inhibitor-1 (C), chemerin (D), and visfatin (E) of MetS subjects with high-normal blood pressure before (Pre) and after (Post) the 1-year experimental period in control (n = 46) and yoga group (n = 53). Statistical significance was accepted at $P < 0.05$. Data are expressed as mean ± standard deviation.

5.4. Discussion

MetS is a serious public health concern due to its intimate link to the pathogenesis of diabetes mellitus, stroke, and cardiovascular diseases. In 2010-2012, the prevalence rate of MetS in Hong Kong was ~27 % according to the NCEP ATP III criteria [2]. While central obesity and insulin resistance have been proposed as the prominent underlying contributors to MetS. Remarkably, as high as 85 % of MetS individuals have been shown to have elevated blood pressure or hypertension [8]. Individuals with both MetS and hypertension are indeed considered to be significantly challenged and at risk for additional morbidities [555]. Adipocytes synthesize and release adipokines such as adiponectin, leptin, angiotensin, perivascular relaxation factors, and resistin, which are all linked with blood pressure control. As adipokines are considered regulators of MetS, the equilibrium and balance of adipokines become an important topic to be explored in studies of obesity, hypertension and MetS. Our present findings reveal that 1-year yoga training increases a circulatory anti-inflammatory adipokine (adiponectin) and decreases pro-inflammatory adipokines (leptin, chemerin, and PAI-1) in MetS participants with high-normal blood pressure. These novel results support the beneficial complementary role of yoga exercise in the management of MetS by illustrating the favourable modulating effects of yoga training on blood adipokines.

Reduction in waist circumference and blood pressure have been shown to be positive health consequences of regular yoga training [2,556] . Waist circumference and visceral fat were decreased after 16 weeks of yoga training compared to a control group of healthy postmenopausal women aged 54.5 ±

2.8 years [556]. Overweight/obese women and breast cancer survivors have also been shown to reduce waist circumference after receiving 6 months of yoga training when compared to breast cancer survivors who did not receive yoga intervention [557]. Another study demonstrated that blood pressure was significantly reduced by yoga exercise intervention in patients with mild hypertension [558]. Consistently, our previous investigation also demonstrated that 1 year of yoga training decreased waist circumference and tended to reduce systolic blood pressure in middle-aged and older adults with MetS compared to the control group who did not receive yoga intervention [2]. Our results are in accordance with the previous findings that the average waist circumference was significantly decreased; however, both systolic and diastolic blood pressures were not decreased in participants with yoga intervention compared to the control participants with MetS and high-normal blood pressure. This study further explored the effect of yoga on the specific subject group with MetS and high-normal blood pressure by investigating the interaction effect between yoga and the 1-year time period. Our results indicated that there was no interaction effect between yoga and the 1-year time period for any of the five risk factors of MetS in our examined participants with high-normal blood pressure. In accordance with our previous study assumptions that the effects of yoga exercise on systolic blood pressure might be dependent on the quantity of the intervention and/or subject compliance to the intervention [2], we again suggested that extra attention should be given to MetS participants with high-normal blood pressure. The controversial results might be explained by the complexities involved in defining MetS. An equal number of MetS risk factors might not indicate that the participants shared the same characteristics and

cardiometabolic risk factor profile. Indeed, the specific outcomes/consequences of MetS are difficult to evaluate without a solid definition and common criteria for diagnosis. Nonetheless, our results support the notion that “MetS in hypertension is an unholy alliance” [555].

Evidence indicates that biomarkers might be valuable in diagnosing and estimating the disease risk for a population and managing many pathological states, especially when clinical signs or obvious anatomic abnormalities are absent or not evident [16]. For example, insulin resistance is one of the major risk factors of type 2 diabetes (T2D). Markers for indicating insulin resistance might be useful for prevention of cardiovascular disease, all-cause mortality, and T2D. Increased concentrations of interleukin-8, monocyte chemotactic protein-1, and interferon γ-induced protein have been associated with the incidence of T2D by hazard ratio risk assessment. One study suggested that chemokines including MCP-1, IL-8 and IP-10 were significantly higher in participants who developed T2D during a follow-up of 10 years compared with those who did not develop T2D [559]. An aerobic exercise (225 min/week) intervention conducted on postmenopausal inactive women resulted in decreases in insulin resistance markers (leptin, adiponectin / leptin ratio, insulin), whereas changes in glucose (one of the risk factors for MetS) were not evident [560]. Another study by the same group suggested that adipokines and systemic inflammation may be associated with the risk of breast cancer in postmenopausal women independent of body mass index [561]. They concluded that after performing long-term aerobic exercise, previously inactive postmenopausal women showed changes in insulin, leptin, and adiponectin / leptin, which might reduce the risk

for postmenopausal breast cancer [560]. Numerous studies support the idea that hypertensive patients with MetS will show early signs of end-organ damage, which are recognized as significant independent predictors of adverse cardiovascular outcomes compared to those without MetS [562]. Therefore, individuals with high-normal blood pressure and MetS should receive special attention.

Researchers suggested that MetS identification should be emphasized when treating patients with hypertension [13,43]. In this study, adipokines were observed to be favourably regulated after yoga intervention in MetS subjects with high-normal blood pressure. These results proposed that an adipokine panel as a circulatory biomarker might be useful for identifying the protective outcomes of interventions, especially in worsening metabolic conditions in which changes in clinical outcomes are not obvious. Biomarkers aid in the diagnosis and management of many pathological states when there are no obvious clinical signs or anatomic abnormalities [16]. Nonetheless, each adipokine has a particular role in maintaining the delicate equilibrium between the pathophysiological effect and protective impact. Although numerous effects of adipocytokines have been reported in recent studies, further investigation of their signalling pathways is still needed to understand how they are eventually integrated.

The regular practice of yoga exercise has been demonstrated to be effective in reducing stress and improving physical and psychological health. Yoga exercise has also been proposed to reduce oxidative stress by at least two

mechanisms [563,564]. The first mechanism is via the suppression of the over-activated sympathoadrenal system and the hypothalamic-pituitary-adrenal (HPA) axis [563,564], which reduces the pro-inflammatory responses by decreasing the levels of stress hormones such as cortisol and epinephrine [563,564]. Yoga exercise has been shown to alleviate inflammatory signalling by downregulating the regulatory molecule that favours the pro-inflammatory microenvironment [529], namely, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [565]. Consistent with the role of NF-κB, the downregulation of the regulatory molecules for the pro-inflammatory microenvironment reasonably explains the principal behind the first mechanism. The second mechanism is attributed to the reactivation of the parasympathetic nervous system by consciously manipulating the breath rhythm (i.e., slow breathing and a long exhalation) during yoga practice [566]. The activities of adenosine monophosphate-activated protein kinase (AMPK) in peripheral tissues and organs are known to be facilitated by the specific regulation of the sympathetic and parasympathetic nervous systems [567]. Intriguingly, AMPK is a regulatory molecule that favours the anti-inflammatory microenvironment. AMPK functions to mediate fat oxidation, reduce circulating fatty acids and triacylglycerol, and increase glucose transport in muscle. In mammals, AMPK has been demonstrated to contribute to glucose homeostasis, appetite regulation, and exercise adaptation [568]. Thus, the upregulation of anti-inflammatory responses by increasing fat oxidation, reducing circulatory fatty acids and promoting insulin sensitivity through the AMPK pathway sensibly contributes to the principal behind the second mechanism. Nevertheless, the precise underlying mechanisms that explain

how yoga practice causes the observed alterations of circulatory adipokines are unclear, and this interesting topic warrants additional research to fully understand the relationship between yoga exercise training and the adipokine profile. Provided that MetS is a condition that is characterized by chronic low-grade inflammation, it is rational that an equilibrium between the anti-inflammatory and pro-inflammatory microenvironments plays a critical role in preventing the development of MetS.

We aimed to provide an estimate of the true efficacy of the intervention, i.e., among those who completed the treatment as planned (per protocol analysis). Nonetheless, the convenience sampling with restricted inclusion criteria and the use of per protocol analysis in our study design might have limited the study generalizability and exaggerated the treatment effect. Future research with the inclusion of both intention to treat analysis and per protocol analysis in the study design might be able to strengthen the present findings. Furthermore, the inclusion of active control group might further enhance the significance of the findings. It remains to be elucidated whether the establishment of a regular gathering group and regular exercise habit might contribute to the beneficial effects of our observed outcome measures other than the yoga intervention. Although the adopted sample size has resulted in a statistical power of 80% or above for most of the outcome measures including visfatin, leptin, BP systolic, BP diastolic and waist circumference, the constraint of sample size might be a limitation in the present study.

In conclusion, 1-year yoga training induces favorable modulation of circulatory adipokines. These findings support the notion that yoga exercise might serve

as an effective lifestyle intervention to reduce chronic inflammation by downregulating the proinflammatory adipokines and upregulating the anti-inflammatory adipokines in individuals with high-normal blood pressure and MetS.

Chapter 6

Doxorubicin induces inflammatory modulation and metabolic dysregulation in diabetic skeletal muscle

(Remark: The findings in this chapter have been published in Frontiers in physiology-Supriya R, Tam BT, Pei XM, Lai CW, Chan LW, Yung BY, et al. Doxorubicin Induces Inflammatory Modulation and Metabolic Dysregulation in Diabetic Skeletal Muscle. Front Physiol 2016 Jul 27;7)

6.1. Introduction

Doxorubicin (DOX) is an effective chemotherapeutic drug for treating various types of cancer [569,570]. Nonetheless, the side effects of DOX on cardiomyocytes leading to life-threatening cardiomyopathy have limited its clinical use [571]. Indeed, the adverse side effects of DOX have also been documented in the brain [572], liver [573], kidney [574], lung [575], blood vessels [576], and skeletal muscle [45,577]. DOX has been demonstrated to exert adverse effects on impairment of muscle quality and reduction of muscle mass [45–47]. In vitro DOX treatment on skeletal muscle cells increases calcium flux from isolated sarcoplasmic reticulum vesicles [578]. DOX has also been shown to reduce calcium sensitivity by interfering with actin-myosin interaction at whole muscle level [579]. Other adverse physiological consequences of DOX in skeletal muscle include muscle atrophy, muscle cell death, muscle mass reduction, and reduction of maximal twitch force [44–47]. DOX is comprised of a quinone moiety in its chemical

structure, which is responsible for causing oxidative stress by interacting with molecular oxygen in skeletal muscle fibers [580]. The resultant elevated oxidative stress disrupts several cellular mechanisms involving calpain and caspase-3 protease (Gilliam & St. Clair 2011), AMP-activated protein kinase (AMPK) [581], and insulin receptor substrate-1 (IRS-1) [582], which impair the processes of apoptosis, autophagy, insulin signaling, and inflammatory pathways.

Inflammation and glycolytic metabolism are the two common molecular mechanisms contributing to the pathogenesis of T2D [583,584] and also to the DOX exposed skeletal muscle [585,586]. Diabetes in skeletal muscle weakens tricarboxylic acid cycle (TCA cycle) [587] and releases enormous amount of ROS by downregulating PGC1-α [588,589]; also, one of the significant contributor of DOX injected skeletal muscle myopathy [590]. ROS/PCG1-α signaling is a key pathway in regulation of mitochondrial function in skeletal muscle and both are inversely related [591]. Hence, we speculated ROS level enhancement leading to down regulation of PGC1-α will contribute to severe pathogenesis in DOX exposed diabetic skeletal muscle. The augmented oxidative stress induced by DOX in diabetic skeletal muscle causes metabolic dysregulation by inhibiting oxidative phosphorylation and upregulating LDH [592]. The upregulated LDH leads to the activation of hypoxia-induced factor 1-α (HIF-1α) [592] and nuclear factor kappa B (NFkβ) [593], which are transcription factor and regulatory molecule favoring proinflammatory microenvironment triggering the secretion of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) [594] and Interleukin 6 (IL-6) [595] respectively. On the other hand, DOX has also been demonstrated to induce oxidative stress in skeletal muscle without altering LDH

[578] but causes insulin resistance in muscle by downregulating IRS-1, glucose transporter type 4 (GLUT4), AMPK and glycogen synthase kinase 3 beta (GSK3β) [596]. While AMPK downregulation is suggested to be involved in insulin resistance development in the DOX-treated skeletal muscle [597]. AMPK also acts as a metabolic master switch that phosphorylates target proteins in fatty acid oxidation metabolic pathways in skeletal muscle [597]. Besides, AMPK acts as a transcription factor favoring the anti-inflammatory microenvironment leading to the shift towards oxidative metabolism, which reduces glycolysis rate by activating PGC1-α that ameliorates inflammatory cytokines/myokines such as TNF-α and IL-6 [598,599] and enhances anti-inflammatory cytokines/myokines such as IL-15 and IL-10 [600,601].

Approximately 8 to 18 % of patients with cancer are diabetic and indeed patients with T2D are at a higher risk for developing tumors including breast, pancreatic, liver, kidney, endometrial, and colon cancers [602]. Though the exact link between diabetes and cancer is not known, the exposure to hyperglycemia, elevated insulin, and growth-promoting IGF-1 have been postulated to be the possible reasons explaining the increased incidence of cancers in diabetic patients [603]. Nevertheless, patients with diabetes and cancer need to be particularly considered for undergoing chemotherapy due to the detrimental side effects of the chemotherapeutic drugs [604]. In regard to the DOX toxicity, the effects of DOX on skeletal muscle of diabetic individuals are largely unknown. DOX has been demonstrated to worsen insulin signaling, engender muscle atrophy, disseminate pro-inflammation, and shift to anaerobic glycolysis metabolism in normal skeletal muscle. The myotoxicity of DOX and the

responsible molecular mechanisms in diabetic muscle has not been comprehensively studied. This study aimed to examine the effects of DOX on insulin signaling, muscle atrophy, pro-/anti-inflammatory microenvironment, and glycolysis metabolic regulation in skeletal muscle of diabetic animals.

6.2. Materials and Methods

6.2.1. Animals

Male 14 to 18-week-old db/db mice were obtained from the Laboratory Animal Services Centre of The Chinese University of Hong Kong. The db/db mouse is a well-established leptin receptor-deficient animal model (homozygous allelic deficient for the leptin receptor gene) that mimics the disease phenotype of human T2D. Non-diabetic db/+ mice (heterozygous allele deficient for the leptin receptor gene) were used as the non-diabetic control because db/db mice and db/+ mice share similar genetic background except that db/db mice exhibit abnormal blood glucose level and show the type 2 diabetic phenotype. Mice were housed in a humidity- and temperature-controlled environment and were exposed to a 12:12-hour light:dark cycle in the Centralized Animal Facilities of The Hong Kong Polytechnic University. Mice were allowed to have access to standard animal diet and water ad libitum. Animal ethics approval was obtained from the Animal Ethics Sub-committee of The Hong Kong Polytechnic University.

6.2.2. Experimental Protocol

Non-diabetic db/+ mice and diabetic db/db mice were randomly assigned to the following groups: db/+CON, db/+DOX, db/dbCON, and db/dbDOX (n = 5 per group). The diabetic status of our examined db/db mice was confirmed by the measurements of fasting blood glucose level (db/db mice vs. db/+ mice: 27.1 ± 1.0 mmol/L vs. 7.8 ± 0.5 mmol/L) and HbA1c level (db/db mice vs. db/+ mice: 7.0 ± 0.4 % vs. 2.8 ± 0.1 %). Mice in db/+DOX and db/dbDOX groups were intraperitoneally injected with doxorubicin (Pharmacia & Upjohn SpA, Milan, Italy) at a dose of 15 mg/kg body weight whereas mice in db/+CON and

db/dbCON groups were injected with the same volume of saline instead of doxorubicin. Gastrocnemius was immediately harvested, weighed, washed with cold phosphate buffered saline (PBS), frozen in liquid nitrogen, and stored at -80 ºC for later analysis.

6.2.3. Protein Fraction Preparation

Protein fractions were extracted from muscle homogenates as previously described Forty mg of sample tissue were minced and homogenized in ice-cold lysis buffer (10 mmol/L NaCl, 1.5 mmol/L $MgCl_2$, 20 mmol/L HEPES, pH 7.40, 20 % glycerol, 0.1 % Triton X 100, and 1 mM dithiothreitol). Homogenates were subject to centrifugation at 875 × g for 5 minutes at 4 ºC. The supernatant was obtained and subject to further centrifugation at 3,500 × g for 5 minutes at 4 ºC in which these procedures were repeated thrice. Finally, the supernatant was collected as the cytoplasmic protein fraction. Then, protease inhibitor cocktail (P8340, Sigma-Aldrich) was added to the cytoplasmic protein fraction. Protein concentration was quantified in triplicates by Bradford assay (Coomassie Protein Assay, Pierce) with bovine serum albumin used as the standard.

6.2.4. Western Blot Analysis

The protein abundances of insulin signaling markers (IRS-1Ser$^{636/639}$, AktSer^{473}, GLUT4), muscle atrophy markers (MuRF1 and MAFBx), markers for proinflammatory favoring microenvironment (TNF-α, IL-6, HIF-1α, pNFkβp65), markers for anti-inflammatory favoring microenvironment (IL-10, IL-15, AMPKβSer^{108}, PGC1-α) and metabolic regulators (PDK4, pACCSer^{79}, LDH) were evaluated by Western blotting. Forty micrograms of protein were denatured at 95 ºC for 5 minutes in Laemmli buffer with 5 % β-mercaptoethanol. The protein samples were subject to gel electrophoresis on 10% SDS-PAGE

gel. Resolved proteins were then transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore) by using the Bio-Rad Mini-Protein II system. The membrane was then blocked with 5 % skimmed milk powder in PBS/0.1 % Tween-20 (PBST) followed by incubation with respective primary antibodies overnight at 4 ºC. The primary antibodies anti-phospho-IRS1 (Ser636/639), anti-phospho-Akt (Ser473), anti-Akt, anti-HIF-1α, anti-phospho-NFkβp65, anti-phospho-AMPKβ (Ser108), anti PGC1-α, anti-PDK4 (Thr410/403), anti-pACC (Ser79) were used from Cell Signaling, anti GLUT4 from Millipore, anti-MURF1, anti-MAFBx, anti-TNF-α, anti-IL-6, anti-IL-10, anti-IL-15 were used from Santa Cruz. Sources and dilution used for primary antibodies are shown in Table 6.1. Membranes were incubated with appropriate secondary antibodies (i.e., anti-mouse, anti-rabbit or anti-goat IgG horseradish peroxidase-conjugated antibodies; Cell Signaling, 1:4000) after washing. The immunoreactivity was determined using the ECL chemiluminescence reaction kit (Perkin Elmer) and the images were captured by Chemi Doc (Bio-Rad camera, USA). GAPDH was used as the internal loading control. The arbitrary units of the blot signal are presented as net intensity x band area, normalized to the signal of GAPDH or the respective total protein for phosphorylation status.

Table 6.1: List of antibodies used

Antibody	Dilution Factor	Source
Anti-phospho-IRS1 (Ser636/639) rabbit polyclonal	1:1000	2388, Cell Signaling Technology
Anti-phospho-Akt (Ser473) rabbit polyclonal	1:1000	9271, Cell Signaling Technology
Anti-Akt rabbit polyclonal	1:1000	9272, Cell Signaling Technology
Anti GLUT4 rabbit polyclonal	1:500	07-1404, Millipore
Anti-MuRF1 rabbit polyclonal	1:500	32920, Santa Cruz
Anti-MAFbx rabbit polyclonal	1:500	33782, Santa Cruz
Anti-phospho-AMPKβ1(Ser108) rabbit polyclonal	1:1000	4181, Cell Signaling Technology
Anti PGC-1α rabbit polyclonal	1:500	13067, Santa Cruz
Anti-IL10 goat polyclonal	1:1000	365858, Santa Cruz
Anti-IL15 goat polyclonal	1:1000	1296, Santa Cruz
Anti-phospho-NFkβ p65 rabbit monoclonal	1:1000	3033, Cell Signaling Technology
Anti-HIF-1α rabbit polyclonal	1:500	10790, Santa Cruz
Anti-TNF-α goat polyclonal	1:1000	52746, Santa Cruz
Anti-IL6 goat polyclonal	1:1000	1265, Santa Cruz
Anti-PDK4(Thr410/403) goat polyclonal	1:1000	14495, Santa Cruz
Anti-phospho-ACC(Ser79) rabbit polyclonal	1:1000	3661, Cell Signaling Technology
Anti-LDH rabbit polyclonal	1:500	33781, Santa Cruz

6.2.5. Lactate Dehydrogenase Activity

Lactate dehydrogenase (LDH) activity was determined by a commercially available kit (ab102526, Lactate Dehydrogenase Activity Colorimetric Assay Kit, Abcam). The assay was performed according to the manufacturer's instruction. In brief, LDH converts pyruvate into lactate that reduces the developer to a colored product with absorbance at 450 nm measured by a microplate reader (Infinite F200, Tecan, Switzerland).

6.2.6. Data Analyses

Statistical analyses were performed by using the SPSS 21.0 software package (IBM, Chicago, IL, USA). Normality test was performed to examine data

distribution. All data were expressed as mean ± standard error of the mean (SEM). Two-way ANOVA, as well as one-way ANOVA, were used to examine the interaction and main effects of the two experimental factors (i.e., diabetes and DOX). Significant results were further analyzed using Tukey's HSD post hoc test and where appropriate, post hoc pairwise comparisons were performed using student's t-test. Statistical significance was set at $P < 0.05$.

6.3. Results

No exacerbating effect of DOX exposure on insulin signaling in diabetic muscle

The abundance of insulin signaling proteins including phosphor-IRS1Ser$^{636/639}$, phosphor-AKTSer^{473} and GLUT4 was measured in gastrocnemius muscle. We observed the main effect of diabetes for phosphor-IRS-1 and AKT but no significant effect of DOX on diabetic muscle was found. The protein abundance of phosphor-IRS1 was significantly increased by 245% in gastrocnemius muscle of db/dbCON mice relative to that of db/+CON mice (Figure 6.1A). The protein abundance of phosphor-AKT was significantly reduced by 61 % in the muscle of db/dbCON mice relative to db/+CON mice (Figure 6.1B). We did not observe any main effect of diabetes or interaction effect of diabetes with DOX for GLUT4 (Figure 6.1C).

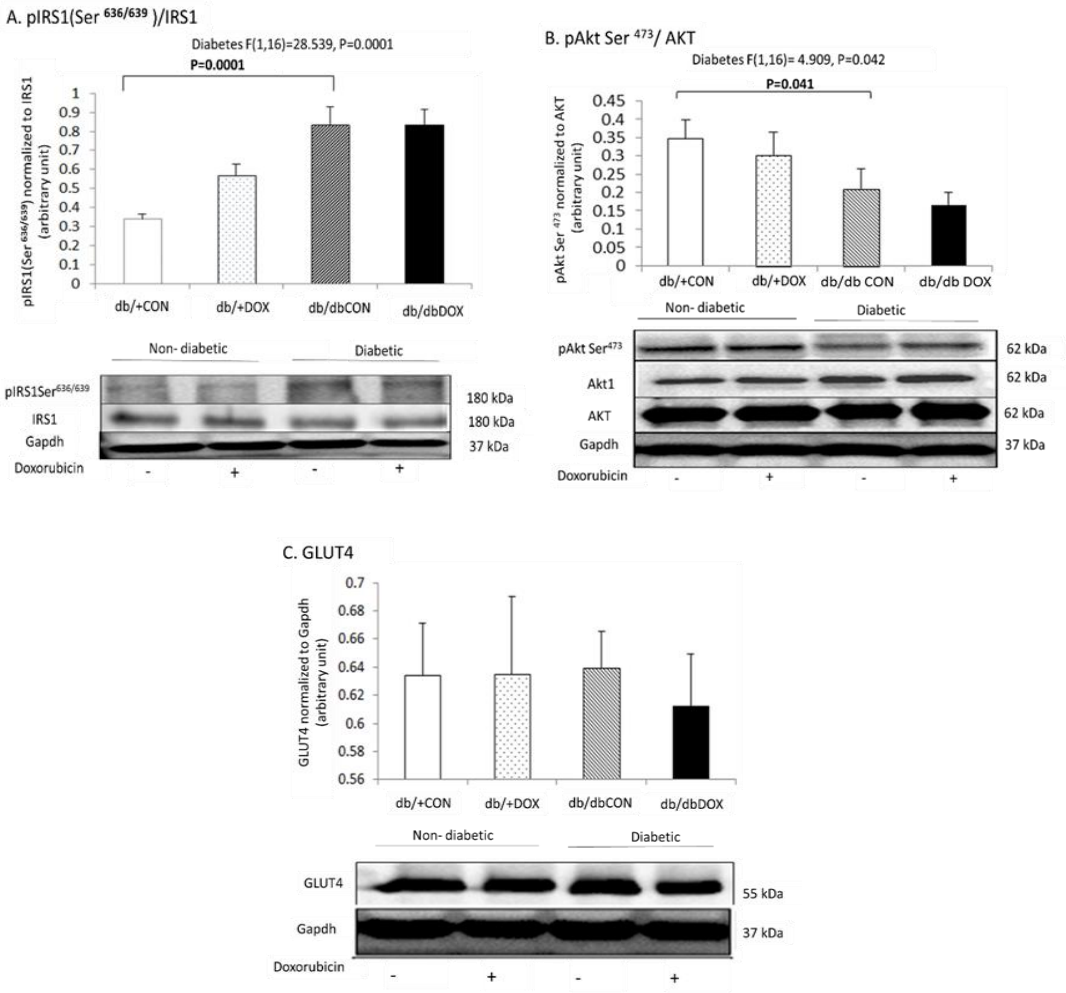

Figure 6.1: Expressions of insulin singnaling markers in non-diabetic and diabetic skeletal muscle after DOX injection.

Non-diabetic and diabetic skeletal muscle insulin signaling markers examined by phosphorylation statuses of IRS1(Ser636/639) (A), Akt (Ser473) (B) and GLUT4 (C) are shown. Data are expressed as mean ± SEM.

No exacerbating effect of DOX exposure on muscle atrophy in diabetic muscle

The abundance of muscle atrophy markers including MuRF1 and MAFBx were measured in gastrocnemius muscle. There was a significant main effect of diabetes for MuRF1 but not MAFBx (Figure 6.2A & B). No interaction effect of diabetes with DOX was found for both MuRF1 and MAFBx. There was a

significant 128 % increase in protein abundance of MuRF1 in muscle of db/+CON mice relative to that of db/dbCON mice. No change in MuRF1 protein abundance was observed in muscles between db/dbCON and db/dbDOX mice (Figure 6.2A). Significant main effect of diabetes was observed on muscle mass reduction. However, we did not observe any interaction effect of diabetes with DOX on muscle mass alteration. Muscle mass was significantly decreased by 42% in gastrocnemius muscle of db/dbCON mice relative to that of db/+CON mice. No significant change in muscle mass was observed in db/dbDOX mice when compared to db/dbCON mice (Figure 6.2C).

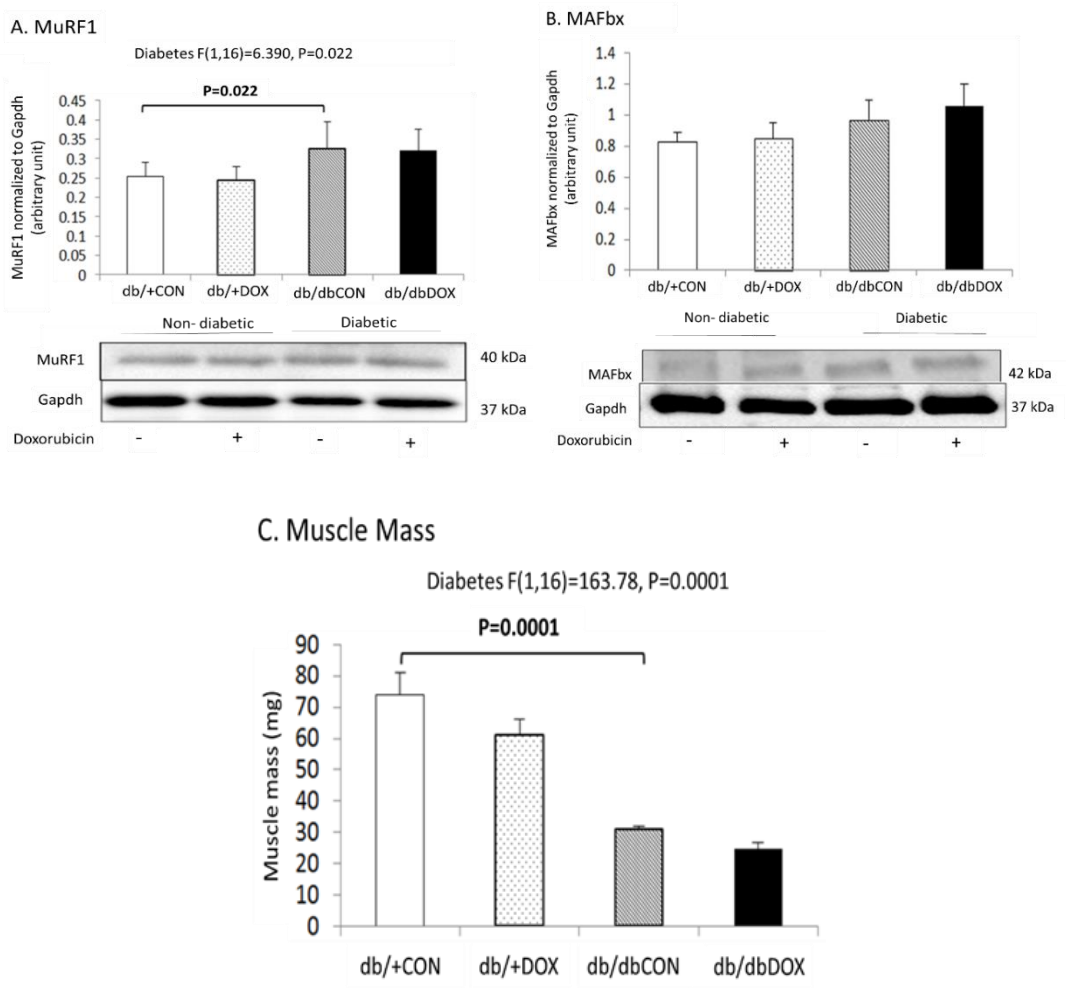

Figure 6.2: Expressions of muscle atrophy markers in non-diabetic and diabetic skeletal muscle after DOX injection.

Non-diabetic and diabetic skeletal muscle atrophy markers examined by phosphorylation statuses of MuRF1 (A) and MAFbx (B) muscle mass (mg) (C) are shown. Data are expressed as mean ± SEM.

DOX causes proinflammatory microenvironment upsurge and anti-inflammatory microenvironment diminution in diabetic muscle

Proinflammatory favoring microenvironment components including muscle specific cytokines, transcription factor and regulatory molecule were measured. Significant main effect of diabetes was observed in IL-6 and TNF-α whereas significant interaction effect of diabetes with DOX was only observed in TNF-α. Significant main effect and interaction effect were found in both proinflammatory favoring microenvironment transcription factor (HIF-1α) and proinflammatory microenvironment favoring regulatory molecule (pNFkβ-P65). IL6 was significantly increased by 123% in muscle of db/dbCON mice relative to that of db/+CON mice (Figure 6.3.B). TNF-α was increased by 149% in muscle of db/dbCON mice relative to that of db/+CON. TNF-α was also increased by 129% in the muscle of db/dbDOX mice relative to db/dbCON mice (Figure 3A). HIF-1α was increased by 568% in muscle of db/dbCON mice relative to db/+CON mice. HIF-1α was also increased by 161% in db/dbDOX mice relative to that of db/dbCON mice (Figure 6.3.C). There was a significant 147% increase in phosphor-Nfkβ in db/dbCON muscle relative to db/+CON muscle. There was a significant 140% increase in phosphor-Nfkβ in db/dbDOX muscle relative to db/dbCON muscle (Figure 6.3.D).

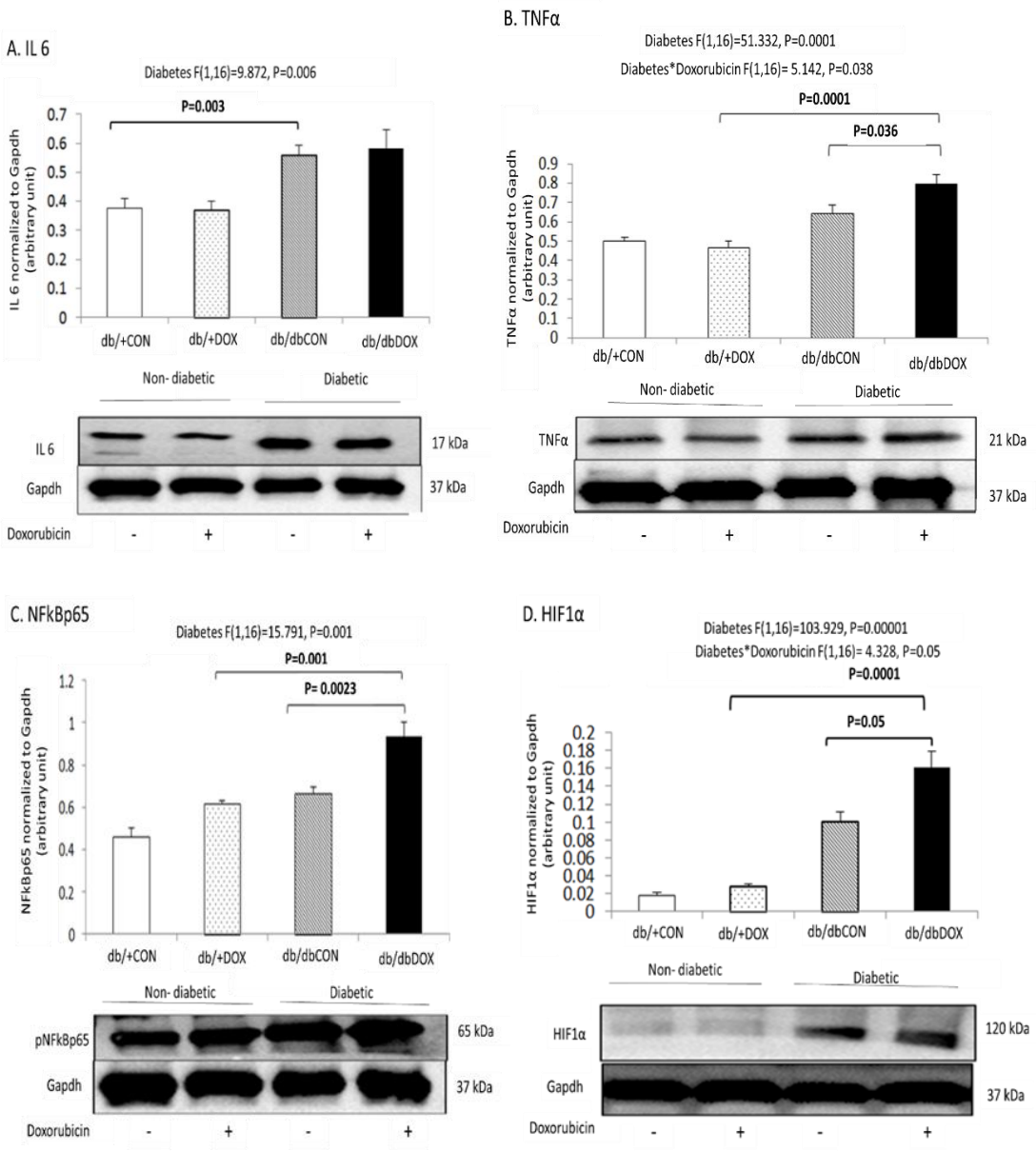

Figure 6.3: Expressions of anti-inflammatory microenvironment markers in non-diabetic and diabetic skeletal muscle after DOX injection.

Non-diabetic and diabetic skeletal muscle anti-inflammatory microenvironment after DOX exposure examined by phosphorylation of myokines: IL10 (A), IL15 (B) AMPKβ1Ser108 (key regulator) (C) and PGC-1α (transcription factor) (D) are shown. Data are expressed as mean ± SEM.

Anti-inflammatory favoring microenvironment components including muscle specific cytokines, transcription factor and regulatory molecule were measured. Significant main effect of diabetes was observed in IL10 and IL15 (Figure 6.4A & B) whereas interaction effect of diabetes with DOX was only observed in IL15. Significant main effect and interaction effect were observed in both anti-inflammatory favoring microenvironment transcription factor (AMPK) and anti-inflammatory microenvironment favoring regulatory molecule (PGC-1α). IL10 was significantly decreased by 22% in the muscle of db/dbCON mice relative to that of db/+CON mice. IL15 was decreased by 55% in muscle of db/dbCON mice relative to the muscle of db/+CON. IL15 was also decreased by 60% in the muscle of db/dbDOX mice relative to db/dbCON mice (Figure 6.4.B). There was a 40% decrease in phosphor AMPKβ1/2 Ser^{108} in muscle of db/dbCON mice relative to db/+CON mice. The phosphor AMPKβ1/2 Ser^{108} was also decreased by 42% in db/dbDOX mice relative to that of db/dbCON mice (Figure 6.4.C). A significant decrease of 76% of PGC-1α in the muscle of db/dbCON relative to the muscle of db/+CON mice was found. There was also a 62% decrease in PGC-1α in the muscle of db/dbDOX relative to that of db/dbCON mice (Figure 6.4.D).

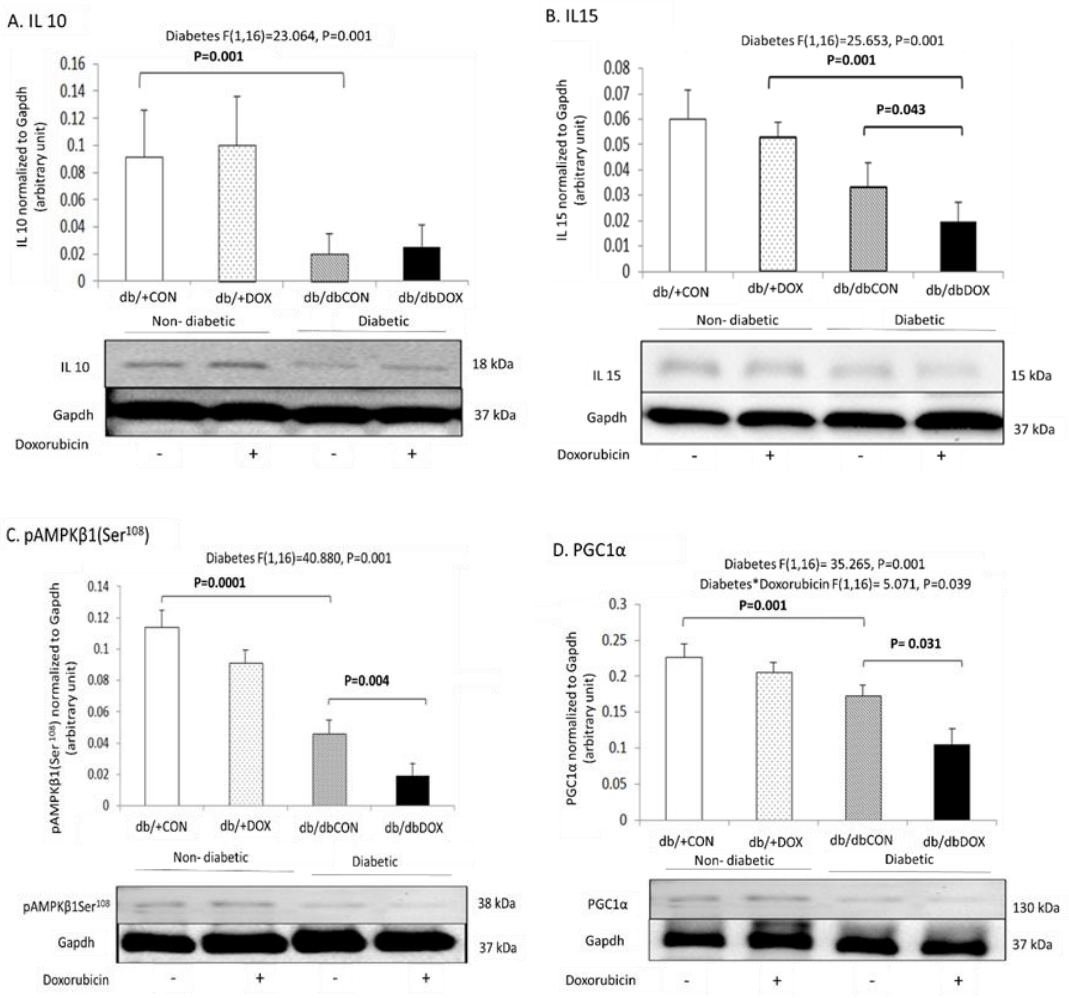

Figure 6.4: Expressions of proinflammatory microenvironment markers in non-diabetic and diabetic skeletal muscle after DOX injection

Non-diabetic and diabetic skeletal muscle proinflammatory microenvironment after DOX exposure examined by phosphorylation statuses of myokines: IL6 (A) TNF-α (B) pNFkBp65 (Key regulator) (C) and HIF-1α (transcription factor) (D) are shown. Data are expressed as mean ± SEM.

DOX causes a shift towards anaerobic glycolysis in diabetic muscle

Anaerobic glycolysis favouring metabolic regulators including PDK4 and LDH were examined. Significant main effect of diabetes and interaction effect of diabetes with DOX were observed in PDK4 (Figure 6.5A). Significant main effect of diabetes and interaction effect of diabetes with DOX were found in LDH (Figure 6.5C). For LDH activity, there was a significant main effect of diabetes (Figure 6.5D). Significant main effect of diabetes and interaction effect of diabetes with DOX were observed in phosphor-ACCSer^{79} (Figure 6.5B). A significant 155 % increase in PDK4 in the muscle of db/dbCON mice relative to the muscle of db/+CON mice was found. A significant 136 % increase in PDK4 in the muscle of db/dbDOX mice relative to that of db/dbCON mice was observed. Both protein abundance and activity of LDH were measured as indicators of anaerobic glycolysis. There was a significant 144 % increase in LDH in the muscle of db/dbCON mice relative to db/+CON mice. There was also a 133 % increase in LDH in the muscle of db/dbDOX mice relative to that of db/dbCON. A significant 321 % increase in LDH activity in muscle of db/dbCON mice relative to that of db/+CON mice was found. There was a significant 94 % decrease in protein abundance of phosphor-ACC in the muscle of db/dbCON relative to that of db/+CON. Muscle of db/dbDOX mice showed a significant 41 % decrease in protein abundance of phosphor-ACC relative to that of db/dbCON mice.

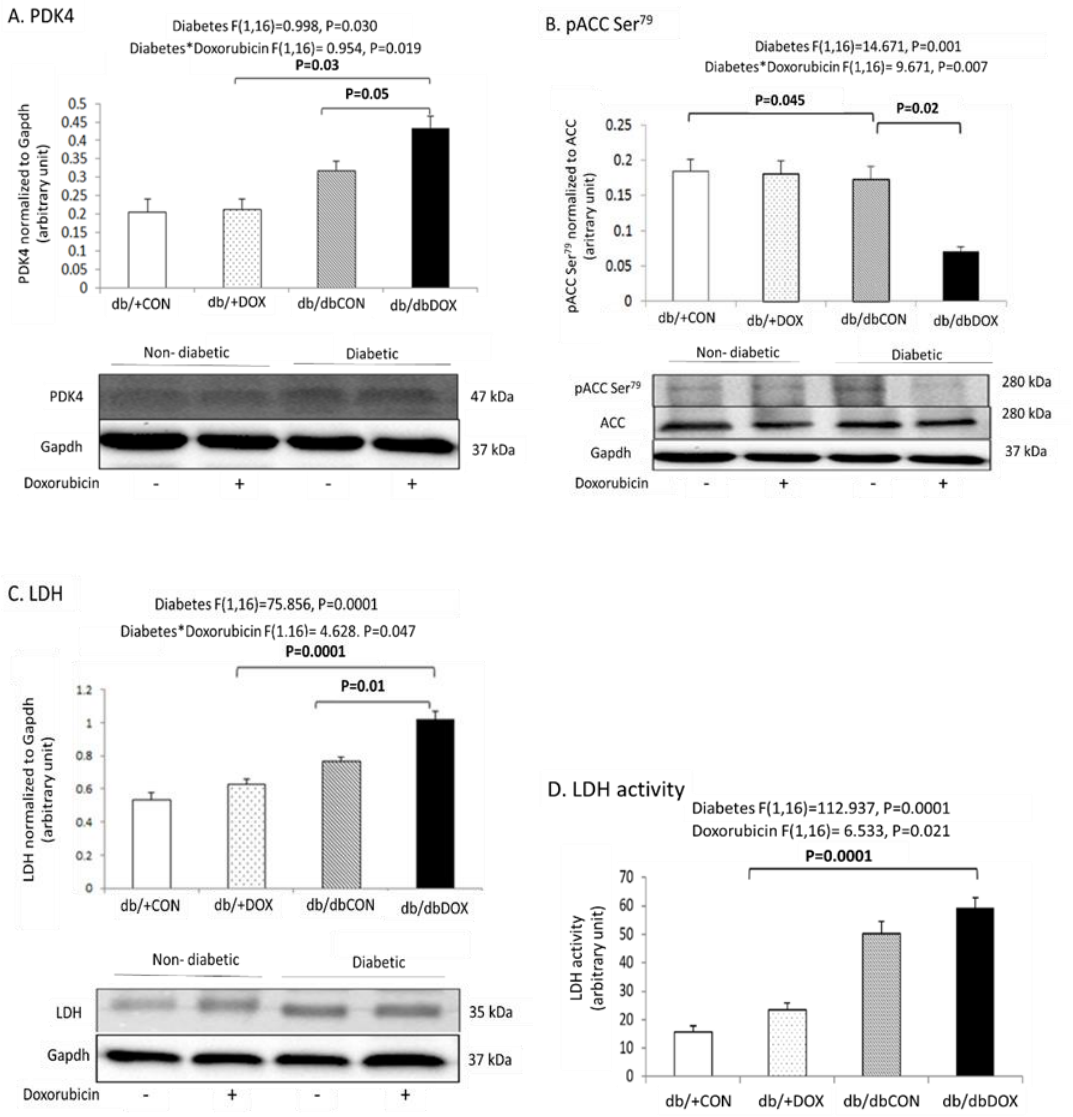

Figure 6.5: Expressions of glycolytic pathway markers in non-diabetic and diabetic skeletal muscle after DOX injection

Non-diabetic and diabetic skeletal muscle glycolytic mechanism after DOX exposure examined by phosphorylation statuses of PDK4 (A) and ACC (Ser79) (B) LDH protein (C) LDH activity (D) are shown. Data are expressed as mean ± SEM.

6.4. Discussions

In this study, we observed DOX treatment on diabetic skeletal muscle does not exacerbate insulin signaling and muscle atrophy as compared to non-diabetic skeletal muscle. However, DOX exposure to diabetic skeletal muscle augments proinflammatory microenvironment - by upregulating transcription factor (HIF-1α), regulatory molecule (pNFκBp65) and cytokine (TNF-α), and reduces anti-inflammatory microenvironment - by downregulating transcription factor (pAMPKβ1/2Ser^{108}), regulatory molecule (PGC-1α) and muscle-specific myokine (IL15). Also, DOX exposure to diabetic skeletal muscle induced dysregulation in glycolytic metabolism, indicated by upregulation of PDK4 and LDH, and downregulation of pACCSer79.

Diminution of anti-inflammatory microenvironment and dysregulated glycolytic metabolism link

AMPK, a transcription factor favoring anti-inflammatory microenvironment [605], directly activates PGC-1α [606] and also regulates IL-15 [607]. Therefore, downregulation of AMPK in diabetic skeletal muscles after DOX treatment leading to the reduction in PGC-1α and IL-15 might indicate the diminution of the anti-inflammatory microenvironment as shown in our results. AMPK activation in skeletal muscle results in muscle fiber's shift from glycolytic fibers to oxidative fibers in a PGC-1α dependent manner [608]. AMPK also stimulates fatty acid oxidation and reduces the activity of acetyl-coenzyme A (CoA) carboxylase 2 (ACC2) which is an enzyme carrying out the conversion of acetyl CoA to malonyl CoA [609] that direct fatty acid oxidation in skeletal muscle [610]. Since pACCSer79 was significantly downregulated in our findings, we

suspected decrease in fatty acid oxidation metabolism in DOX-treated diabetic skeletal muscle. Our results demonstrate downregulation of AMPK also shows the glycolytic shift in PGC-1α dependent manner along with the downregulation of pACCSer79. Our findings also agree with previous investigations based on DNA microarray analysis reporting downregulation of oxidative phosphorylation (OXPHOS) genes, which were strongly correlated with PGC-1α in diabetic skeletal muscle [588]. Also, DOX treatment in the heart inhibits long-chain fatty acid oxidation [611]; so, we speculated that decrease in ACC phosphorylation and AMPK might be the indicator of dysregulation of oxidative phosphorylation mechanisms linking to inflammatory modulation in DOX-treated diabetic skeletal muscle. Our findings are also supported by a study conducted on the white adipose tissue after DOX administration reporting, decrease in ACC after DOX exposure [612], inhibits long chain fatty acid oxidation and leads to the deficiency of adenosine triphosphate (ATP) in cardiac tissue [613]. Also, AMPK activation is proposed to increase ATP generation [614], hence combining the both facts our results showing downregulation of both AMPK (anti-inflammatory regulatory molecule) and pACCser79 may propose a symbiotic link between anti-inflammatory microenvironment and oxidative phosphorylation mechanism.

Upsurge of proinflammatory microenvironment and anaerobic glycolytic metabolism link

NFkβ, a regulatory molecule favoring proinflammatory microenvironment [615] is activated by ROS [616] and TNF-α [593] in skeletal muscle. Additionally, classical activation of NFκβ augments muscle glycolytic metabolism in an HIF-1α dependent manner [593]. The results of the present study support the notion that upregulation of TNF-α activates NFkβ and augments glycolytic mechanism in HIF-1α dependent manner. Nonetheless, we did not found any significant difference in IL-6 content may be because it shows both pro and anti-inflammatory characteristics [360]. Noticeably, DOX induces its cardiotoxicity by decreasing conversion of acetyl-CoA to manolyl CoA that inhibits fatty acid oxidation and decrease in ATP generation [617]. However, an antidiabetic drug (Metformin) prevents cardiotoxicity caused by DOX administration, by increasing fatty acid oxidation and preventing energy starvation [613]. Also, metformin in combination with DOX inhibits the inflammatory pathway by inhibiting NFkβ (proinflammatory regulatory molecule) in mammalian cancer cell line [618]. We suspected if Metformin reduces the effect of DOX toxicity by enhancing phosphorylation of ACC and decreasing NFkβ, then DOX induction along with diabetes might reduce ACC phosphorylation and aggravate inflammation by triggering NFkβ as supported by our findings. Since, NFκβ augments muscle glycolytic metabolism in an HIF-1α dependent manner [593], HIF-1α upregulation in our results may be an indicator of an upsurge in glycolytic metabolism. TNF-α increase in our results indicates NFkβ increase in PGC-1α dependent way as it has been demonstrated that TNF-α increases

NFkβ binding to PGC-1α which downregulates PGC-1α and subsequent dysregulates glucose metabolism [619].

Anaerobic glycolysis metabolism upsurge

Proinflammatory microenvironment has their physiological requirements, and hence they undergo profound metabolic changes. We propose that to mediate metabolic adaptation caused by DOX in diabetic skeletal muscle, upsurge of proinflammatory regulatory molecule stabilizes HIF-1α which leads to lactate release as indicated by upregulation of NFkβ, HIF-1α and LDH in our results. Our results are supported by a study demonstrating that HIF-1α leads to upregulation of anaerobic glycolysis and release of lactate [592]. Glycolysis is the metabolic pathway that converts glucose into pyruvate aerobically and lactate anaerobically [620]. Also, interconversions of pyruvate to lactate and vice versa is carried out by LDH due to brief and intensive activity [621]. In our study, we found upregulation of LDH, indicating the shift of aerobic metabolism to anaerobic metabolism. Our results are supported by a study reporting that diabetic skeletal muscle has increased glycolytic rate with upregulation of LDH [592]. Whereas, DOX injection in skeletal muscle has been reported not to contribute to the release of LDH [622]. Since our study incorporates DOX induction in diabetic skeletal muscle, we suspected worsening in oxidative phosphorylation based on the findings of one study conducted on white adipose tissue reporting that acetyl-CoA carboxylase was inhibited after DOX exposure [612]. We found downregulation of pACCser79 consistent with the previous findings indicating dysregulation in oxidative metabolism. PDK4 increase indicates fatty acid accumulation and has been demonstrated to be upregulated in diabetic skeletal muscle [623], and also in DOX treated skeletal muscle [624]. However, HIF-1α

(proinflammatory transcription factor) can also induce PDK4 [625] consistent with our findings. Our results show upsurge of PDK4, LDH and HIF-1α with downregulation of phosphorylation of ACC, supports fatty acid accumulation and the pro-inflammation upsurge in DOX-treated diabetic skeletal muscle in HIF-1α dependent manner.

In conclusion, we propose there might be a link to modulation of the inflammatory pathway to the shift from oxidative to glycolytic metabolism in diabetic skeletal muscle after DOX exposure. Our results are valuable for the development of new effective strategies to protect skeletal muscle from DOX-induced toxicity in diabetic cancer patients (Figure 6.6).

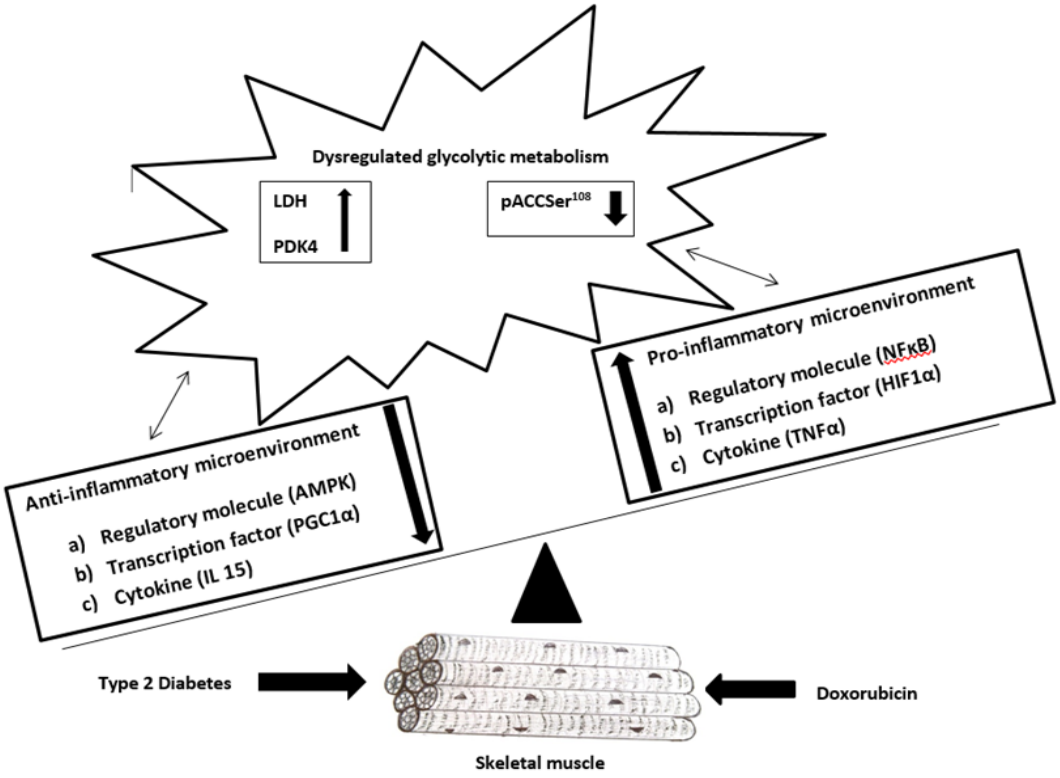

Figure 6.6: Overall mechanism demonstrating inflammatory modulation and metabolic dysregulation in diabetic skeletal muscle after DOX injection.

Doxorubicin administration in diabetic skeletal muscle leads to upsurge of proinflammatory microenvironment (HIF-1α, NFκB, TNF-α) along with upsurge of anaerobic glycolytic metabolic regulators (PDK4, LDH) and diminution of anti-inflammatory microenvironment (PGC-1α, AMPK, IL-15) along with diminution of aerobic metabolic regulator (pACCSer108).

Chapter 7

Overall discussion, limitations and recommendations

Metabolic diseases have become the most important worldwide health concern, not only because they include several diseases that reduce life expectancy, including MetS, insulin resistance, T2D, ischaemic heart disease and atherosclerosis, but also because together, they exert enormous economic burdens and societal consequences. Mounting evidence indicates that systemic metabolic dysfunctions are initiated by obesity, which causes chronic low-grade inflammation [626,627]. It has now been established that adipose tissue stores energy, secretes various bioactive substances (adipokines) and functions as an endocrine organ [628,629]. Excess adiposity and adipocyte dysfunction cause dysregulated expression of these adipokines (such as adiponectin, leptin, and TNF-α) [630]. The dysregulated expression of these adipokines alters the immune response and is linked to the pathogenesis of numerous disease processes. Apart from adipocytes, adipose tissue also contains preadipocytes, fibroblasts, vascular constituents and tissue resident macrophages. Macrophages are recognized as an important contributor to inflammation, but it has been reported that adipocytes also exhibit significant intrinsic inflammatory properties [631]. Adipocytes activate several inflammatory signal transduction cascades that further cause the secretion of numerous acute phase reactants and potent inflammatory cytokines. Adipocyte effects are subtle compared to the effects of TNF-α, which stimulates jun-N-terminal

kinase, NF-κB extracellular signal-regulated kinase, and p38 mitogen-activated protein kinases PI-3 kinase cascades through the p55 and 75 TNF receptors [632]. These factors have been repeatedly reported to be dysregulated with changes in any components of MetS (obesity, hypertension, etc.) but the unique features of their specificity in the regulation of distinct metabolic disease risk factors have never been investigated. Much consideration is needed to develop a better understanding of the immunoregulatory functions of adipose tissue. Recently, a few adipokines (TNF-α, IL-10, IL-8) have been reported either to promote proinflammatory responses to cause metabolic dysfunction or to reduce inflammation and exert beneficial effects on metabolic disorders [633]. The findings of this thesis provide additional support to the hypothesis that an imbalance of pro- and anti-inflammatory adipokines secreted by adipose tissue contributes to metabolic dysfunction and can help in differentiating the risk factors related to metabolic syndrome and may assist in indicating the disease severity. Although metabolic disorders may be the result of a combination or the overlap of several factors, determining the mechanisms concerning adipose tissue derivatives (adipokines and cytokines) would enhance our understanding of their contribution to the pathogenesis of this dangerous combination of metabolic disease risk factors.

Several overlapping functions of adipokines, including regulation of appetite and satiety, endothelial function, energy expenditure, haemostasis, insulin sensitivity, blood pressure, energy metabolism in insulin-sensitive tissues, adipogenesis, fat distribution and insulin secretion by pancreatic β-cells, have been reported. These adipokines are clinically relevant as biomarkers for fat

distribution, adipose tissue function, liver fat content, insulin sensitivity and chronic inflammation and have potential as future pharmacological treatment strategies for obesity and its related diseases. However, few studies on adipokines provide evidence for their non-overlapping functions, for instance, some adipokines cause altered appetite and satiety (e.g., vaspin and leptin), whereas a few are specifically known for impairing insulin sensitivity (e.g., RBP4, adiponectin and leptin). Therefore, functionally characterizing the specific roles of adipokines in metabolic disorders and elucidating the molecular mechanisms through which adipokines are secreted from adipocytes are the next biggest challenges for a more inclusive understanding of the role of adipose tissue in the physiology of metabolic diseases and whole-body energy homeostasis.

To date, clinicians rely on biomarkers to assist in diagnosis and to manage many pathological states. The biomarkers are especially needed when evident clinical signs or overt anatomic abnormalities are not visible or are absent. Additionally, biomarkers can identify persons within a population susceptible to a disease based on their "genotype" instead of their recorded history. Notably, only biomarkers can quantify the susceptibility to diseases and help to estimate the disease risk for a population [16]. Metabolic dysregulation has been reported as the cause of most diseases, including MetS, T2D [14] and cancer [15]. Therefore, metabolic disease biomarkers may provide relatively easy, negligibly invasive means of detecting those people who are at a higher risk for developing metabolic syndrome and its following complications. A panel of biomarkers, instead of an individual biomarker, would be advantageous. Since biomarkers

play multiple roles in multiple pathways in which they are involved, it would be difficult to interpret whether only one biomarker is specific and sensitive for the diagnosis of metabolic diseases. Furthermore, knowing the relations between biomarkers and metabolic disease risk factors would be helpful in assessing patients. Early detection would aid in early intervention and could be an effective means to reduce the widespread effects of metabolic diseases. A biomarker panel would also facilitate a way to personalize treatment based on aetiology differences amongst individuals [406].

Although numerous articles have been published listing both established and novel adipokines as biomarkers for metabolic diseases, the first two studies of this thesis compiled a panel of the most explored adipokines to provide a comprehensive adipokine profile and explore their non-overlapping functions for differentiating the clinical manifestations of metabolic syndrome. MetS is a cluster of cardiometabolic risk factors, including obesity, hypertriglyceridemia, reduced high-density lipoprotein cholesterol, hypertension, and insulin resistance [1]. To date the definition of MetS is not well established. Among the cardiometabolic risk factors of MetS, central obesity has been emphasized for decades [504]. Mortality due to cardiovascular disorders is higher in obese subjects even when all other MetS cardiometabolic risk factors are absent [634]. The findings of the first study of this thesis demonstrate the important role of central obesity in interacting with other MetS cardiometabolic risk factors to affect circulating levels of adipokines. The interaction between the components of the clinical phenotype (e.g., central obesity, insulin resistance, hypertension, and dyslipidaemia) of MetS might contribute to the development of a pro-

inflammatory state [493]. Researchers have suggested that a single adipokine may not induce MetS but that the interaction between pro-inflammatory and anti-inflammatory adipokines induces a systemic metabolic abnormality. Physiologically, a disproportion in the expression of anti-/pro-inflammatory adipokines results in the increased size of adipocytes. These hypertrophied adipocytes result in a shift towards the dominance of the pro-inflammatory adipokines [494]. The imbalance in the production of pro-inflammatory and anti-inflammatory biomolecules precedes increased immune cell infiltration and the induction of a macrophage phenotype switch in visceral adipose tissue [495]. The findings of the first study demonstrate the significant role of the interaction between central obesity and the other MetS cardiometabolic risk factors in affecting the circulatory levels of adipokines. Therefore, it is proposed that the interaction between central obesity and the cluster of all 4 MetS risk factors would favour a dominance of pro-inflammatory adipokines that might enhance the enlargement of adipocytes.

Renal insufficiency, cardiovascular disorders, cerebrovascular disease and metabolic syndrome are among the common sequelae of hypertension and obesity [186,191,193,505]. Obese hypertensive individuals have higher mortality rates and a higher risk of cardiovascular diseases than non-obese hypertensive subjects due to inappropriately increased cardiac output from relatively restricted arterial capacity [506,507]. A high rate of mortality due to cardiovascular disorders (myocardial infarction) has been observed more frequently in female adults compared to male adults [635], and the association between the waist circumference and blood pressure is also higher in females than in males [521].

Adipokines (secreted from adipose tissue) have been associated with high blood pressure, indicating their role as a link between abdominal obesity and hypertension [41]. Therefore, the second study of this thesis demonstrated the differential consequences of hypertension and abdominal obesity on proinflammatory and anti-inflammatory adipokine profiles in Hong Kong Chinese female adults with abdominal obesity, hypertension or both abdominal obesity and hypertension. The adipokine profile data demonstrated a decrease in anti-inflammatory adipokines (adiponectin) and an increase in proinflammatory adipokines (TNF-α) due to the interaction between abdominal obesity and hypertension. The circulating adiponectin concentration has been shown to be negatively associated with proinflammatory cytokines such as IL-6 and TNF-α [524,525]. The increase in adiponectin has also been shown to inhibit the expression of adhesion molecules and suppress adherence of TNF-α-stimulated endothelial cells to monocytes [527] , which is accompanied by reduced NFκB activation [528]. NFκB is a regulatory molecule that regulates proinflammatory cytokines such as TNF-α [529]. Cross-talk between angiotensin II and proinflammatory adipokines (TNF-α) participates in self-amplifying and sustaining positive feedback loops, resulting in progression of hypertension and cardiac remodeling [518]. Therefore, according to the results of the second study, the interaction between central obesity and hypertension might lead to the diminution of anti-inflammatory adipokine, adiponectin, and further exacerbation in proinflammatory adipokine, TNF-α, when compared to subjects with only central obesity or hypertension. Adiponectin was demonstrated to diminish M1 phenotypes of macrophages (which favor proinflammatory microenvironment) by downregulating proinflammatory cytokines including

TNF-α, lL-6, and MCP-1. Besides, adiponectin has been shown to upgrade endothelial cell function by increasing nitric oxide (NO) and prostaglandin I2 production in endothelial cells [636]. Collectively, our results drive us to propose that the diminution of adiponectin might exacerbate TNF-α production and subsequently affect the vascular functions when obesity interacts with hypertension. Our results might provide hint for explaining the high mortality rate documented in central obese individuals with hypertension.

Adipokines have been shown to be associated with the severity of liver disease [637] and the severity of kidney disease [638]. For instance, patients with overt diabetic nephropathy were observed to have elevated plasma adiponectin levels, regardless of increased urinary adiponectin excretion compared to diabetic patients with normal microalbuminuria and normal renal function [639]. The study suggested that increases in adiponectin levels in the blood plasma of patients with overt diabetic nephropathy occurred to mitigate microvascular damage [639]. On the other hand, diabetic patients with overt diabetic nephropathy have been demonstrated to have low plasma adiponectin levels, suggesting the progression of kidney disease [640]. Although adiponectin has anti-inflammatory and anti-atherogenic properties, some studies have shown that higher levels of adiponectin versus lower levels of adiponectin predict poor patient outcomes, indicating a complex relationship between various adipokines. Therefore, in the third and fourth study of this thesis, adipokines were profiled in worsening metabolic conditions, including MetS combined with high-normal blood pressure in human subjects and diabetic skeletal muscle with DOX administration in mouse models.

Reduction in waist circumference and blood pressure have been shown as positive health consequences of regular yoga training [2,641]. Consistently, our previous investigation has also demonstrated that 1-year yoga training decreased waist circumference and tended to reduce systolic blood pressure when compared to control group without receiving yoga intervention in middle-aged and older adults with MetS [2]. The results of the third study are in accordance with the previous findings that average change in waist circumference was significantly decreased but both systolic and diastolic blood pressure were not decreased in subjects with yoga intervention as compared to the control subjects with MetS and high-normal blood pressure. Additionally, the results indicated that there was no interaction effect between yoga and 1-year time on any of the five risk factors of MetS in our examined subjects with high-normal blood pressure. However, the results demonstrated that 1-year yoga training reduced the abundances of circulatory proinflammatory adipokines (leptin, chemerin, and PAI-1) and increased the concentration of circulatory anti-inflammatory adipokine (adiponectin) in adults with high-normal blood pressure and MetS. Numerous studies go for the idea that hypertensive patients with MetS would show early signs of end-organ damage, which are recognized as significant independent predictors of adverse cardiovascular outcomes as compared to those without MetS [562,642]. Therefore, individuals with high-normal blood pressure and MetS should require special attentions in whom the MetS risk factors did not reflect the protective effect of yoga. In accordance to our previous study assumptions, that the effects of yoga exercise on systolic blood pressure might be depended on the quantity of the intervention and/or subject compliance to the intervention[2], we again suggest

that extra attention should be given to MetS subjects with high-normal blood pressure.

In the fourth study of this thesis, we observed that DOX treatment in diabetic skeletal muscle does not exacerbate insulin signalling and muscle atrophy compared to non-diabetic skeletal muscle. However, DOX exposure in diabetic skeletal muscle supports a proinflammatory microenvironment by upregulating a transcription factor (HIF-1α), a regulatory molecule (pNFκBp65) and a cytokine (TNF-α) and hinders an anti-inflammatory microenvironment by downregulating a transcription factor (pAMPKβ1/2Ser^{108}), a regulatory molecule (PGC-1α) and a muscle-specific myokine (IL-15). Additionally, DOX exposure in diabetic skeletal muscle induced the deregulation of glycolytic metabolism, indicated by the upregulation of PDK4 and LDH and the downregulation of pACCSer79. The results of both the third and fourth studies of this thesis suggest that adipokines may be a useful biomarker to assist in the diagnosis and management of worsening metabolic conditions when evident clinical signs or obvious anatomic abnormalities are not apparent or absent.

Adipose tissue is an active endocrine organ, and its secretions are involved in kidney damage, changes in immune response, endothelial dysfunction, inflammation and oxidative stress and have been linked to renal sympathetic nerve activity. There are no specific roles or impacts of individual adipokines that seem to induce an imbalance in pathophysiology. The results of this thesis support the notion that each adipokine plays a particular role in maintaining the delicate equilibrium depending on its pathophysiological and protective impacts [638,639].

Limitations and recommendations:

Adipose tissues can communicate and influence many other organs, including the brain, heart, vasculature, liver, and muscle, through the production of various secretory factors or adipokines. Adipokines have both proinflammatory and anti-inflammatory roles, and the balance between the different factors is important for determining homeostasis throughout the body based on nutritional status. When adipocyte dysfunction occurs because of adipose tissue expansion (which may be due to physical inactivity or over-nutrition), the dysregulation of adipokine production can have local or systemic effects on inflammatory responses, which further contribute to the initiation and progression of obesity-induced metabolic and cardiovascular complications. Adipokines are numerous in number, and new ones are still being discovered. The collective change in adipokine expression is part of coordinated mechanisms, but the differential regulation of the release of each adipokine is due to the response of each fat cell to altered metabolism [643]. The key adipokines on which we should focus remain unclear. Currently, this is a hard question to answer as several adipokines and their emerging functions are still being discovered. Therefore, we profiled only a few well-described adipokines to determine their non-overlapping functions, which might be one of the limitations of this thesis. Therefore, it is suggested that a study profiling all adipokines might help to determine their overall functional mechanisms in metabolic diseases.

The second limitation of the present thesis is related to its cross-sectional design. A cross-sectional design examines the relationship between exposure

and outcome prevalence in a defined population at a single point in time. A cross-sectional design has the advantage that it is less time consuming than case-control or cohort studies, but it has its limitations. In cross-sectional studies, it is difficult to determine the temporal relationship between exposure and outcome because a time element is lacking [644]. Therefore, the present thesis cannot distinguish whether the adipokine dysregulations are the cause or effect of the abnormalities in the MetS risk factors. Therefore, a cohort study or case-control study is suggested as these designs can help to understand causes, incidence, and prognosis because they measure events in chronological order and can be used to distinguish between the cause and effect [644].

Further, the prevalence of MetS is high and varies greatly among ethnicities. For instance, a Suriname Health Study (a national survey) including 2946 participants was designed based on WHO guidelines. The authors determined the prevalence of MetS and its components for all ethnicities. The regression analysis of this study revealed that there were associations of ethnic origin, age, marital status, sex, educational level, employment, income status, smoking status, physical activity, residence, and fruit and vegetable intake with MetS [645]. Ethnic discrepancies in the risk of metabolic diseases may result in differences in circulating adipokines and inflammatory markers related to ethnic variations in obesity and body fat distribution [37]. In comparison to Caucasians, significant ethnic differences were observed for all biomarkers except TNF-α. Japanese-American men and women had significantly lower CRP and leptin levels than Caucasians, and Japanese-American women also had lower adiponectin

levels. Leptin was reported to be substantially higher in African-American women ($P < 0.01$), and adiponectin was significantly lower in African-American men and women ($P = 0.02$ and $P < 0.001$) [37]. Our thesis focused mainly on Hong Kong Chinese adults. Therefore, the results of this thesis are ethnic (Hong Kong Chinese)-specific and may not apply to the general population. It is suggested that adipokine profiling for all ethnicities would be useful to determine the normal physiological levels of a panel of adipokines and their correlations with ethnic parameters. Therefore, knowledge about ethnic-specific adipokine profiles would further help to gain an unambiguous picture about the role of adipokines.

Additionally, one of the studies of this thesis focused specifically on female subjects. Therefore, the first limitation of this study is that its results cannot be generalized. Second, female subjects have been reported to have fluctuating levels of sex hormones and some adipokines [646]. The fluctuations are modulated by menopausal status [647]. However, the mean age of the female subjects included in this thesis was 58 ± 11 years, which is much greater than the reported mean age (51 years) for the onset of menopause in Chinese females [648]. Still, the unclear menopause status of the female subjects is a limitation of this thesis. Therefore, it is suggested that further studies on adipokines in female subjects should include their menopause status.

One of the limitation of the thesis is small saple size. Although the adopted sample size has resulted in a statistical power of 80% or above for the assessments on most of our noteworthy results, the constraint of sample size might be a limitation in both the studies. Also, there is great species difference

between human and animal model. Although DOX exposure resulted in the enhancement of a proinflammatory favouring microenvironment, the conclusions of the fourth study 4 may not be generalized to human species. Therefore, it is suggested that further studies on micro environment shift in diabetic human subjects injected with DOX should be investigated.

Chapter 8

Conclusions and recommendations

Studies on metabolic diseases, such as metabolic syndrome and T2D, are crucial to the health of the world's population and the global economy. This thesis may be a substantial step towards enhancing the knowledge of the specific role of each adipokine in maintaining a delicate equilibrium that depends on their pathophysiological and protective impacts [638,639]. The first two studies of this thesis explored adipokine profiles for differentiating the clinical manifestations of metabolic syndrome. The results of the third and fourth studies of this thesis suggested that adipokines may be a useful biomarker to assist in the management and diagnosis of worsening metabolic conditions when clinical signs or anatomic abnormalities are not obvious.

The conclusions of this thesis are as follows:

1) The interaction effect between central obesity and a cluster of MetS cardiometabolic risk factors on increasing TNF-α and leptin levels and decreasing adiponectin levels reveals the inter-individual variability that exists in the obese phenotype. Our findings are consistent with the notion that central obesity is an independent and important risk factor that deserves special attention when characterizing the cardiometabolic profiles of MetS patients. Additional research is needed to fully understand the precise relationship between obesity and other MetS cardiometabolic risk factors. Further explanation of the molecular mechanisms through which these adipokines interact might be the next

biggest challenge towards a more inclusive understanding of the role of adipose tissue in the physiology of metabolic diseases and whole-body energy homeostasis.

2) The interaction between abdominal obesity and hypertension leads to a decrease in adiponectin that may exacerbate TNF-α production, affecting vascular function in adult females. In line with our results, research suggests that high adiponectin levels protect against ischaemia-induced vascular injury in the retina by decreasing the TNF-α inflammatory response [649]. Although obesity-related hypertension may be the result of a combination or overlap of several factors, determining the mechanisms of adipose tissue derivatives (adipokines and cytokines) would enhance our understanding of their contribution to the pathogenesis of this dangerous combination of obesity and hypertension. Hence, the presence of obesity-related outcomes such as hypertension may be an indication of the necessity for more exhaustive treatment options, especially in females.
3) Yoga training is a potential lifestyle intervention that reduces chronic inflammation by downregulating proinflammatory adipokines and upregulating anti-inflammatory adipokines in individuals with high-normal blood pressure and MetS. This is in line with the notion that MetS is a condition characterized by chronic low-grade inflammation [467]; hence, an equilibrium between both anti-inflammatory and proinflammatory microenvironments is important in preventing MetS as they have a central and peripheral effect on both metabolism and energy balance. It is suggested that metabolic syndrome subjects with high-

normal blood pressure have a dangerous combination so they should be given additional care.

4) There might be a link between modulation of the inflammatory pathway and a shift from an oxidative to glycolytic metabolism in diabetic skeletal muscle after doxorubicin (an anti-cancer drug) exposure. Our results are valuable for the development of new, effective strategies to protect skeletal muscle from DOX-induced toxicity in diabetic cancer patients. Additional research is required regarding the inflammatory and metabolic pathways to better understand worsening T2D conditions.

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