

To the Graduate Council:

I am submitting here with a thesis written by Christina Marie Caserio entitled “Calcitriol and the Renin Angiotensin System, and Adipose Tissue Inflammation.” I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Michael Zemel, Major Professor

We have read this thesis and
recommend its acceptance:

Jay Whelan

Jang Han Kim

Accepted for the Council:

Carolyn R. Hodges
Vice Provost and Dean of the Graduate School

(Original signatures are on file with student records)

Calcitriol, the Renin Angiotensin System, and Adipose Tissue Inflammation

A Thesis

Presented for the Masters of Science Degree

The University of Tennessee, Knoxville

Christina Marie Caserio

December 2009

Copyright © Christina Marie Caserio 2009 All Rights Reserved

Dedication

This thesis is dedicated in loving memory to my grandparents,
James Caserio and Jolanda Caserio

Acknowledgements

I wish to thank all those who helped me complete my Master of Science degree in Nutritional Science.

I would like to thank Dr. Zemel for his guidance and introduction to calcitriol and adipose tissue metabolism. I would like to thank Dr. Whelan and Dr. Kim for serving on my research committee. I would like to thank Dr. Sun for her valuable instruction in laboratory techniques and interpretation of data. I would also like to thank Beth Wilson for her statistical support.

Lastly, I would like to thank my family, whose encouragement and support made this work possible.

Abstract

Adipose tissue is well recognized as an endocrine organ and a source of proinflammatory molecules¹. We recently demonstrated calcitriol stimulates adipocyte reactive oxygen species (ROS) production and inflammatory stress (IS), while dietary calcium suppression of calcitriol exerted the opposite effect. These effects are mediated, in part, by calcitriol modulation of intracellular calcium (Ca^{2+}) signaling and mitochondrial potential. However, adipocytes contain a functional RAS, and angiotensin II (ANGII) modulates ROS and IS. Accordingly, we investigated the role of ANGII in mediating calcitriol effects. Calcitriol (1 nM) stimulated NOX4 expression and ROS production in 3T3-L1 adipocytes by 67% ($p < 0.01$), while these effects were reversed by ACE inhibition (enalapril) or antagonism of either ANGII type 1 receptor (AT1R) or ANGII type 2 receptor (AT2R), antagonism. Similarly, ANG (0.1-1.0 nM) stimulated NOX4 expression ($p < 0.03$) and this effect was reversed by AT1R or AT2R antagonism. Calcitriol and ANGII both suppressed adiponectin expression ($p < 0.04$) and increased IL6 and MCP-1 expression ~2 fold ($p < 0.03$), and these effects were reversed with enalapril or AT2R, but not AT1R, antagonism. These data demonstrate that calcitriol modulation of adipocyte ROS production and IS is modulated, in part, by the adipocyte RAS.

Table of Contents

Chapter I	1
Introduction and Background Information	1
The Obesity Epidemic.....	1
Function of Adipose Tissue	2
Effects of Obesity on Adipose Tissue.....	5
Vitamin D: A multifunctional Hormone	7
Synthesis and Metabolism of Vitamin D	7
Classical Function of Calcitriol: Bone Mineralization.....	8
Structure of Calcitriol	9
Nonskeletal Functions of Calcitriol	11
Calcitriol Modulates Adipose Tissue Metabolism.....	13
Energy Metabolism.....	16
Production of Cellular Energy	16
Uncoupling Proteins.....	17
Calcitriol Modulates Uncoupling Protein Expression.....	19
Glucocorticoids Modulate Adipose Tissue Metabolism	20

The Role of Glucocorticoids in the Development of Obesity	20
Calcitriol Up-regulates Glucocorticoid Activity in Adipose Tissue	22
The Renin Angiotensin System	24
Adipose RAS and Adipose Tissue Dysfunction	24
Dysregulation of Adipocytokine Production	26
The AngII Receptor	27
Inflammation in Obese Adipose Tissue	30
Stimulators of Reactive Oxygen Species Production.....	30
Macrophage Accumulation and Adipose Tissue Inflammation	32
Calcitriol Modulates Macrophage Activity.....	37
Pharmacological Inhibition of RAS	38
Additional Benefits	38
Improvement in Metabolic Parameters	38
Special Attributes of Telmisartan	39
Irbesartan Prevents Degradation of Adiponectin	41
Remodeling of Adipose Tissue	43
Negate Effects of Obesity Induced Hypoxia.....	44
Antioxidant Effect.....	45

Pancreatic Beta Cell Function and Insulin Sensitivity.....	47
Down-Regulate Inflammatory Signaling.....	50
Problem Statement.....	55
Chapter II.....	56
Research Paper.....	56
The Adipocyte Renin Angiotensin System Mediates the Effects of Calcitriol on Oxidative Stress and Cytokine Production in Cultured 3T3-L1 Cells	56
Introduction.....	56
Methods	59
RNA Extraction	59
Quantitative Real Time Polymerase Chain Reaction.....	59
Gene Silencing with Small Interfering RNA	60
Chemicals.....	61
Statistical Analysis.....	61
Results.....	62
Effects of Calcitriol.....	62
Effect of RAS.....	62
Effect of AT1R Knockdown.....	63

Discussion.....	64
Conclusions.....	68
Future Research	69
List of References	70
Appendix.....	117

List of Figures

Figure 1A. Effects of calcitriol and RAS on IL-6 and 18S expression ratio with differentiated 3T3-L1 adipocytes.	118
Figure 1B. Effects of calcitriol and RAS on MCP-1 and 18S expression ratio with differentiated 3T3-L1 adipocytes.	118
Figure 1C. Effects of calcitriol and RAS on NOX4 and 18S expression ratio with differentiated 3T3-L1 adipocytes.	118
Figure 1D. Effects of calcitriol and RAS on adiponectin and 18S expression ratio with differentiated 3T3-L1 adipocytes.	118
Figure 2A. Effects of RAS on IL-6 and 18S expression ratio in differentiated 3T3-L1 adipocytes.	119
Figure 2B. Effects of RAS on MCP-1 and 18S expression ratio in differentiated 3T3-L1 adipocytes.	119
Figure 2C. Effects of RAS on adiponectin and 18S expression ratio in differentiated 3T3-L1 adipocytes.	119
Figure 3A. Effects of AT1R small interfering RNA knockdown and RAS on AT1R and 18S expression ratio in differentiated 3T3-L1 adipocytes.	120

Figure 3B. Effects of AT1R small interfering RNA knockdown and RAS on MCP-1 and 18S expression ratio in differentiated 3T3-L1 adipocytes.	120
Figure 3C. Effects of AT1R small interfering RNA knockdown and RAS on NOX-4 and 18S expression ratio in differentiated 3T3-L1 adipocytes.	121
Figure 4A. Effects of AT1R small interfering RNA on AT1R and 18S expression ratio	122
Figure 4B. Effects of AT1R small interfering RNA on MCP-1 and 18S expression ratio	123
Figure 4C. Effects of AT1R small interfering RNA on NOX-4 and 18S expression ratio	124

Abbreviations

ACC2	Acetyl-coA carboxylase-2
ACE	angiotensin converting enzyme
ADD	adipocyte determination and differentiation
AGEs	advanced glycated end products
AGT	Angiotensinogen
ANGII	angiotensin II
AP	activator protein
aP2	adipocyte lipid binding protein
ARBs	ANGII receptor blockers
ATP	adenosine triphosphate
BAT	brown adipose tissue
BMI	body mass index
Ca ²⁺	intracellular calcium
cAMP	cyclic adenosine monophosphate
CD14	macrophage surface specific protein
C/EBP	CCAAT/enhancer binding protein
CREB	cAMP response element binding protein
CYP27A1	25-hydroxylase
CYP27B1	1- α -hydroxylase
DEPC	diethylpyrocarbonate
DMEM	Dulbecco's modified Eagle medium
ERK1/2	extracellular signal regulated kinase1/2
FAS	fatty acid synthase
FBS	fetal bovine serum
GLUT	glucose transporter
GPCRs	G protein coupled receptors
GRK	G-protein coupled receptor kinase
GST-p65	Glutathione-S-transferase p65
HIF	hypoxia inducible factor
IBMX	Iso-butyl-1-methyl-xanthine
ICAM	intracellular adhesion molecule
IGF	insulin like growth factor
IKB	I-kappa beta
IKKB	IKB kinase beta
IKK	IKB kinase
IL	Interleukin
IR	insulin receptor
IRS	insulin receptor substrate
JNK	c-jun N terminal kinase

LPL	lipoprotein lipase
M1	classically activated macrophages
M2	alternatively activated macrophage
MAPK	mitogen activated protein kinase
MCP	monocyte chemoattractant protein
M-CSF	macrophage colony stimulating factor
MEK	Ras/mitogen activated kinase
MIF	macrophage inhibitory factor
mVDR	membrane VDR
NADPH	nicotinamide adenine dinucleotide phosphate
NEFA	non-esterified free fatty acids
NIK	NFKB inducing kinase
NFKB	nuclear factor kappa beta
NO	nitric oxide
NOS	nitric oxide synthase
NOX4	NADPH Oxidase isoform
nVDR	nuclear VDR
OD	optical density
PAI	plaminogen activator inhibitor
PCR	polymerase chain reaction
PD	PD123177 AT2R chemical inhibitor
PKC	protein kinase C
PI3-K	phosphoinositide 3- kinase
PPAR	peroxisome proliferator-activated receptor
PS	penicillin/streptomycin
PTH	parathyroid hormone
RANKL	receptor activator for NFKB ligand
RAS	renin angiotensin system
RAW264.7	clonal murine monocyte/macrophage cell line
ROS	reactive oxygen species
RSK	ribosomal S6 kinase
RXR	retinoid-X-receptor
SD	standard deviation
SOD	superoxide dismutase
SPARMS	Selective PPAR modulators
SREBP	sterol regulatory element binding protein
T2DM	type 2 diabetes mellitus
TLR	toll-like receptor
TM	telmisartan AT1R chemical inhibitor
TNF	tumor necrosis factor
TZDs	Thiazolidinediones
UCP	uncoupling protein

VDR	vitamin D receptor
VEGF	vascular endothelial growth factor
WAT	white adipose tissue
1,25D ₃ MARRS	membrane associated rapid response steroid binding protein
11βHSD-1	11-β-hydroxysteroid dehydrogenase type 1
24OHase	25-hydroxyvitamin D ₃ -24-hydroxylase

Chapter I

Introduction and Background Information

The Obesity Epidemic

Obesity is a multifactorial disease that increases the risk for type 2 diabetes mellitus, cardiovascular disease, osteoarthritis, chronic obstructive pulmonary disease and development of numerous forms of cancer². Obesity has reached a pandemic status, becoming a top public health concern due to the increased morbidity and mortality associated with obesity². According to the International Obesity Task Force, more than 1.1 billion adults worldwide are overweight and 312 million adults are considered obese³. Both developed and developing countries are confronted with an obesity epidemic with the emergence of multifactorial cluster of metabolic diseases³. While the etiology of obesity is complex, the root cause is quite simple, energy excess. Although genetics may influence an individual's susceptibility towards obesity, the increased prevalence of obesity is most likely attributed to environmental factors².

Environmental factors that influence increased caloric consumption include increased portion sizes, increased consumption of high sugar beverages, refined carbohydrates, restaurant dining, and a sedentary lifestyle². Weight gain reflects a long term positive energy balance resulting from energy consumption being greater than energy expenditure⁴. Decreased energy expenditure has been an issue of concern dating back to the 1950's when President Eisenhower initiated the council on fitness and health to promote the importance of physical activity as a component to a healthy lifestyle⁵. However, decreased physical activity has remained a problem and has been compounded by the popularity of sedentary activities such as television viewing, increase usage of computers and the convenience of time and energy saving transportation

methods⁴. Numerous studies investigating the impact of physical activity on body weight have uniformly concluded that body fat increases proportionally with a decrease in the level of physical activity⁶⁻⁹. The major driving forces of obesity are environmental factors that shift the energy balance creating an increase in energy input and a decrease in energy output⁴.

The International Obesity Task Force of the World Health Organization developed an index for classifying degrees of obesity based on the body mass index (BMI)¹⁰. $BMI = (\text{weight [kg]} / \text{height [m]}^2)$. Using BMI as an index, grade 1 consists of a BMI of 25-29.9 kg/m² and is defined as overweight¹⁰. Grade 2 is classified by a BMI of 30-39.9 kg/m² and is called obese¹⁰. Grade 3 consists of a BMI of greater than or equal to 40 kg/m² and is defined as morbid obesity¹⁰. In the United States it has been estimated greater than 60% of adults are categorized as overweight or obese⁴. In order to gain control of this epidemic, it is critical to clarify the mechanisms responsible for modulating lipid metabolism and thereby provide therapeutic anti-obesity strategies.

Function of Adipose Tissue

Due to the prevalence of obesity, adipose tissue has gained scientific interest in the biology of adipose tissue lipid metabolism and its effects on systemic metabolism and physiology¹¹⁻¹³. Two types of adipose tissue exist, white and brown adipose tissue with dichotomous physiological function¹⁴. White adipose tissue (WAT) is found in all vertebrates and functions as a storage depot for times of energy excess¹⁴. In contrast, brown adipose tissue (BAT) functions in energy dissipation instead of storage and is essential for thermal homeostasis of hibernating mammals¹⁵. The classical function of WAT was perceived to be a passive storage site for excess fatty acids after ingestion of food^{12, 16, 17}. However, later research emerged showing that adipose

tissue is an endocrine organ that synthesizes and secretes a plethora of adipokines in addition to fatty acids ^{11, 12, 17-19}.

Adipokines have a wide range of physiological and metabolic effects contributing to energy balance, insulin sensitivity, vascular hemodynamics, lipid metabolism, angiogenesis, blood pressure regulation, and inflammation ²⁰. In mammals the predominate form of adipose tissue is WAT ¹. WAT is composed of a heterogeneous mixture of cell types including mature adipocytes, pre-adipocytes, endothelial cells, vascular smooth muscle cells, leukocytes, monocytes and macrophages ^{16, 21}. WAT demonstrates substantial fluidity in its ability to increase or decrease its mass over a 10-fold range to accommodate lipid storage ²⁰. During lipogenesis, enzymes responsible for import of fatty acids, glucose, and intra-adipocyte lipid synthesis stimulate accumulation of triglyceride in lipid droplets ²². Lipid droplets coalesce together distending the size of the adipocyte up to 120 μm in diameter ²². The tendency for enlarged adipocytes and increased number are directly regulated by circulating hormones and nutrients²². The process of overfilling lipid droplets, as a consequence of obesity, has been demonstrated to modulate adipocyte metabolism, resulting in altered function ²³⁻²⁵.

Excess fatty acids can be secreted from hypertrophic adipocytes, potentially impairing the functional activity of peripheral tissues promoting the development of obesity-induced morbidities ²⁶. A pattern of body fat distribution localized to the abdominal region is highly correlated with a significant increase in risk factor for the development of obesity associated metabolic complications and pathologies ²⁷⁻²⁹. In the 1950's, the French physician, Jean Vague, was the first to recognize the direct correlation between body fat distribution and metabolic morbidities ^{28, 30}. Vague identified that android (upper body) obesity was highly associated in patients presenting with diabetes, gout and atherosclerosis and less evident in patients with gynoid (lower body) obesity ^{28, 30}. Later studies confirmed Vague's initial clinical observations

demonstrating the significance of the anatomical distribution of adipose tissue to development of metabolic disease^{27, 29, 31-36}.

WAT is a critical regulator in buffering the uptake of dietary fat acids from circulation^{16, 19}. This buffering action is maintained by inhibiting the release of non-esterified fatty acids into circulation while increasing the removal of triglycerides from circulation^{16, 37}. In the obese state, the buffering capacity for lipid storage is diminished due to overfilling of lipids¹. Consequently a redirection in the storage of fatty acids in non-adipose tissues may occur, resulting in ectopic lipid accumulation^{11, 16, 38, 39}. Deposition of lipids within the cytoplasm of non-adipose tissue cells, such as skeletal muscle and liver has been demonstrated to impair cell signaling and tissue function^{21, 39}. The occurrence of insulin resistance in obese subjects has been demonstrated to be a consequence of hypertrophic fat cells^{40, 41}. Hypertrophic adipocytes have a tendency to be insulin resistant corresponding to a diminished clearance of plasma triglycerides and increased leakage of free fatty acids⁴¹. In contrast, a lack of adipose tissue, known as lipodystrophy, has also been shown to lead to ectopic lipid accumulation, insulin resistance, and development of type 2 diabetes mellitus (T2DM)^{42, 43}.

Genetically modified fatless mice, A-ZIP/F-1 have extreme insulin resistance and excessive ectopic lipid accumulation in skeletal muscle and liver⁴³. Surgical transplantation of adipose tissue from healthy donor mice into lipodystrophic mice reduced hyperglycemia, normalized insulin levels, and improved insulin sensitivity⁴³. High levels of circulating free fatty acids induce accumulation of intracellular lipids in non-adipose tissues increasing peripheral insulin resistance⁴⁴. In addition, excess circulating fatty acids may also cause pancreatic dysfunction and possibly apoptosis of pancreatic beta cells, resulting in a diminished insulin supply⁴⁵. Consequently, these conditions enhance insulin resistance, glucose intolerance, hyperlipidemia, and hyperinsulinemia⁴⁶. These findings demonstrate that impairment in the

storage of fatty acids may promote ectopic fat accumulation impairing metabolism. Therefore, maintaining the buffering function of adipose tissue is critical for the prevention of obesity-induced metabolic disturbances.

Effects of Obesity on Adipose Tissue

Multiple cellular processes, including adipocyte hypertrophy, recruitment of adipocyte precursors, adipocyte differentiation, and neovascularization are all coordinated in the development of obesity⁴⁷. Recent evidence has emerged demonstrating that cellular inflammation is a primary mediator of obesity associated adipose tissue dysfunction⁴⁸. Obesity is associated with an up-regulation in inflammatory signaling pathways such as c-jun N terminal kinase (JNK) and nuclear factor kappa beta (NFkB)⁴⁹. Stimulation of inflammatory pathways significantly increase expression of downstream inflammatory cytokines, including tumor necrosis factor- α (TNF)- α , interleukin-6 (IL)-6, and monocyte chemoattractant protein-1 (MCP)-1⁵⁰⁻⁵². Furthermore, a comparison of tissues from obese people and lean people demonstrated that obese subjects had an increase in classically activated macrophages (M1) which are associated with secreting inflammatory cytokines TNF- α and IL-6¹⁷.

Local pro-inflammatory paracrine loop has been suggested to result from adipocyte secreted free fatty acids and macrophage production of TNF- α ^{17, 53}. As free fatty acids are released from enlarged adipocytes, the free fatty acids bind to macrophage-toll-like receptor-4 (TLR)-4 which leads to the activation of NFkB resulting in an increased production of TNF- α ^{17, 53}. The up-regulation of TNF- α then leads to activation of numerous other pro-inflammatory molecules including; intracellular adhesion molecule-1 (ICAM)-1, IL-6, and MCP-1^{17, 53, 54}. In addition to an up-regulation of pro-inflammatory molecules, enlarged adipocytes have reduced levels of the

anti-inflammatory molecule, adiponectin^{17, 54, 55}. Adiponectin is an important adipokine in the regulation of metabolism by reducing hepatic gluconeogenesis and increasing lipid oxidation in muscle⁵⁶. Reduced levels of adiponectin have been suggested to augment the vicious paracrine cycle between pro-inflammatory state of macrophages and adipocytes^{17, 53, 55}.

The excessive abundance of adipose tissue requires an increase in nutrients and oxygen for the transport of fatty acids. Consequently, an expansion of the microvasculature is required and is an intricate part in the development of obesity¹⁷. Hypertrophic adipocytes are also associated with local adipocyte hypoxia as the diffusion limit of oxygen (~100 μ M) is unable to keep up with the rate of growth of the adipocyte^{12, 17, 53}. Hypoperfusion activates the expression of hypoxia-inducible transcription factors resulting in the expression of angiogenic factors including vascular endothelial growth factor (VEGF), angiopoietins, and plasminogen activator inhibitor-1 (PAI)-1⁵⁴. The up-regulation of angiogenic factors inhibits adiponectin gene transcription, as observed in obese, insulin resistant mice¹⁷. Adiponectin functions to enhance insulin sensitivity via reducing hepatic glucose production and augmenting fatty acid oxidation in muscle and liver^{17, 55}. Consequently, a reduction in the expression of adiponectin increases insulin resistance augmenting the vicious pro-inflammatory paracrine loop between adipocytes and macrophages¹⁷.

Vitamin D: A multifunctional Hormone

Synthesis and Metabolism of Vitamin D

Vitamin D is a fat soluble pro-hormone found in two major forms, ergocalciferol D₂ and cholecalciferol D₃⁵⁷. Ergocalciferol D₂ is synthesized from irradiation of ergosterol occurring in plankton and has only 1/3 the biological activity as vitamin D₃ in humans⁵⁸. Cholecalciferol vitamin D₃ is the natural form of vitamin D that can be endogenously synthesized by the skin given appropriate wavelengths of UV light⁵⁸. Vitamin D₃ is produced from 7-dehydrocholesterol, a derivative of cholesterol, and then photolyzed by UV light to produce pre-vitamin D₃⁵⁸. Pre-vitamin D₃ spontaneously isomerizes into biologically inert vitamin D₃⁵⁸. Whether vitamin D₃ is obtained from the diet or endogenously synthesized by the skin it must be converted into its biologically active form via a two step hydroxylation process⁵⁸.

The first hydroxylation occurs in the liver by the enzyme 25-hydroxylase (CYP27A1) to produce the metabolically inactive 25-hydroxycholecalciferol (calcidiol)⁵⁸. A second hydroxylation occurs in the kidney by the enzyme 1- α -hydroxylase (CYP27B1) to produce two dihydroxylated metabolites the primary hormone 1,25-dihydroxycholecalciferol (calcitriol) and the candidate hormone 24R,25(OH)₂ D₃⁵⁸. Plasma vitamin D levels are measured using the primary circulating metabolite, 25-hydroxycholecalciferol (calcidiol)⁵⁹. Calcidiol has a longer half life than calcitriol suggesting that its concentration is more stable and a better predictor of plasma vitamin D status⁵⁹. The average plasma concentration of calcidiol in a healthy individual averages around 25-125 nM and while circulatory levels of calcitriol range from 0.1 nM to 10 nM^{60 59}. Calcitriol is released into circulation bound to the carrier protein, vitamin D

binding protein and transported to target organs ⁵⁸. Binding of calcitriol to a nuclear receptor or plasma membrane receptor of target cells generates the biological responses of calcitriol ⁵⁸.

Classical Function of Calcitriol: Bone Mineralization

The classic physiological function of calcitriol is mineralization of the skeleton by increasing plasma calcium levels ⁵⁸. Extracellular calcium concentration is strictly maintained at 5 mM while intracellular concentration is significantly lower around 0.05-10 μ M ^{61, 62, 63}. Calcitriol stimulates plasma calcium levels via three distinct mechanisms ⁵⁸. First calcitriol induces active intestinal absorption of both calcium and phosphate ⁵⁸. Second, in response to hypocalcemic plasma levels, calcium sensitive proteins on parathyroid cells stimulate secretion of parathyroid hormone (PTH) ⁵⁸. In a concerted mechanism, PTH and calcitriol activate osteoblasts generating receptor activator nuclear factor kappa beta ligand (RANKL) a critical factor in bone metabolism ⁵⁸. RANKL activates osteoclasts resulting in calcium mobilization from bone, raising serum calcium levels ⁵⁸.

PTH also activates CYP27B1 in the distal renal tubule stimulating the synthesis of calcitriol ⁵⁸. Calcitriol and PTH synergistically stimulate the reabsorption of the 1% of the last filtered load of calcium ⁵⁸. The increase in plasma calcium levels beyond the set point of the calcium sensing system suppresses PTH production completing the negative feedback loop ⁵⁸. If plasma calcium levels become hypercalcemic, the “C” cells of the thyroid gland synthesize the peptide calcitonin ⁵⁸. Calcitonin blocks mobilization of calcium from bone and calcium reabsorption from the kidney ⁵⁸. Under normocalcemic conditions calcitonin up-regulates renal CYP27B1 for synthesis of calcitriol ⁵⁸.

A critical function of calcitriol is maintaining the parathyroid status in normal individuals⁵⁸. Calcitriol suppresses the preproparathyroid gene and thereby inhibits proliferation of parathyroid gland cells regulating PTH production⁵⁸. Degradation of the vitamin D metabolites is induced by the enzyme, 25-hydroxyvitamin D₃-24-hydroxylase (24OHase) to produce either 24,25(OH)₂D₃ or 1,24,25(OH)₃D₃⁶⁴. Following hydroxylation at carbon number 24, the metabolites are further degraded and excreted as calcitroic acid or 23-carboxyl derivatives⁶⁴. This degradation pathway is directly monitored by levels of calcitriol which stimulates expression of 24OHase, thereby inhibiting excessive production of the hormone⁶⁴.

Structure of Calcitriol

The molecular structure of calcitriol is similar to that of the classic steroid hormones estradiol, cortisol and aldosterone⁵⁷. Structurally they are related as they share the same root ring structure cyclopentanoperhydrophenanthrene⁵⁷. However, calcitriol differs in that it is a secosteroid with one root ring structure broken⁵⁷. Steroid hormones function as chemical messengers transmitting signals to produce genomic and rapid responses⁶⁵. The presence of cognate vitamin D receptors in target tissues and organs enables calcitriol to generate its biological effects⁶⁵. In 1969, the discovery of the nuclear vitamin D receptor spurred extensive research into the physiological function of the vitamin D endocrine system⁶⁵.

The ligand activated transcriptional effects of calcitriol occur upon binding of calcitriol to the nuclear vitamin D receptor (nVDR) inducing heterodimerization of nVDR with the retinoid X receptor (RXR)⁶⁵. Calcitriol induced heterodimerization of nVDR-RXR is the functionally active transcription factor in calcitriol targeted gene activation⁶⁴. The heterodimer DNA binding to the vitamin D response elements results in a concomitant recruitment of numerous co-activator

proteins initiating formation of multi-protein complexes that together with basal transcriptional machinery and histone modifiers stimulate transcription of vitamin D responsive elements of well over 200 genes ⁶⁴⁻⁶⁶.

In the late 1980's, evidence emerged demonstrating that calcitriol could quickly, within seconds generate a multitude of cellular responses via a plasma membrane receptor ⁶⁵. This membrane receptor was later identified as the membrane associated rapid response steroid binding protein (1,25D₃-MARRS) ⁶⁵. Activation of the membrane vitamin D receptor (mVDR) is associated with signal transduction pathways leading to stimulation of calcium channels with an influx of intracellular calcium ⁶⁷. Evidence of the non-genomic actions of calcitriol in various cell types include; rapid transcellular movement of calcium across chick enterocytes, rapid calcium mobilization from internal stores of osteoblasts and mitogen activated protein kinase (MAPK) stimulated calcium release from sarcoplasmic reticulum of skeletal muscle cells ⁶⁷⁻⁷¹. In addition to location on the plasma membrane, the mVDR has also been demonstrated on the endoplasmic reticulum ⁷².

Treatment with calcitriol stimulated a redistribution of the 1,25D₃-MARRS from both the plasma membrane and endoplasmic reticulum to the nVDR ⁷². It has been suggested that the traditional nVDR may also have non-transcriptional functions ⁷³. Localization studies demonstrate that calcitriol stimulates a rapid nuclear translocation of 1,25D₃-MARRS to the plasma membrane ⁷³. This finding suggests that the 1,25D₃-MARRS may therefore be a recycling of the nVDR to the plasma membrane ⁷⁴. Additional evidence indicating the recycling of the nVDR was demonstrated by Zanello et al. who suggested that the membrane initiated events of calcitriol are linked to a functional nVDR ⁷⁵. Zanello et al. found that gene knockout of the nVDR in mice inhibits the calcitriol mediated activation of calcium channels on osteoblasts ⁷⁵.

This finding demonstrates that the calcitriol mediated activity of calcium channels is fundamentally linked to the nVDR⁷⁵.

Nonskeletal Functions of Calcitriol

The demonstration of nVDR and mVDR in tissues and cells not associated with bone-mineral homeostasis indicated additional roles for vitamin D in endocrine function⁷⁶⁻⁷⁹. Substantial evidence demonstrates that calcitriol alters cellular differentiation and metabolism impacting various disease states^{76, 78, 79}. Adipose tissue is a target organ for the calcitrophic hormones, calcitriol and PTH^{60, 80-87 88, 89}. Substantial evidence has demonstrated a dysregulation in vitamin D metabolism related to obesity^{90, 91}. Decreased levels of serum calcidiol and increased levels of PTH have been reported in obese subjects⁹²⁻⁹⁹. Intriguingly, elevated levels of calcitriol serum concentrations have been confirmed in obese subjects compared to non-obese subject controls^{92, 100, 101}. It has been suggested that up-regulated PTH levels in obesity stimulate CYP27B1, possibly accounting for increased calcitriol levels with obesity¹⁰². Furthermore, it has been proposed that increased calcitriol levels in obese subjects is a negative feedback regulator on the synthesis of calcidiol in the liver accounting for reduced levels of calcidiol associated with obesity¹⁰².

Elevated PTH and calcitriol levels have both been indicated to increase intra-adipocyte calcium levels altering adipocyte metabolism^{89, 103}. Alteration of intracellular calcium levels play a critical function in modulating adipocyte metabolism in favor of adipogenesis⁸⁷. Stimulation of receptor or voltage mediated calcium channels in adipocytes alters key lipid metabolizing enzymes enhancing lipogenesis and suppressing lipolysis^{86, 104-106}. Influx of intracellular calcium increases expression and activity of a key lipogenic enzyme, fatty acid synthase (FAS), enhancing

triglyceride content of adipocytes and suppressing lipolysis^{104, 106, 107}. Addition of a calcium channel antagonist blocked these effects demonstrating that modulation of intracellular calcium is a key factor in adipocyte metabolism^{104, 105}. Calcitriol (5 nM) has been shown to stimulate an influx of intracellular calcium, resulting in upregulation of FAS expression and activity and inhibition of lipolysis⁸⁶. This calcitriol mediated effect was repeatable using a vitamin D membrane agonist, $1\alpha,25\text{-(OH)}_2\text{-lumisterol}_3$, and blocked by a vitamin D membrane antagonist, $1\beta,25\text{-dihydroxyvitamin D}_3$ ⁸⁶. These findings demonstrate that calcitriol stimulates an influx of intracellular calcium modulating adipocyte lipid metabolism via the $1,25\text{ D}_3$ MARRS⁸⁶.

PTH stimulates an increase in intra-adipocyte intracellular calcium associated with reduced sensitivity of insulin stimulated glucose uptake in adipocytes¹⁰⁸. Adipocytes and muscle cells are the primary targets of insulin-stimulated glucose uptake and are key determinants of overall insulin sensitivity¹⁰⁹. Insulin is a critical regulator of systemic energy homeostasis, controlling the storage, mobilization and utilization of free fatty acids and glucose¹¹⁰. A coordinated orchestration of a cascade of phosphorylation/dephosphorylation signaling events are responsible for the action of insulin stimulated glucose uptake by adipocytes¹⁰⁹. The initial events of insulin signaling include tyrosine phosphorylation of the insulin receptor (IR), tyrosine phosphorylation of the insulin receptor substrates (IRS)-1 and (IRS)-2 and activation of phosphatidylinositol 3-kinase (PI3-K)^{109, 111}.

PI3- K induces the translocation of the primary insulin responsive glucose transporter- 4 (GLUT)-4 to the plasma membrane for insulin stimulated uptake of glucose^{109, 111}. High levels of intra-adipocyte calcium have been shown to inhibit the translocation of GLUT-4 from the intracellular vesicles to the plasma membrane resulting in insulin-resistant adipocytes¹¹². High intra-adipocyte levels of calcium have been suggested to alter the phosphatase activity involved in the dephosphorylation of GLUT-4, reducing intrinsic activity of GLUT-4 and impairing

response of adipocytes to insulin¹¹³. In vitro induced insulin response has been demonstrated to be abolished by the addition of a calcium channel antagonist, nitrendipine, as a result of decreasing intracellular calcium concentration¹¹⁴. Dietary calcium supplementation has also been demonstrated to reduce intracellular calcium in adipocytes^{89, 115-117}. This anti-obesity effect of high dietary calcium supplementation is mediated, in part, by suppressing calcitriol and possibly PTH induced influxes of intracellular calcium (Ca^{2+})^{89, 115-117}. Thus, it remains possible that the calcitrophic hormones may play a pivotal role in the regulation of adipocyte metabolism relative to the pathogenesis of obesity^{89, 104, 106}.

Calcitriol Modulates Adipose Tissue Metabolism

Adipocytes are derived from fibroblastlike cells during normal mammalian development and under various pathological conditions^{118, 119}. A shift in adipocyte gene expression patterns results in morphological alterations in adipocyte shape and an increase in lipid accumulation stimulate adipocyte differentiation¹²⁰. Adipocyte differentiation requires three transcriptional factors, CCAAT/enhancer binding proteins (C/EBP), peroxisome proliferator-activated receptor- γ (PPAR)- γ , and adipocyte determination and differentiation factor-1 (ADD)-1/sterol regulatory element binding protein-1c (SREBP)-1c^{118, 120, 121}. Cross regulatory and auto-regulatory mechanisms drive and maintain C/EBP and PPAR- γ protein expression, inducing expression of various other genes responsible for differentiation of adipocytes^{109, 120, 121}. Differentiation of preadipocytes into adipocytes can be induced by insulin like growth factor-1, (IGF)-1 glucocorticoids, and agents that increase cyclic adenosine monophosphate (cAMP) levels^{122, 123}. The process of differentiation is characterized by re-entry into the cell cycle and expression of a cascade of transcription factors regulating adipogenesis¹²⁴.

C/EBP- β is a key transcription factor in preadipocyte differentiation and is rapidly increased following induction of differentiation ¹²⁵. Transcriptional activation of C/EBP- β is regulated via phosphorylation of the two cAMP response element binding protein (CREB) sites ¹²⁶. Following phosphorylation, acquisition of DNA-binding by C/EBP- β occurs initiates the entry of preadipocytes into the G₁-S cell check-point, a growth-arrested stage, stimulating terminal differentiation of preadipocytes into mature adipocytes ¹²⁷. Phosphorylated C/EBP- β induces additional adipogenic transcription activators including C/EBP- α , PPAR- γ , ADD-1/SREBP-1c leading to the expression of enzymes responsible for stimulating fatty acid synthesis, binding, uptake, and storage ¹²⁴.

Evidence for the role of calcitriol modulation in adipocyte metabolism has been conflicting ^{60, 80, 88, 128-131}. Research suggests that intracellular calcitriol levels have dual effects on adipocyte differentiation ^{60, 88, 128}. Calcitriol has been demonstrated to inhibit adipocyte differentiation based on reduced activity of glycerophosphate dehydrogenase, suppression of triglyceride accumulation, and inhibitory effect on PPAR- γ , a transcription factor required for differentiation of adipocytes ¹³²⁻¹³⁴. The molecular mechanisms by which calcitriol inhibits adipogenesis have been suggested to antagonize the C/EBP- β signaling pathway ^{128, 129}. Specifically, calcitriol has been demonstrated to inhibit the expression of C/EBP- β mRNA and the levels of C/EBP- β protein present during the induction of differentiation ¹²⁸. Consequently, reduced levels of C/EBP- β are incapable of inducing C/EBP- α and PPAR- γ , two critical transcriptional activators required for expression of adipogenesis related genes ^{128, 129}. Moreover, very high doses of calcitriol (100 nM) have demonstrated anti-proliferative activity inducing pro-apoptotic factors initiating adipocyte apoptosis ⁶⁰.

In contrast, physiological doses of calcitriol (0.1-10 nM) has been demonstrated to induce a cascade of events that trigger adipocyte proliferation and differentiation ^{60, 130, 135-137}.

Lipoprotein lipase, (LPL) is a key enzyme involved in lipogenesis and differentiation of adipocytes¹³⁶. Treatment of 3T3-L1 adipocytes with calcitriol resulted in a 3-fold increase in LPL mRNA expression and LPL activity compared to control¹³⁶. Calcitriol has also been reported to stimulate an increase in expression of adipocyte lipid binding protein (aP2), a specific adipocyte marker of differentiation^{135, 136}. Up-regulation of aP2 mRNA expression was associated with increased induction of adipocytic cells in rat bone marrow stromal cell cultures¹³⁵. In addition, calcitriol treated adipocytes morphologically appeared rounder with increased number of adipocyte foci compared to control^{136, 138}.

Dace et al. demonstrated that physiological levels of calcitriol are capable of inducing terminal differentiation in Ob 17 cells, a clonal cell line established from epidymal fat pad of the obese mice, C57BL/6J¹³⁵. The adipogenic effect of calcitriol was evident from the finding that no additional factors were required to stimulate differentiation in Ob 17 cells¹³⁵. Dace et al. concluded that calcitriol induced differentiation is mediated through the VDR due to increased expression of the VDR mRNA during the differentiation process¹³⁵. The impact of VDR signaling on adipose tissue metabolism is evident from the finding that ablation of the VDR in mice resulted in significant atrophy of adipose tissue mass¹³⁹. In addition, mice lacking CYP27B1, also demonstrate a reduction in adipose tissue mass¹³¹. These findings suggest that calcitriol-VDR signaling plays a pivotal role in modulating adipocyte lipid metabolism regulating adiposity.

Energy Metabolism

Production of Cellular Energy

The cellular organelle of energy in adipocytes, as with any cell, is the mitochondria^{41, 140}. In vitro studies have demonstrated that adipose tissue metabolism is regulated by the energy charge of the adipocyte^{141, 142 143}. Mitochondrial efficiency, also known as the coupling efficiency, refers to the proportion of calories burned and oxygen consumed coupled to the synthesis of adenosine triphosphate (ATP)¹⁴⁴. Coupling efficiency can be approximated in cells by using oligomycin, a specific antagonist for ATP synthase¹⁴⁴. At high coupling efficiency there is an increased conversion rate of calories consumed to production of ATP¹⁴⁴. Increased production of ATP can be used for cellular processes or stored for later use in the form of fat¹⁴⁴. In contrast at low coupling efficiency, there is a reduced ATP synthesis and increased conversion of energy into heat¹⁴⁴.

As electrons are reduced to water and oxygen via the mitochondrial electron transport chain, protons are pumped from the mitochondrial matrix into the inner mitochondrial membrane^{142, 144}. The pumping of protons into the inner mitochondrial membrane generates an energy potential and protonmotive force^{142, 144}. This source of potential energy is harvested when protons are driven back into the mitochondrial matrix passing through ATP synthase^{142, 144}. A high coupling efficiency is achieved when the majority of protons from the inner membrane are passed back into the mitochondrial matrix generating ATP¹⁴⁴. However, the coupling efficiency can be reduced by leakage of protons back across the inner membrane through alternative proton conductance pathways not associated with the synthesis of ATP¹⁴⁴. Inducible proton leak utilizes

uncoupling proteins (UCP), located in the inner mitochondrial membrane, that dissipate the proton gradient generated by the respiratory chain ¹⁴⁴.

Uncoupling Proteins

The classic uncoupling protein, UCP-1 primarily functions in brown adipose tissue of rodents and is involved in cold-induced thermogenesis ^{144, 145}. UCP-1 homologues (UCPs 2-5) have been found in non-thermogenic mammalian tissues, prompting the re-examination of their function beyond thermogenesis ^{144, 146}. UCP-2 is expressed in white adipose tissue and is 59% homologous to UCP-1 ¹⁴⁷. UCP-3 is primarily expressed in skeletal muscle and was found to be 57% homologous to UCP-1 and 73% homologous to UCP-2 ¹⁴⁸. UCP-4 and UCP-5 are highly expressed in the central nervous system and as a result less emphasis has been focused on them with regards to altering metabolism ¹⁴⁴. In addition UCPs partially uncouple electron transport from ATP synthase, reducing the rate of the mitochondrial respiratory chain and thus diminishing production of reactive oxygen species (ROS), possibly guarding against oxidative stress ¹⁴⁹.

Accumulating evidence suggests that UCPs are key targets in regulating energy metabolism ^{60, 80, 85}. In vitro studies have indicated that mitochondrial uncoupling may contribute to the control of lipid metabolism and adiposity due to a reduction in ATP production ^{40, 41}. Synthesis of UCP from the fat specific promoter of the aP2 gene in C57BL/6J transgenic mice serve as a useful model for investigating the role of mitochondria in WAT metabolism ¹⁴. Diet or genetic induced obesity in aP2-UCP1-1 transgenic mice were found to have a reduction in adiposity associated with ectopic expression of UCP-1 ⁴¹. Analysis of transgenic aP2-UCP-1 adipocytes indicated a decreased intracellular charge to be the driving force for the alteration in lipid metabolism and protection from obesity ⁴¹. Therefore, mild uncoupling of mitochondrial

respiration from oxidative phosphorylation may provide a therapeutic target for counteracting obesity-induced oxidative stress ^{144, 150}.

Investigation into the mechanism of UCPs and decreased adiposity was examined by overexpressing UCP-1 in adipose tissue of aP2-UCP-1 transgenic mice ⁴¹. Results indicate that the mitochondrial electron transport chain has three coupling sites that require at least one molecule of UCP-1 to achieve a reduction in the mitochondrial membrane potential ¹⁴⁵. Flow cytometry experiments using fluorescent dye showed that the mitochondrial membrane potential of adipocytes from aP2-UCP-1 transgenic mice was decreased compared to wild-type controls ¹⁴⁵. In addition, augmented expression of UCP-1 generated a significant reduction in accumulation of lipids in adipose tissue under diet-inducing obesity ¹⁴⁵. This phenotypic anti-obesity effect suggests that transgenic modification of adipose tissue by uncoupling may affect lipid metabolism by altering lipid metabolizing enzymes ⁴¹. Indeed, ectopic expression of UCP-1 in adipose tissue demonstrated an augmentation in LPL, indicating that respiratory uncoupling increased LPL-stimulated clearance of triglycerides ⁴¹. Furthermore, transgenic mice were found to have reduced plasma triglyceride levels compared to matched wild type controls ^{41, 151}. These findings suggest that uncoupling respiration in adipocytes may provide beneficiary control over lipid metabolism ⁴¹.

Overexpression of UCP-1 was also associated with stimulating mitochondrial biogenesis and increasing mitochondrial content ¹⁴¹. Electron microscopy revealed changes in mitochondrial morphology with ectopic expression of UCP-1 in adipocytes, indicating that UCP-1 induces mitochondrial biogenesis ¹⁴¹. These mitochondrial characteristics included a thick peripheral rim surrounding the adipocytes with inclusion of larger sized mitochondria that were oval shaped with a high degree of cristae per mitochondria ¹⁴¹. In contrast, mitochondria from control group had a thin peripheral rim surrounding the adipocyte, elongated mitochondria with sparse

distribution of cristae¹⁴¹. Structural changes in mitochondria associated with overexpression of UCP-1 in adipocytes demonstrate an intermediate phenotype between brown and white adipocytes^{141, 152}. These findings indicate that white adipocytes can acquire some brown adipocytes characteristics and thereby augment energy expenditure. UCP-2 was first identified when UCP-1 knock out mice failed to become obese resulting in a search for a UCP-1 homolog¹⁵³. UCP-2 is upregulated in WAT in response to high fat feeding of mice¹⁴⁷. UCP-2 and UCP-3 mediate the transport of fatty acids and oxidation during situations of fatty acid oversupply¹⁵⁴⁻¹⁵⁶. These findings suggest a key role of UCP-2 in energy metabolism and protection from development of obesity.

Calcitriol Modulates Uncoupling Protein Expression

Calcitriol has been demonstrated to act via the adipocyte nVDR to inhibit the expression of UCP-2^{60, 80}. Treatment of human adipocytes with 1 nM calcitriol resulted in a 50% reduction in UCP-2 mRNA and protein expression⁸⁰. Anti-sense nuclear oligodeoxynucleotide knockout of the nVDR prevented his effect, confirming that calcitriol inhibits UCP-2 expression via the nVDR⁸⁰. Thus, suppressing calcitriol levels and upregulating UCP-2 expression may inhibit lipogenesis and thereby regulate adipocyte lipid metabolism⁸⁰. Increasing dietary calcium suppresses circulating calcitriol levels resulting in increased expression of UCP-2 and a reduction in metabolic efficiency and adiposity in mouse models of obesity^{117, 157, 158}.

Glucocorticoids Modulate Adipose Tissue Metabolism

The Role of Glucocorticoids in the Development of Obesity

Adipose tissue function can be modulated by changes in intracellular cortisol levels ¹⁵⁹. The function of glucocorticoids in fat accumulation is demonstrated in Cushing's syndrome, in which hypercortisolemia results in a significant increase intraabdominal fat accumulation ¹⁶⁰. Cortisol is a potent stimulator of adipocyte differentiation ¹⁶¹. In the presence of insulin, the addition of cortisol resulted in a 70-fold increase in the number of developing adipocytes, indicating a role for glucocorticoids in the development of hyperplastic obesity ¹⁶¹. A critical determinant of triglyceride accumulation and adipocyte expansion is the activity level of LPL ¹⁶². LPL catalyzes the hydrolysis of circulating triglycerides into free fatty acids for re-esterification and storage in adipocytes ¹⁶². In the presence of insulin, cortisol enhances lipid accumulation by activating LPL and inhibiting lipolysis ¹⁶³. The expression of glucocorticoid receptors have been demonstrated to be increased in visceral versus subcutaneous adipocytes, resulting in a heightened response to glucocorticoid stimulation ¹⁶⁴.

Excess visceral adipose tissue has been identified as the most powerful predictor for the development of the metabolic syndrome ¹⁶⁵. Visceral adipocytes are notorious for increased fatty acid turnover and lipolysis compared to subcutaneous adipocytes and are less responsive to antilipolytic effects of insulin ¹⁶⁶. The increased supply of free fatty acids may be directed to the liver via the portal circulatory system, interfering with insulin signaling and increasing hepatic insulin resistance ¹⁶⁵. Consequently, insulin resistant hepatocytes, a contributor to metabolic syndrome, may result in increased glucose output by the liver inducing hyperglycemia ¹⁶⁷. In addition, gluconeogenesis may be up-regulated as a result of the increased supply of glycerol from

the high oxidation rate of fatty acids in visceral adipose tissue ¹⁶⁵. Gluteofemoral adipose tissue releases stored fatty acids primarily under circumstances of metabolic glycogen depletion in which fatty acids are released and rapidly oxidized to meet the requirement as a fuel source ¹⁶⁸. In contrast, visceral adipose tissue releases excessive amounts of fatty acids even when glycogen availability is high and insulin levels are reduced, resulting in inhibition of free fatty acid oxidation and increased intramuscular accumulation of lipids, potentially creating metabolic dysfunction ¹⁶⁸.

A key factor in the development of visceral obesity is glucocorticoid level ¹⁵⁹. Visceral adipocytes have increased expression of glucocorticoid receptor levels compared to subcutaneous adipocytes ¹⁶⁹. Moreover, 11- β -hydroxysteroid dehydrogenase type-1 (11 β HSD-1) expression is significantly higher in visceral adipose tissue, with only negligible amounts detected in subcutaneous adipose tissue ¹⁷⁰. 11 β HSD-1 regenerates active cortisol from inactive cortisone ¹⁵⁹. 11 β HSD-1 is located in the endoplasmic reticulum and exhibits bidirectional functioning as a dehydrogenase in the conversion of cortisol to cortisone and a reductase in the conversion of cortisone to cortisol ¹⁷¹. However, in adipose tissue 11 β HSD-1 functions exclusively as a reductase, producing active cortisol ¹⁷¹.

Activity of adipose tissue 11 β HSD-1 is tightly controlled by various growth factors, cytokines and pharmacological agents ¹⁷²⁻¹⁷⁷. In human adipose tissue IGF-1 inhibits activity of 11 β HSD-1 suppressing cortisol levels ¹⁷³. In contrast, the cytokines TNF- α and IL-1 β stimulate enzyme activity and increase local production of cortisol ¹⁷². Moreover, the product of 11 β HSD-1, cortisol, promotes a positive feedback mechanism on intracellular availability of 11 β HSD-1, magnifying the effects of cortisol ^{159, 170}. Pharmacological agents such as thiazolidinediones (TZDs) for the treatment of diabetes and protease inhibitors used in the management of human immunodeficiency virus infected patients inhibit activity of 11 β HSD-1 ¹⁷⁴⁻¹⁷⁷. Intriguingly, both

of these medications dichotomously modulate location of adipose tissue fat depots^{174, 175, 177, 178}. Protease inhibitors increase fat deposition centrally while TZDs decrease visceral adiposity but increase subcutaneous adiposity^{174-176, 178}.

Increased levels of local glucocorticoid production has been reported in adipose tissue and skeletal muscle in human models of obesity and metabolic syndrome¹⁷⁹⁻¹⁸³. Numerous studies have demonstrated that the expression of 11 β HSD-1 enzyme activity is significantly up-regulated in adipose tissues from obese humans and rats^{180, 181, 184, 185}. These findings indicate a direct role for local regeneration of cortisol and amplification of glucocorticoid production in obesity and the metabolic syndrome^{180, 181, 184, 185}. The metabolic function of 11 β HSD-1 in mice was examined using gene knockout and transgenic overexpression^{159, 186-189}. 11 β HSD-1 null mice demonstrated an insulin sensitive response with phenotypic resistance to the development of visceral adiposity even when challenged with a high fat diet¹⁸⁷⁻¹⁸⁹. In contrast, transgenic mice overexpressing 11 β HSD-1 in adipose tissue expressed a stimulatory effect in enzyme activity with phenotypic visceral adiposity and insulin resistance, comparable to that observed in the metabolic syndrome^{159, 186}.

Calcitriol Up-regulates Glucocorticoid Activity in Adipose Tissue

Calcitriol has been demonstrated to directly up-regulate the expression and activity of 11 β HSD-1 in human adipocytes and to increase the production and release of cortisol from adipocytes¹⁹⁰. Cortisol itself enhances activity of 11 β HSD-1, creating a positive feedback mechanism on glucocorticoid activity^{170, 190-192}. Long term treatment of 48 hours with the murine precursor of active glucocorticoid, 11 β -dehydrocorticosterone, stimulated expression of the nVDR in 3T3-L1 cells⁸⁸, and the combination of calcitriol and 11 β -dehydrocorticosterone

further enhanced expression of the nVDR ⁸⁸. Therefore, it has been suggested that inhibition of 11 β HSD-1 would down-regulate expression of nVDR, suppressing calcitriol stimulated activity of glucocorticoid activity ⁸⁸. Indeed, siRNA knockdown of 11 β HSD-1 suppressed nVDR expression ⁸⁸. However, calcitriol still stimulated production of corticosterone in 11 β HSD-1 siRNA treated adipocytes, suggesting that calcitriol may modulate glucocorticoid release by a nVDR-independent mechanism ⁸⁸.

Although calcitriol stimulated 11 β HSD-1 expression is nVDR mediated, calcitriol stimulated cortisol release is mediated through the rapid non-genomic action of 1,25D₃ MARRS ¹⁹⁰. Calcitriol mediated calcium signaling augmented cortisol release, an effect that was also achieved by treatment of adipocytes with lumisterol, a 1,25D₃ MARRS agonist, calcitriol, calcium channel depolarizer and L-type calcium channel agonist ^{88 190}. Thus, calcitriol increases local glucocorticoid activity in visceral adipose tissue by both nVDR and MARRS-mediated mechanisms ¹⁹⁰. It has been established that calcitriol regulates glucocorticoid activity by a positive feedback mechanism ⁸⁸. Calcitriol stimulates increased activity of 11 β HSD-1 which increases corticosterone levels and up-regulates expression of nVDR ⁸⁸. An increase in levels of nVDR enhances ligand binding ability to the nVDR increasing glucocorticoid activity ⁸⁸. Diets high in calcium have been indicated to suppress calcitriol levels attenuating intra-adipocyte calcium levels ^{87, 190}. Increasing dietary calcium may antagonize activity of 11 β HSD-1 reducing local cortisol production regulating visceral adiposity ^{87, 190}.

The Renin Angiotensin System

Adipose RAS and Adipose Tissue Dysfunction

Dysfunctional adipose tissue is associated with an up-regulation in the secretion of bioactive molecules such as angiotensinogen (AGT), pro-inflammatory cytokines, and reactive oxygen species¹⁹³. It has been proposed that dysfunctional adipose tissue may lead to activation of the sympathetic nervous system stimulating the renin angiotensin system (RAS), promoting vasoconstriction and influencing the development of obesity-induced hypertension^{193, 194}. Classically RAS functions in salt and extracellular fluid homeostasis for blood pressure regulation¹⁹⁵. Activation of the classic RAS components angiotensinogen, renin and angiotensin converting enzyme result in production of the bioactive peptide hormone angiotensin II (ANGII)¹⁹⁵. The primary effect of activation of the classic RAS is vasoconstriction, which is achieved via binding of ANGI, an octapeptide, to its receptor¹⁹⁵. The normal physiological concentration of ANGI in plasma is around 10 pM¹⁹⁶. It should be noted that local production of ANGI may result in higher intracellular concentrations compared to circulation¹⁹⁷. For example, in the rat kidney, renal interstitial fluid concentrations of ANGI have been demonstrated to be around 3.76 nM, about 30 fold higher than circulating levels¹⁹⁷.

In addition to the classic RAS, local production of all RAS components have been confirmed in a multitude of tissues indicating the existence of a functional tissue RAS^{198 199, 200 201}. Mature adipocytes produce and secrete vasoactive factors that enhance adipogenesis and may contribute to obesity-induced hypertension^{202, 203}. Mature adipocytes express all molecules, enzymes, and receptors that comprise a functional RAS^{11, 202, 203}. The main effector molecule of RAS, ANGI, is a vasoactive and proatherogenic peptide that stimulates production and release of prostacyclin,

which has been suggested to stimulate differentiation of preadipocytes into mature adipocytes^{11, 203, 204}. It has been proposed that ANGII enhances activity of glycerol-3-phosphate dehydrogenase, a marker of adipocyte formation, in preadipocytes as a response to prostacyclin release from adipocytes²⁰⁵. In addition, ANGII was demonstrated to stimulate adipocyte lipid accumulation by up-regulating expression and activity FAS and glycerol-3-phosphate dehydrogenase in both murine and human adipocytes²⁰⁶. These findings suggest that ANGII coordinately stimulates genes responsible for adipocyte expansion contributing to an increase in adipose tissue mass.

Research discrepancies demonstrating an anti-adipogenic role of ANGII has created ambiguity as to the true effect of ANGII on adipocyte differentiation²⁰⁷⁻²⁰⁹. ANGII has been demonstrated to block in vitro recruitment of undifferentiated cells from visceral adipocytes during adipocyte differentiation as evident by a reduction in glycerol-3-phosphate dehydrogenase activity²¹⁰. In addition, this effect of ANGII was found to be greater in visceral adipocytes from obese subjects than subcutaneous adipocytes²¹⁰. In accordance with this finding, other studies have reported that visceral adipose tissue significantly expresses higher levels of RAS transcripts compared to subcutaneous adipose tissue²¹¹⁻²¹³. It has been postulated that an up-regulation in visceral ANGII production may inhibit the number of newly differentiated adipocytes contributing to the development of hypertrophic adipocytes creating a link between ANGII generation and visceral fat dysfunction²¹⁰.

The increased production of ANGII resulting in a decreased recruitment of newly differentiated adipocytes indicates the presence of a paracrine negative feedback loop²⁰³. Accordingly, the selective over-expression of AGT in murine adipose tissue resulted in adipocyte hypertrophy, suggesting that the effects of ANGII are trophic rather than adipogenic¹³. In addition, the over-expression of adipose AGT in mice was demonstrated to augment circulating

levels of AGT resulting in hypertension compared to wild-type mice ¹³. Up-regulation in the expression of adipose AGT may also influence an elevation in systemic levels of AGT, demonstrating that cross talk may exist between adipose tissue RAS and the systemic RAS ³⁷. ANGII inhibits nitric oxide (NO), resulting in a diminished vasodilator capacity in the vessel wall, modulating vascular tone and promoting hypertension ²⁰⁴. Adipocytes from obese subjects produce and secrete increased levels of AGT and ANGII which have been found to elevate systemic blood pressure levels ¹⁷. These findings support a role for the adipose tissue RAS to activate systemic RAS contributing to obesity-induced hypertension.

The promoter region of the ANGII gene contains two glucocorticoid response-elements, and glucocorticoids have been demonstrated to induce expression of AGT in murine adipocytes ²¹⁴⁻²¹⁶. Glucocorticoid stimulated expression of ANGII mRNA was associated with an increase in ANGII synthesis and secretion from adipocytes ²¹⁶. Calcitriol stimulates activity of 11 β HSD-1 enhancing cortisol production in adipose tissue indicating a regulatory function of calcitriol in glucocorticoid activity ¹⁹⁰. Transgenic over-expression of 11 β HSD-1 in mice was found to stimulate the systemic RAS system contributing to a significant rise in blood pressure ¹⁸⁶. In addition, transgenic over-expressing of AGT specific to adipose tissue also produced hypertensive effects ¹³.

Dysregulation of Adipocytokine Production

ANGII has been demonstrated to promote inflammation through upregulation of inflammatory cytokines or through increased oxidative stress ^{217, 218}. Tsuchiya et al. was the first to demonstrate that exogenous infusion of ANGII in rats resulted in increased expression of MCP-1 in adipose tissues ²¹⁷. Inflammation in adipose tissue has been suggested to be a link between obesity and the development of inflammatory-associated disease states of diabetes and

cardiovascular disease^{56, 202, 219}. Obesity promotes a dysregulation in the expression and production of adipokines, resulting in an up-regulation of inflammatory cytokine expression²²⁰.

Hypertrophic adipocytes are associated with an increase in the expression of inflammatory adipocytokines TNF- α and IL-6^{56, 220}. It has been suggested that obesity-induced up-regulation of inflammatory cytokines may result in dysfunctional adipose tissue, leading to oxidative stress in accumulated fat²²¹. ANGII has also been demonstrated to stimulate oxidative stress by binding to the AT1R resulting in activation the membrane bound, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a major contributor to ROS production²²². Obesity induced oxidative stress has been demonstrated to result in a dysregulation in production of local adipocytokines as a consequence of increased activation of NADPH oxidase and a reduction in cellular antioxidant system^{83, 221}.

The AngII Receptor

Due to the vast physiological and pathological effects of the AT1R, pharmaceutical research has placed immense importance on determining the molecular mechanism essential for AT1R activation²²³. Current AT1R research has yet to fully identify the exact mechanistic approach but has proposed that AT1R stimulation occurs via G-protein dependent and G-protein independent signaling events²²⁴. The AT1R is comprised of 359 amino acids and belongs to class A family of G-protein coupled receptors (GPCRs) better known as seven transmembrane 7(TM) receptors due to the molecular organization composed of 7TM helical structures²²⁵. Research with mutated receptors and modified ligand binding has demonstrated that the ANGII binding site involves the interaction of amino acid residues in the TM helical segments of TM3, TM5, and TM6 enabling ligand binding and receptor activation^{226, 227}.

Although the precise mechanistic mode of activation has not been fully elucidated, a “global toggle switch” has been suggested to characterize the general and conserved features of the conformational transformations between the inactive and activated state of 7TM receptors²²⁸. According to this model, receptor activation requires an inward movement of the upper part of the extracellular segments of the TM helices with an oppositional movement of the lower cytoplasmic part of the helical bundle^{229, 230, 231}. Research suggests that it is the separation of the cytoplasmic parts of TM3 and TM6 and the outward movement of TM6 away from the central axis of the helical bundle that makes the intracellular loops flexible enough to present epitopes for binding of G- proteins²³². This restructuring initiates G- protein coupling as well as uncovering sites that can be modified via phosphorylation on the intracellular domains^{233, 234}.

In the classical model of AT1R activation via G- protein coupling, ANGII binds the AT1R stimulating the binding of regulatory G proteins resulting in second messenger signaling activating phospholipase A2, C and D which stimulates the primary transduction pathway, inositol triphosphate (the frequently used indicator of AT1R activation) and calcium signaling^{225, 235}. The activated AT1R is phosphorylated by the G- protein coupled receptor kinase (GRK) or second messengers such as PKC^{225, 235}. Second messenger signaling promotes beta-arrestin recruitment which physically uncouples G -protein from the receptor and terminates further G- protein activity²³⁵. The recruitment of beta-arrestins function as scaffolding mediating receptor internalization via clathrin coated pits initiating a second phase of activation defined as G- protein independent, beta-arrestin dependent²³⁵. Although receptor desensitization is considered the primary role of beta-arrestins for altering signal transduction pathways, several studies demonstrate that beta-arrestins are capable of initiating signaling independent of G protein activation^{236, 237, 238}.

The carboxy terminal tail of the AT1R favors binding and stabilization of beta-arrestins as it contains epitopes that enable direct binding and activation of beta-arrestins²³⁹. Exposing sites for beta-arrestin binding on the carboxy terminal tail of the AT1R requires conformational changes of the helical bundle in favor of G-protein independent signaling^{235, 236}. Research demonstrates that separation of the cytoplasmic parts of TM3 and TM6 are required for G-protein activation of 7TM receptor, while inhibiting this movement abolished G-protein coupling^{229, 240}. Mutational studies using zinc binding sites in which zinc cross links with TM3 and TM6, did not interfere with GRK phosphorylation and beta-arrestin recruitment^{240, 241}.

In addition, ANGII binding to AT1R has been demonstrated to induce beta-arrestin dependent signaling with receptor internalization and stimulation of ERK1/2 without inositol triphosphate generation²²⁴. Research has suggested that receptor conformations with high affinity for beta-arrestins would favor G-protein independent signaling while simultaneously inhibiting G-protein dependent signaling²⁴². In addition, it has been proposed that beta-arrestin degradation of second messengers may promote a switch from G-protein dependent to G-protein independent signaling²²⁵. Moreover, pre-assembled beta-arrestin scaffolding proteins interacting with the AT1R can prevent the receptor from adopting various conformations and as a result this constraint on conformation would discriminantly regulate activity of signaling pathways²⁴³.

ERK1/2 is the most well known example of beta-arrestin activated signaling pathway^{238, 242, 244}. In cells overexpressing AT1R, G-protein activation occurred rapidly within two minutes and stimulated nuclear and cytoplasmic localization of activated ERK1/2²⁴⁵. In addition, G-protein stimulated nuclear ERK1/2 localization promoted transcriptional activity of growth response 1²⁴⁵. Alternatively, beta-arrestin had delayed and prolonged pattern of AT1R activation with only cytoplasmic localization²⁴⁵. Accordingly, G-protein and beta-arrestin demonstrate differential regulation of ERK1/2 signaling down stream of AT1R activation²⁴⁵.

These findings suggest that modulation of AT1R activation by G-protein dependent or G-protein independent mechanisms can functional select signaling pathways and potentiate regulating cellular consequences.

Inflammation in Obese Adipose Tissue

Stimulators of Reactive Oxygen Species Production

Furukawa et al. demonstrated that increased ROS production in the adipose tissue of obese mice was responsible for modulating adipocytokine gene expression and enhancing systemic oxidative stress²²¹. It was recently confirmed that the adipose tissue of mice on a high fat diet demonstrated an up-regulation in activity of the NADPH oxidase complex, which contributes to adipose tissue ROS production²⁴⁶. Calcium signaling is required for activation of ROS generating enzymes²⁴⁷. Influx of calcium through voltage-gated channels has been demonstrated to activate protein kinase C (PKC) contributing to the assembly of NADPH oxidase complex^{83, 247}. Furthermore, numerous ROS-generating enzyme activities are calcium dependent such as myeloperoxidase which contains a calcium binding site that is critical for activation²⁴⁷.

It has been suggested that ROS stimulates intracellular calcium levels and likewise high intracellular calcium levels favors production of ROS creating a positive feedback mechanism⁸³. This suggestion was confirmed by the ability of the calcium channel blocker, nifedipine, to coordinately suppress intracellular calcium influxes simultaneously decreasing ROS formation⁸³. Calcitriol has been reported to stimulate intracellular calcium levels, via 1,25D₃ MARRS, enhancing ROS production and up-regulating gene expression of NADPH oxidase in 3T3-L1 adipocytes, whereas nifedipine inhibited these effects⁸³. Calcitriol has also been suggested to act as a pro-oxidant, down-regulating cellular anti-oxidant defense systems²⁴⁸. In addition, ANGII

has been reported to enhance ROS production via influx of intracellular calcium, elevating mitochondrial calcium uptake resulting in an increase in mitochondrial ROS production ^{249, 250}.

ROS are byproducts of normal physiological respiration and mitochondria contribute a significant proportion of ROS generation to total cellular ROS ^{83, 247}. ROS have been suggested to function as signaling molecules stimulating in vitro cellular proliferation and growth ^{83, 251}. In adipocytes, a low dose of hydrogen peroxide was found to increase cell DNA synthesis and inducing adipocyte proliferation while the addition of the anti-oxidant, α -tocopherol, antagonized these effects ⁸³. In addition, the mitochondrial uncoupling inhibitor, guanosine 5'-diphosphate, was found to augment the hydrogen peroxide proliferative effects while nifedipine, a calcium channel blocker antagonized the proliferative effects of hydrogen peroxide ⁸³. ROS production is regulated in part by the mitochondrial potential, as a slight decrease in the mitochondrial potential of 10 mV was sufficient to abolish 70% of mitochondrial ROS formation ²⁵². In addition, mild activation of UCP via transfection was also found to diminish the mitochondrial potential and suppress ROS production ⁸³. Calcitriol has been shown to increase adipocyte ROS production by inhibiting mitochondrial uncoupling while overexpression of UCP-2 attenuated this effect ^{80, 83}.

It is well accepted that ROS are key factors involved in oxidative damage to cellular structure and dysregulation of redox-sensitive signaling pathways. A moderate increase in ROS can act as intracellular signaling molecules to regulate protein kinase activity and transcription factor activity production to stimulate mammalian cellular growth and proliferation ²⁵¹. ROS modulate cellular signal transducers of MAPK such as the extracellular signal regulated kinase $\frac{1}{2}$ (ERK $\frac{1}{2}$) that activates the downstream transcriptional effector complex activator protein-1 (AP)-1 dimer consisting of the transcription factors c-fos and c-jun ^{253, 254}. Calcitriol contributes to ROS production by increasing cytosolic calcium levels and inhibiting mitochondrial uncoupling

⁸³. Calcitriol has been found to alter cellular signaling by stimulating PKC leading to activation of MAPK signaling pathways to induce AP-1 dimer complex enhancing gene expression to positively modulate cellular proliferation ^{83, 253, 255}.

In addition, calcitriol was demonstrated to stimulate adipocyte proliferation as indicated by an increase in expression of cyclin a, required for entry into the synthesis stage of the cell cycle ⁸³. ANGII also activates MAPK signal transduction pathways of ERK ½ and JNK to positively modulate cellular proliferation ²⁵⁶. Furthermore, ANGII has been demonstrated to reduce the number of preadipocytes in the growth arrested phase of the cell cycle and increase the proportion of 3T3-L1 cells in the synthesis phase of the cell cycle ²⁵⁷. Calcitriol and ANGII independently alter signaling transduction pathways to increase ROS that function as second messengers in the modulation of MAPK pathways that activate transcription factors responsible for cellular proliferation ^{255, 256, 258}.

Macrophage Accumulation and Adipose Tissue Inflammation

A critical component to the inflammatory state of obese adipose tissue is the presence of activated macrophages ²⁵⁹. Macrophages are leukocytes which are derived from bone marrow and mature and enter the circulation as monocytes ^{260, 261}. The circulating monocytes are then recruited into various tissues where they reside and differentiate into macrophages to function in phagocytic and microbial cellular activities ^{260, 261}. Macrophages are recruited from bone marrow and infiltrated into adipose tissue via the residential adipose tissue MCP-1 ²⁵⁹. Adipose tissue of obese individuals exhibit an increased infiltration of macrophages ^{262 259}. Genetic analysis of gene transcripts from mouse adipose tissue revealed that 30% of 1,304 transcripts that were

highly correlated with body mass in genetic and diet-induced models of obese mice compared to lean control ⁵⁶.

Annotation of the transcripts indicated a characteristic upregulation of genes that are associated with enhancing the expression of macrophage transcripts in the models of obesity ⁵⁶. This finding suggests that a positive correlation exists between the infiltration of macrophages with increasing adiposity ⁵⁶. Infiltrated macrophages recruit haemopoietic cells to areas of local inflammatory sites to mediate the innate and adaptive immune responses ²⁶⁰. Macrophages are classically activated by interferon gamma, or in combination with lipopolysaccharide to produce the the classically activated macrophage, M1 ²⁶³. M1 macrophages release numerous cytokines such as TNF- α and IL-6 which in turn activate inflammatory cell signaling pathways to produce acute phase inflammatory molecules such as C-reactive protein and PAI-1 ^{56, 260}. In contrast, an alternative pathway of macrophage activation occurs by the cytokines IL-4 and IL-13 which lead to activation of the alternatively activated macrophage, M2 ²⁶⁴. The M2 phenotype has been suggested to function in reducing the inflammatory effects of M1 macrophages and to participate in repair processes ²⁶⁴.

The significant contrast in properties of macrophage polarization states are due to the regulation of arginase and nitric oxide synthase (NOS), which both require the substrate L-arginine ^{265, 266}. Competition between NOS and arginase for the substrate L-arginine results in significantly contrasting effects²⁶⁶. Arginase requires L-arginine as a substrate for the production of L-ornithine, a precursor for proline, required for collagen synthesis ^{265, 266}. NOS utilizes L-arginine for the production of NO, a ROS, that increases inflammation ^{265, 266}. In addition, ANGII antagonizes NO resulting in promotion of macrophage adhesions and increasing monocyte/macrophage accumulation in the vessel walls promoting endothelial dysfunction and oxidative stress ²⁰⁴. Comparison of adipose tissue macrophages from obese and lean mice

revealed that arginase activity is augmented in lean mice due to an increase in M2 phenotype²⁶⁶. This finding led to the suggestion that maintaining a positive balance in favor of arginase may function to downregulate the production of ROS via NOS thus reducing inflammatory effects and preventing adipocyte dysregulation²⁶⁶. Furthermore, gene expression analysis of adipose tissue macrophages between lean and obese mice found that lean mice have an increased expression of the cytokine, IL-10²⁶⁶. IL-10 is a critical mediator for insulin sensitivity which has been indicated to attenuate the proinflammatory effects of TNF- α ²⁶⁶.

The chronic low-grade inflammatory state associated with obesity is a result of infiltration and activation of M1 macrophages in adipose tissue²⁶¹. Obesity favors a shift in macrophage phenotype towards the “classically” activated M1 macrophage²⁶⁶. M1 activated macrophages produce proinflammatory cytokines (TNF- α , IL-6, IL-12) and increase reactive oxygen species production such as nitric oxide²⁶⁶. It has been suggested that factors such as MCP-1, TNF- α , and saturated fatty acids induce the expression of TLR-4, leading to an alteration in circulating monocytes in favor of the proinflammatory phenotype^{266, 267}. Therefore, the “classically activated”, M1 macrophage, has been characterized as the primary producer of inflammatory molecules that have been detected in the pathogenesis of obesity²⁶². Augmented production of MCP-1 from hypertrophied adipocytes enhances the release of free fatty acids, promoting inflammatory modulation in the adipocyte metabolism⁵³. Takahashi et al. observed that obese mice have a 7.2 fold increase in the inflammatory expression of MCP-1 compared to normal mice²⁶⁸. More importantly, Takahashi et al. also demonstrated that a positive correlation in the upregulation of MCP-1 protein levels was associated with a concomitant increase in the CD11b-positive monocyte/macrophage in adipose tissue of obese mice²⁶⁸.

Recent evidence confirmed that consumption of high fat diets results in a phenotypic switching of adipose tissue macrophages resulting in an alteration in the cytokine expression of

murine adipose tissue macrophages from M2 to M1, as a result of the decreased expression of IL-10 and increased expression of TNF- α ²⁶³. Weisberg et al. analyzed the expression profile of macrophage and nonmacrophage cell populations isolated from adipose tissue and found that adipose tissue derived macrophages are the primary source of adipose tissue TNF- α and MCP-1 ⁵⁶. Weisberg et al. concluded that an increase in quantity of adipose tissue macrophages activates inflammatory pathways in the adipose tissue of obese individuals ⁵⁶. Furthermore, macrophages appear to inhibit adipocyte differentiation, potentially influencing adipocyte hypertrophy, adipokines secretion, and ectopic lipid accumulation ²⁶².

Lumeng et al. demonstrated that the phenotypic switching of macrophages in obese adipose tissue is due to spatial differences in macrophage subtypes ²⁶⁹. Resident adipose tissue macrophages from lean C57Bl/6 mice expressed markers associated with M2 phenotype localized to interstitial spaces between adipocytes ²⁶⁹. The “alternatively” activated macrophage, M2, has been suggested to function as a tumor-associated macrophage and in parasitic infections with the potential to regulate tissue repair and inhibit inflammation ²⁶⁶. Diet induced obesity in C57Bl/6 mice resulted in a high concentration of M1 gene expression localized to clusters of necrotic adipocytes with a significant reduction in markers for M2 gene expression ²⁶⁹. This differences in macrophage subtypes demonstrates that there is an obesity induced switch in activation of adipose tissue macrophages with a concomitant recruitment of inflammatory M1 macrophage subtype from the circulation to surround local necrotic adipocytes in obese adipose tissue ²⁶⁹.

Resident macrophages in adipose tissue are surrounded by adipocytes that are continually releasing free fatty acids that have the potential to activate adipose tissue macrophages ²⁷⁰. The excessive production of free fatty acids via lipolysis have the ability to activate inflammatory pathways primarily through the JNK cascade signaling system and increased I-kappa-B-kinase-beta (IKKB) in adipose tissue ²⁷⁰. Nguyen et al. demonstrated that clonal murine

monocyte/macrophage cells (RAW264.7) treated with a mixture of saturated and unsaturated free fatty acids invoke a proinflammatory response by activation of JNK and IKKB signaling²⁷⁰. The proinflammatory effects of free fatty acids were found to be time and concentration dependent²⁷⁰. At approximately 5 minutes after treatment of RAW264.7 cells with 500 μ M free fatty acids, JNK and IKKB activation was confirmed by western blot analysis for components of the JNK and IKKB signaling pathway²⁷⁰. Furthermore, 500 μ M of free fatty acids at approximately 3 hours induced activation of JNK pathway-mediated proinflammatory genes including IL-1 β , IL-6, MCP-1 and TNF- α ²⁷⁰.

Xu et al. demonstrated that the up-regulation of macrophages in white adipose tissue in the obese mouse model initiates an inflammatory response and interferes with insulin signaling, resulting in systemic insulin resistance²¹⁹. Xu et al. hypothesized that the excessive release of free fatty acids from adipocytes into systemic circulation impairs insulin signaling pathways in muscle and liver²¹⁹. Coculturing of differentiated 3T3-L1 adipocytes and RAW264.7 macrophage cells resulted in a dramatic increase in expression of TNF- α and a concomitant inhibition in the expression of the anti-inflammatory cytokine adiponectin⁵³. It has been suggested that a paracrine loop exists between adipocytes and macrophages, resulting in a vicious cycle that enhances inflammation in obese adipose tissue^{53, 271}.

A large scale gene expression analysis between the common features shared by preadipocytes, adipocytes, and macrophages revealed that the preadipocyte profile is more intimately related to the macrophage than the adipocyte²⁷². Moreover, given appropriate environmental conditions, preadipocytes can display cellular plasticity with the potential to efficiently be converted into macrophages²⁷². Charriere et al. indicated that direct contact between preadipocytes and macrophages appears to be required for phenotypic conversion between two distinct cell lineages²⁷². Accordingly, preadipocyte cellular plasticity may be a

contributor to the enhanced macrophage activity and inflammatory state of obese adipose tissue
219, 272 .

Calcitriol Modulates Macrophage Activity

Calcitriol has been found to induce adipocyte-macrophage cross-talk, resulting in modulation of adipose cytokine production ⁸¹. Calcitriol was found to enhance the expression of two macrophagic factors in differentiated adipocytes; macrophage inhibitory factor (MIF) and macrophage surface-specific protein (CD14) ⁸¹. This effect was inhibited by the addition of the calcium channel blocker, nifedipine, indicating an intracellular calcium dependent mechanism in the calcitriol mediated macrophagic activity ⁸¹. In addition, calcitriol increased expression of macrophage colony stimulating factor (M-CSF), macrophage inflammatory protein, IL-6, TNF- α and also MCP-1 ⁸¹. These effects were attenuated with the addition of either a calcium channel blocker, (nifedipine), or a mitochondrial uncoupler (dinitrophenol) ⁸¹.

Furthermore, co-culturing of RAW264.7 macrophages and 3T3-L1 adipocytes significantly up-regulated expression and production of inflammatory cytokines compared to individual cultures ⁸¹. These findings indicate a key role of calcitriol in increasing adipocyte inflammation by modulating the interaction between adipocytes and macrophages ⁸¹. It has previously been demonstrated that increasing dietary calcium attenuates obesity-induced oxidative stress by suppressing inflammatory markers ⁸¹. The principle factor responsible for this effect is a suppression of calcitriol resulting in regulation of calcium signaling and mitochondrial uncoupling leading to a reduction in the inflammatory status of adipose tissue ⁸¹.

Pharmacological Inhibition of RAS

Additional Benefits

Recent studies have indicated that anti-hypertensive treatment via pharmacological blockade of the RAS guards against the development of T2DM in high risk patients with or without hypertension²⁷³⁻²⁸². AT1R blockade has been demonstrated to enhance insulin sensitivity and thereby reduce the incidence of T2DM in hypertensive patients²⁸³. Moreover, elevated levels of plasma concentrations of ANGII have been reported in diet induced obese, hypertensive rats²⁸⁴. In contrast, the administration of AT1R blockers such as telmisartan to diet induced obese mice resulted in a reduction of weight gain and a decrease in plasma levels of glucose, insulin, and triglycerides compared to non-treated, control mice²⁸⁵. Various mechanisms have been proposed to explain how the inhibition of RAS provides these therapeutic metabolic effects independent of the primary hypotensive effects. These include enhanced muscle glucose uptake, protection of beta cell function, remodeling of adipose tissue structure, suppression of NADPH oxidase activity, increased activation of insulin signaling, and an up-regulation in expression of PPAR- γ by selective subclasses of pharmacological AT1R blockers²⁸⁵⁻²⁹¹. Collectively, these observations have stimulated a significant amount of interest to be placed on ANGII receptor blockers (ARBs) as potential therapeutic agents for the management of obesity related metabolic complications.

Improvement in Metabolic Parameters

Clinical studies have indicated that ANGII inhibition by subclasses of ARBs significantly reduce the incidence of T2DM when compared to other anti-hypertensive medications^{273, 274, 292-}

²⁹⁵. In addition, several studies collectively indicate that sub-types of ARBs including, telmisartan, irbesartan, and losartan, stimulate the nuclear hormone receptor, PPAR- γ ^{285, 296, 297}. PPAR- γ is considered a master regulator for adipocyte differentiation ^{118, 298, 299}. The strength of PPAR- γ stimulation by ARBs (in descending order) is telmisartan, irbesartan and losartan ^{297, 300}. The additional subtypes of ARBs, valsartan, candesartan, olmesartan, and eprosartan have little or no effect on PPAR- γ activity ^{285, 300}. However, clinical studies indicate that these ARBs do provide metabolic improvements regardless of their effects on PPAR- γ ^{293, 295, 296, 301-303}. Moreover, the ARBs, losartan, telmisartan, and irbesartan have been shown to up-regulate plasma levels of adiponectin ^{280, 285, 292, 293, 296}. Adiponectin levels are suppressed with increasing adiposity and are positively correlated with insulin sensitivity ^{292, 293, 304-306}.

PPAR- γ has been demonstrated to be a key factor responsible for increased expression levels of adiponectin ³⁰⁷. It is generally accepted that the clinical use of pioglitazone results in increased serum adiponectin secondary to transcriptional regulation mediated by PPAR- γ ³⁰⁷. However pre-treatment with a PPAR- γ inhibitor under basal conditions had no effect on the level of adiponectin expression, indicating that basal transcriptional activity of adiponectin may occur independently of PPAR- γ stimulation ³⁰⁸. Intriguingly, investigation into the mechanism responsible for the associated increase in adiponectin levels via ARB treatment has also proposed both PPAR- γ dependent and PPAR- γ independent regulatory mechanisms ^{296, 308}.

Special Attributes of Telmisartan

Telmisartan has been demonstrated to be the most potent PPAR- γ agonist ^{285, 297, 309}. Treatment with telmisartan increased PPAR- γ expression, and this effect was not antagonized by a PPAR- γ inhibitor ^{285, 308, 310}. This finding suggests that telmisartan's effect on adiponectin

expression may be independent of PPAR- γ stimulation³⁰⁸. Investigation into this unique effect of telmisartan identified specific biological features applicable only to telmisartan, and not the additional ARBs, and traditional inducers of PPAR- γ , TZDs^{285, 297, 300, 311}. These unique properties are a result of the molecular structure of telmisartan which is significantly different from the structures of other ARBs³¹². Molecular modeling studies have identified distinct features of the structure of telmisartan which include, a carboxyl substituent in place of the tetrazole group, the heterocyclic substituent of the benzimidazole moiety is absent, and the imidazole moiety is lacking a carboxyl group³¹². It is these structural differences that have been proposed to provide telmisartan with specific attributes unrelated to the additional ARBs^{300, 312, 313}.

In vitro studies comparing cellular diffusion levels of telmisartan to losartan reported that the intracellular concentration of telmisartan was 10 fold higher than the concentration in the culture medium while losartan was undetectable intracellularly compared to culture medium³¹⁴. This effect of telmisartan was suggested to be due to its higher lipophilicity and higher membrane permeability compared to losartan³¹⁵. As a result, these properties have been suggested to endow telmisartan with a unique fully penetrable cellular structure non-existent to the other ARBs^{309, 314, 315}. Molecular characterization of telmisartan within the LBD of PPAR- γ has indicated a unique orientation which produces a highly stabilized complex³¹⁶. Crystallography studies indicate that the binding mode of telmisartan in the receptor pocket is stabilized by hydrophobic interactions between helices H3 and H7 and by hydrogen bonding with an amide proton^{285, 316}. These conformational arrangements within the receptor pocket do not appear to exist with the additional ARBs or traditional TZDs^{285, 316}. These structural differences between telmisartan and the other ARBs may explain the underlying differences in the selective PPAR modulators (SPARMs) by ARBs²⁹⁷.

In comparison to TZDs, telmisartan was found to act as a partial agonist of PPAR- γ , resulting in differential modulation of PPAR target gene expression patterns compared to a full agonist of PPAR- γ such as, rosiglitazone ²⁸⁵. This differential regulation is evident by investigation of the gene expression pattern of acetyl-CoA carboxylase-2, (ACC2), a primary regulator of muscle fatty acid metabolism ²⁸⁵. Treatment of murine muscle myotubes with telmisartan resulted in a down-regulation of ACC2 gene expression while treatment with rosiglitazone, irbesartan, and valsartan had no effect ²⁸⁵. Reducing ACC2 expression has been suggested to promote fatty acid oxidation in muscle with a reduction in adiposity, possibly explaining the observed in vivo weight loss via treatment with telmisartan ³¹⁷. As a result this raises significant interest in the ability of SPPARMs to regulate carbohydrate and lipid gene expression without activating expression of genes associated with weight gain, fluid retention and additional adverse effects associated with the treatment of traditional PPAR- γ modulators ^{285, 318, 319}.

Irbesartan Prevents Degradation of Adiponectin

Irbesartan appears to function as another SPPARM which increases adiponectin levels, ²⁹⁶. Mechanistically irbesartan has been suggested to prevent cellular adiponectin protein depletion while significantly augmenting the half life of adiponectin dependently on PPAR- γ activity ²⁹⁶. However, irbesartan had no effect on mRNA adiponectin levels ²⁹⁶. Instead, this appears to be a result of abolishment of the ubiquitin-proteasome pathway, upstream of the 26S-proteasome complex ²⁹⁶. The 26S-proteasome is a multi-subunit enzyme complex located in the nucleus and cytoplasm of the cells which plays a key role in the degradation of proteins ³²⁰. The 26S-proteasome utilizes a unique substrate identification mechanism which results in covalent modification of substrates which are then tagged with the well recognized protein, ubiquitin ³²⁰.

In vitro treatment of adipocytes with peptide aldehyde proteasome inhibitors prevented adiponectin protein depletion as effectively as treatment with irbesartan ²⁹⁶. As a result, cellular adiponectin levels are stabilized and enhanced via treatment with irbesartan (10 μ mol/L). In addition to stimulation of PPAR- γ , irbesartan also was found to induce the PPAR- γ target gene, aP2 ²⁹⁶. Collectively, these findings suggest that the PPAR- γ inducing effects of the ARB, irbesartan, protects against degradation of adiponectin protein at the post-transcriptional level ²⁹⁶.

To investigate PPAR- γ activating and non-PPAR- γ activating ARBs on adiponectin level in vivo, obese Zucker fa/fa fatty rats were administered Irbesartan (50 mg/kg) orally for 21 days or given an oral saline solution for duration of treatment ²⁹⁶. Metabolic comparison at day 0 and day 21 of treatment showed that the irbesartan group had a 36% decrease in fasting insulin levels and a significant reduction in serum triglyceride levels, demonstrating a marked improvement in metabolic parameters compared to saline (control) group ²⁹⁶. Serum adiponectin levels of the irbesartan treated group remained stable through duration of treatment period while the control group had a 20% reduction in serum adiponectin levels at end of treatment period ²⁹⁶. These findings suggest that treatment with irbesartan provides protection against the degradation of serum adiponectin levels.

Irbesartan has been demonstrated to activate the insulin sensitizing nuclear transcription factor PPAR- γ , independent of inhibition of AT1R ^{285, 300}. This finding is supported by in vitro studies demonstrating that pharmacological antagonism of PPAR- γ suppresses irbesartan's effects on adiponectin expression ²⁹⁶. Moreover, obese rats treated with irbesartan showed an improvement in insulin sensitivity associated with a stabilization in serum adiponectin levels ²⁹⁶. These findings suggest that irbesartan maintains adiponectin levels via stimulation of PPAR- γ ²⁹⁶. In contrast non PPAR- γ activating ARBs such as eposartan failed to have an effect on adiponectin expression ²⁹⁶.

Remodeling of Adipose Tissue

The remodeling of adipose tissue mediated by TZDs is associated with newly formed small clusters of adipocytes, an increase in the expression of key lipogenic enzymes with a increased response to the effects of insulin ³²¹. Despite the quantitative increase in adipose tissue mass by treatment with TZDs, there is an associated improvement in the metabolic state ³²¹. In contrast, in vivo treatment with telmisartan has demonstrated an insulin sensitizing effect without the simultaneous stimulation in adiposity ³²²⁻³²⁴. The insulin sensitizing effects of specific ARBs have been suggested to result from up-regulation in the expression of insulin sensitizing adipocytokines ^{296, 324-326}. The associated insulin sensitizing effects of adiponectin have been attributed to the stimulation of AMP-kinase, leading to an enhancement in glucose disposal and fatty acid oxidation, reducing tissue triglyceride accumulation ³²⁷.

The apparent decrease in adipose tissue mass with telmisartan results from enhanced expression of adipose tissue UCP-1 ³²⁴. This is in agreement with a prior study reporting an up-regulation in adipose UCP-1 expression via AT1R knock-out mice ³²⁸. Moreover, the telmsiartan increase in UCP-1 expression was accompanied with an increase in oxygen consumption and a decrease in respiratory quotient compared to control ³²⁴. This evidence indicates the ability of specific ARBs such as, telmisartan, to be regulators of adipose tissue metabolism ³²⁴.

Up-regulation of UCP-1 expression via telmisartan increases oxygen consumption and decreases the respiratory quotient indicates a switch from carbohydrate to lipid as a metabolic fuel source ³²⁴. This metabolic switch has been proposed to be responsible for the phenotypic decrease in body adiposity observed with treatment of diet induced obese mice with the ARB, telmisartan ³²⁴. The apparent decrease in adiposity while retaining PPAR- γ modulating activity

associated with selective ARBs such as telmisartan and irebesartan demonstrates the efficacy of using ARBs as therapeutic treatment agents for obesity associated metabolic disorders^{296, 324-326}.

Negate Effects of Obesity Induced Hypoxia

Increasing adipose tissue mass is significantly correlated with alterations in the endocrine and metabolic functions of adipose tissue⁵⁶. Specifically, adipose tissue hypoxia has been recognized as a key consequence of obesity³²⁹. In obesity, adipocytes become hypertrophic, resulting in a significant increase in diameter of up to 140-180 μM ³³⁰. The capacity for adipocyte hypertrophy is limited by the 100 μM diffusion limit of oxygen and consequently oxygen availability does not meet oxygen demand during obesity^{331, 332}. Previous reports have demonstrated that adipocyte hypoxia stimulates adipocytokine dysregulation secondary to up-regulation in oxidative stress induced dependent posttranscriptional mechanisms³²⁹. The effects of hypoxia are mediated by the transcription of hypoxia inducible factor (HIF) responsible for stimulating expression of hypoxia sensitive markers, such as GLUT-1³³³.

Hypoxia up-regulates the expression of inflammatory adipocytokines such as IL-6 and MCP-1 and down-regulates expression of anti-inflammatory adipocytokines^{334, 335}. Moreover, obesity stimulates an increase in HIF which has been suggested to negatively regulate insulin signaling, contributing to insulin resistant adipocytes³³⁶. HIF expression has been shown to stimulate insulin resistance whereas HIF inhibition via HIF siRNA restored insulin stimulation of the IR³³⁶. The inhibitory effects of hypoxia were found to occur at the step of insulin receptor tyrosine autophosphorylation³³⁶. Chronic up-regulation in HIF has been reported to also stimulate infiltration of macrophages, leading to a further dysregulation in adipocytokine expression^{329, 337}.

HIF proteins are stimulated not only by hypoxia but also by various growth factors and inflammatory cytokines such as ANGII^{336, 338, 339}. A primary adaptive change associated with chronic hypoxia is angiogenesis and ANGII is a potent angiogenic factor^{338, 340, 341}. Mounting evidence indicates that therapeutic intervention of obesity induced hypoxia may be achieved via targeting the vasculature^{342, 343}. ARBs have been reported to reduce the expression of HIF with a concomitant attenuation in the expression of inflammatory cytokines and abrogation of the angiogenic response to chronic hypoxia^{338, 341, 344, 345}.

Antioxidant Effect

It is well accepted that activation of the AT1R by ANGII is associated with induction of ROS formation mediated by the AT1R³⁴⁶⁻³⁴⁹. In vitro studies have demonstrated a time and dose dependent induction of superoxide production in response to treatment with ANGII³⁵⁰⁻³⁵³. The molecular source of ANGII induced superoxide production has been attributed to an up-regulation in the expression of NADPH oxidase subunits^{349, 354-357}. Treatment with NADPH oxidase inhibitors attenuated the dysregulation of adipocytokine expression suggesting that the redox state of adipose tissue is a powerful regulator of local oxidative stress²²¹.

Several studies have indicated that the AT1R inhibitor, valsartan, acts as an anti-oxidant^{358, 359}. This anti-oxidant activity of valsartan has been suggested to be a result of its molecular structure³⁵⁹. The presence of phenolic rings or conjugated double bonds has been suggested to be the determinant for the scavenging properties of anti-oxidant like molecules³⁶⁰. Valsartan is composed of several aromatic rings which have been suggested to provide the anti-oxidant effect associated with its usage^{358, 359}. Additional studies suggest alternative mechanisms for the observed anti-oxidant effect of ARBs^{314, 350, 361-365}. Telmisartan was found to directly suppress

reactive oxygen species production without up-regulating intracellular anti-oxidant molecules³¹⁴. Further investigation into the anti-oxidant ability of telmisartan revealed a non-receptor-mediated anti-oxidant effect of telmisartan, but not losartan, in AT1R knockout mouse mesangial cells³¹⁴. These findings indicate that telmisartan's anti-oxidant properties occur in both receptor-independent and receptor-dependent fashion³¹⁴.

A major advantage of in vitro studies using ARBs is that the results observed can not be mediated by any additional cellular intermediates making clear the in vitro effects of ARB agents. In vivo studies with hypertensive patients have been reported to have increased ROS production accompanied by an up-regulation in superoxide dismutase (SOD) activity³⁵⁸. This finding indicates that increased expression of endogenous anti-oxidant enzymes may signify cellular defense mechanisms for combating oxidative stress³⁵⁸. Interestingly, administration of 180 mg daily of valsartan for three months to hypertensive patients resulted in a 2 fold decrease in SOD activity and protein expression when compared to pre-treatment measurements³⁵⁸. This finding is consistent with other findings demonstrating an antioxidant effect associated with pharmaceutical blockage of AT1R^{359, 366}.

In vivo, ARBs have the ability to target all three significant sources of oxidative stress such as; suppression of glycation, oxidative metabolism, and chelation of transition metals respectively^{367, 368}. ARBs are the only hypotensive agent demonstrated to inhibit advanced glycation³⁶⁸. Valsartan and additional ARBs have been shown to successfully suppress the inflammatory action of advanced glycation end products (AGEs) providing an additional defense against oxidative damage³⁶⁶. Mechanistically it has been suggested that AT1R inhibitors reduce glycation by chelating transition metals and inhibiting oxidative steps such as carbon centered and hydroxyl radicals at the pre and post stages of the Amadori reaction^{366, 369}. These effects of AT1R inhibitors result in a reduction of reactive carbonyl precursors generated by auto-

oxidation of carbohydrates or lipid peroxidation ³⁷⁰. The ability of AT1R inhibitors to chelate transition metals holds great therapeutic potential in combating cross linking of proteins and formation of AGEs ³⁷¹.

Highly potent sources of ROS are generated by the metal catalyzed glucose autooxidation and oxidation of glycated residues resulting in the cross link of proteins, formation of AGEs and incurring insults to cellular structures and tissue functions ³⁷¹. The ability of AT1R antagonists to chelate transition metals has been reported to down-regulate the cross-linking of proteins demonstrating an alternative, off label approach for the use of AT1R antagonists ³⁶⁸. Iron deposition stimulates the fenton reaction and hydroxyl radical generation exacerbating oxidative stress ³⁷². Chronic infusion of ANGII into rats has been reported to accelerate the deposition of iron and infiltration of inflammatory cells ^{373, 374}. All ARB agents have been shown to share the same characteristic of transition metal chelation ^{367, 369, 375}.

Pancreatic Beta Cell Function and Insulin Sensitivity

The pancreas also appears to have a complete functional RAS which is up-regulated as a result of hyperglycemia and oxidative stress ^{288, 290, 376-380}. Moreover, exogenously administered ANGII has been demonstrated to directly inhibit insulin release as a result of decreasing islet blood flow and suppressing (pro) insulin biosynthesis ³⁸¹. Stimulation of the pancreatic RAS mediated by hyperglycemia was demonstrated to induce pancreatic stellate cells responsible for contributing to pancreatic inflammation, fibrosis, insulin resistance, and pancreatic oxidative stress ³⁸⁰. Pancreatic oxidative stress via RAS mediated ROS production is a key factor initiating beta cell dysfunction ³⁸². Moreover, beta cell dysfunction is considered the primary metabolic defect in T2DM ³⁸³.

Reducing the decline and eventual failure of beta cells is a critical step in preventing the development of diabetes ³⁸⁴. Therapeutic and prophylactic treatments with ARBs have been shown to block NADPH oxidase activity attenuating oxidative modification of pancreatic proteins ³⁸². A decrease in pancreatic oxidative damage, via treatment with ARBs, has been shown to improve beta cell function and glucose tolerance in part by increased islet blood flow ³⁷⁸. Collectively, these findings indicate that antagonism of the RAS may play a significant role in the protection and preservation of pancreatic beta cell structure and function ³⁸⁰.

Obesity induced hypertrophic adipocytes has been indicated to be a key factor in the development of adipose tissue dysfunction ^{385, 386}. Adipocyte hypertrophy is associated with a significant increase in the rate of lipolysis suggesting that there is an increase in circulatory levels of non-esterified fatty acids (NEFA) in obese individuals ^{387, 388}. The increased rate of lipolysis due to adipocyte hypertrophy has been proposed to be the driving force of whole body insulin resistance ³⁸⁸. AT1R blockade was shown to decrease plasma levels of NEFA in obese mice, stimulate formation of smaller, more insulin sensitive adipocytes, and ameliorate adipocytokine dysfunction ²⁸⁶.

Administration of AT1R pharmacological inhibitors in insulin resistant, hypertensive rats and hypertensive human subjects results in a therapeutic improvement in insulin sensitivity ³⁸⁹⁻³⁹¹. It has been suggested that the determining factor in the attenuation of skeletal muscle glucose transport by AT1R antagonism is mediated in part by an up-regulation in GLUT-4 protein expression ^{288, 390}. Accordingly, AT1R blockade with irbesartan was shown to increase the translocation of GLUT-4 to the plasma membrane, augmenting whole body insulin sensitivity in obese Zucker rats ³⁹⁰. This effect of irbesartan was suggested to be a result of abrogating ANGII induced suppression of insulin signaling ²⁸⁸.

ANGII has been shown to impair insulin signaling by suppressing IRS-1 tyrosine phosphorylation and activation of PI3-K, thus diminishing the insulin signaling cascade system²⁹¹. This effect of ANGII was attenuated by addition of saralasin, a specific inhibitor of ANGII²⁹¹. Selective AT1R blockade via treatment with valsartan reportedly increased insulin sensitivity and glucose uptake in skeletal muscle of T2DM mice, thereby increasing GLUT-4 translocation to the plasma membrane²⁸⁷. Valsartan treatment intensified insulin induced tyrosine phosphorylation of IRS-1, enhancing PI3-kinase activity, and GLUT-4 translocation to the plasma membrane²⁸⁷.

Research demonstrates that TNF- α suppresses insulin signaling leading to a reduction in GLUT-4 translocation to the plasma membrane³⁹². Moreover, ANGII is considered to be a primary factor upregulating skeletal muscle TNF- α ³⁹³. Accordingly, AT1R blockade with valsartan reportedly decreased TNF- α expression in skeletal muscle of diabetic mice²⁸⁷. These findings indicate that suppressing production of TNF- α via ARBs in skeletal muscle improves insulin sensitivity²⁸⁷. In addition, it has been demonstrated that ROS play a critical role in the development of insulin resistance and ANGII is a well known stimulator of ROS production³⁹⁴. ANGII activates NADPH oxidase to produce superoxide, which leads to phosphorylation of IRS-1, resulting in inhibition of IRS-1 activity and down-regulation of GLUT-4 translocation³⁹⁵. Administration of valsartan was found to significantly decrease production of superoxide in skeletal muscle of diabetic mice²⁸⁷. These findings suggest that tissue superoxide production is modulated by AT1R activation and inhibition also enhances insulin sensitivity by decreasing ROS production²⁸⁷. Collectively, these findings indicate that AT1R blockade has potential to ameliorate inhibition of insulin signaling thereby improve insulin signaling.

Down-Regulate Inflammatory Signaling

ANGII exerts pro-inflammatory effects in a multitude of cell types including endothelial cells, vascular smooth muscle cells, and also adipocytes³⁹⁶⁻⁴⁰⁰. A critical component linking ANGII and inflammation is the transcription factor, NF κ B³⁹⁶. NF κ B is a redox sensitive nuclear transcription factor, composed of two protein subunits, p65 and p50⁴⁰¹. NF κ B plays a key role in mediating the inflammatory effects of ANGII and increased expression of inflammatory cytokines including, TNF- α , IL-6, IL-1, adhesion molecules, and chemokines^{353, 396}. In the cytoplasm, NF κ B is bound to an inhibitory protein, I κ B, resulting in the inactive conformation of NF κ B⁴⁰². The I κ B proteins consist of three functional proteins, I κ B- α , I κ B- β , and I κ B- ϵ residing in the cytoplasm of un-stimulated cells and coupled to NF κ B in the inactive conformation⁴⁰³. The regulatory factor of NF κ B activation is the phosphorylation of I κ B proteins by the IKK complex⁴⁰³. The IKK complex is comprised of two homologous kinase subunits, IKK- α and IKK- β and the regulatory subunit IKK- γ ⁴⁰⁴.

Inducers of NF κ B activation include the pro-inflammatory cytokines and chemokine families such as, TNF- α , IL-1, IL-6, ROS and ANGII⁴⁰⁵. These inducers of the NF κ B pathway stimulate the IKK complex to phosphorylate I κ B proteins resulting in the ubiquitylation and proteosomal degradation⁴⁰³. The degradation of I κ B proteins transforms NF κ B into its activated form resulting in the translocation of NF κ B to the nucleus⁴⁰³. In the nucleus, NF κ B binds to promoters of target genes including I κ B- α which terminates transcriptional activity by binding to NF κ B in the cytoplasm^{403, 406}. The signaling pathway of NF κ B has proven to be quite complex as post-translational modifications of NF κ B enable this transcription factor to both stimulate and inhibit the expression of target genes⁴⁰³.

Previous research has demonstrated that infusion of ANGII in rats up-regulated renal and VSMC NF κ B binding activity⁴⁰⁷⁻⁴⁰⁹. Accordingly, ANGII mediated activation of NF κ B

stimulated inflammatory cell infiltration and tubule-interstitial inflammatory responses^{407, 410}. In cultured adipocytes, ANGII has been demonstrated to also up-regulate expression of pro-inflammatory mediators, IL-6, IL-8, and PAI-1, mediated by activation of NFKB signaling pathway^{400, 411}. Antagonism of redox sensitive NFKB mediated inflammation has been shown to be effective by treatment with the ARB, candesartan⁴¹². Attenuation of NFKB signaling, by candesartan, was suggested to result in suppressing TNF chemokine expression and maintain redox homeostasis in cultured renal tubular epithelial cells⁴¹². This decrease in oxidative stress was suggested to be a result of the anti-oxidant effect of candesartan⁴¹². This effect was dose dependent, and a dose five times the standard therapeutic dose significantly reduced renal inflammation and blocked NFKB activity in spontaneously hypertensive rats⁴¹². Moreover, an ultra dose, fifteen times the therapeutic standard, intensified this renal anti-oxidant effect⁴¹².

ANGII was demonstrated to up-regulate MCP-1 mRNA expression in rat pre-adipocytes via AT1R mediated and NFKB dependent pathway²¹⁷. This effect was attenuated by the ARB, valsartan, and also by a NFKB inhibitor²¹⁷. Immunocytochemical studies revealed that ANGII induces translocation of the NFKB subunit p65 from the cytoplasm to the nucleus, characterized as the hallmark of NFKB activation²¹⁷. MCP-1 expression is associated with a reduction in insulin stimulated glucose up-take and decreased expression of adipogenic genes such as PPAR- γ , aP2, adipsin, and LPL⁴¹³. Mechanistically these findings show that ANGII induces NFKB activation, resulting in transcription of inflammatory cytokines such as, MCP-1^{217, 413}.

The up-stream signaling pathways regulating NFKB activation have been well characterized and consist of the canonical (classical) and noncanonical (alternative) pathways^{405, 414}. The classical NFKB pathway is the primary regulator of NFKB activation, with the associated up-regulation of inflammatory cytokines, chemokines, and growth factors and is well characterized for the inflammatory process of NFKB in cells and tissues^{415, 416}. A key feature

required for stimulation of the canonical pathway is activation of the IKK complex ⁴¹⁴. In the classical pathway, inducers of NF κ B bind to their cell surface receptors along with attachment of adaptor proteins to the cytoplasmic domains of the inducers ⁴⁰⁵. This binding pattern stimulates recruitment and activation of the catalytic subunits IKK- α and IKK- β and the regulatory subunit IKK- γ ⁴⁰⁵. Activated IKK phosphorylates I κ B at its serine residues which are then ubiquitinated resulting in degradation of the inhibitory protein I κ B ⁴⁰⁵. Removal and degradation of I κ B releases NF κ B allowing for translocation to the nucleus and regulation of target genes ⁴⁰⁵. In contrast, while not fully elucidated, the alternative pathway responds to B-cell related signaling playing an important role in premature B-cell survival and lymphoid organ development ⁴¹⁷.

Interestingly, the alternative pathway acts independent of I κ B but rather an IKK complex consisting of only two IKK- α subunits are recruited and no regulatory subunit IKK- γ ⁴¹⁷. This IKK complex is then activated via phosphorylation by an up-stream NF κ B inducing kinase (NIK) releasing NF κ B ⁴¹⁷. NF κ B activation by ANGII is not well characterized, but it is suggested to require AT1R activation and G protein coupled receptors (GPCRs), following a series of complex cellular mechanisms ^{396, 399, 418}. Binding of ANGII to the AT1R results in a cascade of signaling events starting with activation of phospholipase C (PLC)/Ca²⁺/PKC signaling, transactivation of epidermal growth factor receptors and activation of receptor tyrosine kinase consisting of ERK1/2 and p38 MAPK ⁴¹⁹⁻⁴²¹. Activation of the AT1R signaling cascades recruits adaptor proteins for the activation of IKK complex, phosphorylating I κ B followed by proteosomal degradation of I κ B ⁴²²⁻⁴²⁴.

Studies indicate that ANGII stimulation of NF κ B occurs by both canonical and noncanonical signaling pathways ^{422, 424}. Moreover, research suggests that ANGII can induce cross-talk between both pathways achieving maximal stimulation of NF κ B ^{422, 425}. This noncanonical cellular signaling pathway has been proposed to involve Ras/mitogen activate

kinase-1 (MEK-1)/ ERK1/2/ribosomal S6 kinase (RSK) resulting in phosphorylation and activation of p65 subunit of NFkB ⁴²². This signaling pathway is supported by the finding that addition of MEK-1 inhibitor to ANGII treated VSMC suppressed transcriptional activity of NFkB ⁴²². In addition, ANGII was found to up-regulate RSK resulting in direct phosphorylation of p65 and activation of NFkB ⁴²². This RSK mediated phosphorylation of p65 was further investigated by an in vitro assay using recombinant glutathione-S-transferase p65 (GST-p65) ⁴²². Immunopurified RSK from ANGII treated VSMC resulted in phosphorylation of recombinant GST-p65 ⁴²². These findings confirm that ANGII induces activation of NFkB by a noncanonical signaling pathway that is MEK-1 dependent leading to RSK mediated phosphorylation of p65 ⁴²².

ANGII has been demonstrated to stimulate the expression and release of the inflammatory cytokines, IL-6 and IL-8 in cultured adipocytes ⁴⁰⁰. This inflammatory effect was demonstrated to be due to activation of the AT1R and stimulation of the NFkB pathway ⁴⁰⁰. Treatment with the ARB, candesartan and the NFkB inhibitor suppressed expression of IL-6 and IL-8 ⁴⁰⁰. To examine ANGII stimulatory effects of NFkB in adipocytes a western blot analysis was performed ⁴⁰⁰. Approximately five minutes post-incubation with ANGII (10^{-5} M) resulted in a two fold increase in phosphorylation of NFkB p65 subunit ⁴⁰⁰. An electromobility shift assay was used on nuclear extracts from cells treated with or without ANGII to determine if ANGII translocates NFkB to the nucleus ⁴⁰⁰. As expected, ANGII catalyzed the translocation of NFkB to the nucleus in adipocytes ⁴⁰⁰. This activity of NFkB mediated by ANGII was abolished by addition of the ARB, candesartan, while the AT2R blocker, PD123319 had no effect ⁴⁰⁰. These findings demonstrate that adipose AT1R activation acts via NFkB signaling pathway to contribute to an inflammatory response ⁴⁰⁰.

Additional research also confirmed a key role for the activation NFkB in mediating ANGII induced inflammatory effects in adipocytes ²¹⁷. ANGII was shown to positively effect the

mRNA and protein expression of MCP-1 in rodent preadipocytes via an AT1R mechanism ²¹⁷. MCP-1 expression was also up-regulated via exogenously administered ANGII in vivo to rodents ²¹⁷. This finding is in accordance with current research supporting the notion that inflammatory adipocytokines such as ANGII and MCP-1, secreted from obese adipose tissue promotes and exacerbates cardiovascular disease by favoring an atherogenic effect via NFkB dependent mechanism in cardiovascular cells ⁴²⁶⁻⁴²⁸.

ANGII induced up-regulation of MCP-1 expression in adipocytes was abolished by the addition of a NFkB blocker, a direct inhibitor of IKB- α phosphorylation ²¹⁷. Moreover, luciferase assay using rat MCP-1 promoter constructs indicated two NFkB binding sites in the MCP-1 enhancer region required for the ANGII stimulated MCP-1 transcription ²¹⁷. Elevated expression of adipose tissue MCP-1 is positively associated with macrophage infiltration and intensification of the inflammatory response in adipocytes ⁴²⁹. Treatment of diet induced obese rats with ARBs potently inhibited the expression of MCP-1 and down-regulated the level of macrophage infiltration leading to an overall reduction in systemic inflammation ⁴³⁰. Accordingly, pharmacological RAS inhibition by ARBs may serve as therapeutic targets to suppress the molecular mechanisms underlying the inflammatory changes in obese adipose tissue and thereby attenuate obesity induced diseases ^{400, 430, 431}.

Problem Statement

The RAS via ANGII generates inflammatory and oxidative stress altering the redox status of adipose tissue. Calcitriol has also been demonstrated to alter the redox status of adipose tissue, contributing to oxidative stress. However, data are lacking to determine whether or not calcitriol up-regulates the adipocyte RAS to generate local inflammatory cytokine expression in adipose tissue. This research aims to investigate; the role of RAS inhibition in the modulation of inflammatory cytokine expression in adipocytes, determine role of calcitriol modulation of the adipocyte RAS in the regulation of ROS and inflammatory cytokine expression, and evaluate discrepancies in inflammatory cytokine expression from the pharmaceutical ARBs using siRNA to knockdown expression of AT1R.

Chapter II

Research Paper

The Adipocyte Renin Angiotensin System Mediates the Effects of Calcitriol on Oxidative Stress and Cytokine Production in Cultured 3T3-L1 Cells

Introduction

Adipose tissue is a multifunctional endocrine organ that plays a central role in modulating metabolic function and inflammation^{19, 38, 432}. Excess adipose tissue, specifically in the visceral region, is a critical contributor to systemic oxidative stress and metabolic dysfunction^{82, 220, 433}. The recognition of adipose tissue as an endocrine organ suggests that adipose tissue derived biological molecules may be a key factor in the metabolic disturbances associated with obesity³⁷. Adipocytes synthesize and secrete a variety of inflammatory and anti-inflammatory cytokines (adipocytokines)^{11, 219, 434, 435}. Obesity is associated with a dysregulation in adipocytokine production which is characterized by increased expression of inflammatory cytokines such as TNF- α , IL-6, and IL-8 and a decrease in the anti-inflammatory cytokines such as adiponectin and IL-15^{82, 221, 346, 436-439}.

Adipose tissue possesses all components of a functional RAS including local production of the main effector molecule ANGII^{11, 440}. Traditionally the RAS was identified as a critical regulator of blood pressure, electrolyte balance, and a critical factor of hypertension^{441, 442}. Obesity is frequently associated with hypertension and the discovery of an active adipose tissue RAS suggests the possibility that locally formed ANGII may contribute to the pathophysiology of obesity providing a link connecting obesity with the development of hypertension^{37, 190, 443}. Locally formed ANGII has been demonstrated to modulate adipocyte lipid metabolism by up-

regulating key lipogenic enzymes, FAS and glycerol-3-phosphate dehydrogenase, stimulating preadipocyte differentiation and accumulation of lipids ²⁰⁶.

Obesity is recognized as a low grade inflammatory state associated with macrophage infiltration in adipose tissue and an increase in circulating levels of inflammatory molecules ^{262, 432, 444}. Increasing adiposity is accompanied with an up-regulation in adipocyte monocyte chemotactic factors that facilitate macrophage infiltration and local oxidative stress in adipose tissue ^{56, 219, 445-448}. Increased oxidative stress has been demonstrated in obese versus lean individuals and it has been suggested that pro-inflammatory chemokines, cytokines, and hormones released from adipose tissue are significant mediators of systemic oxidative stress in obesity ^{445, 447, 449, 450}. ANGII increases oxidative stress in adipose tissue by up-regulating expression of a key ROS generating enzyme, NADPH oxidase (NOX4) and stimulating superoxide release ^{11, 202, 221, 451}. ANGII alteration of adipose tissue redox state increases expression of pro-inflammatory adipocytokines, exacerbating the inflammatory state of adipose tissue ^{219, 449}.

We previously have demonstrated that dietary calcium modulates adipocyte metabolism by inhibiting lipogenesis, stimulating lipolysis and thermogenesis, and increasing adipocyte apoptosis ^{82, 103, 116}. We have also shown that oxidative stress due to diet induced-obesity can be inhibited by increasing dietary calcium and decreasing adiposity ⁸⁴. These effects are accomplished by suppressing both the genomic and non-genomic actions of calcitriol ^{80, 83, 86, 88, 116}. Adipocyte intracellular calcium signaling is a key factor in the modulation of adipocyte metabolism ⁸⁶. Increasing intradipocyte calcium stimulates expression of key lipogenic enzymes and suppresses lipolysis ^{63, 452}. We previously demonstrated calcitriol induced calcium influx in adipocytes, resulting in increased expression of lipogenic enzymes and expansion of adipocyte triglycerides ⁸⁶.

Calcitriol also acts via the adipocyte nVDR to suppress expression of UCP-2⁸⁰. UCPs diminishes the mitochondrial proton gradient resulting in a thermogenic effect and a potential role in energy metabolism¹⁴⁹. We previously showed calcitriol to suppress expression of adipocyte UCP-2⁸⁰. We also demonstrated that calcitriol increases ROS production and that this effect is mediated by both an increase in calcium influx and a decrease in UCP-2 expression^{82, 83, 88}. Furthermore, we previously demonstrated that calcitriol directly stimulates local inflammation by modulating the interaction between adipocytes and macrophages to synergistically up-regulate production of inflammatory cytokines⁸¹. In the present study we investigated the role of the adipocyte RAS in mediating these effects of calcitriol in cultured 3T3-L1 cells. We hypothesize that calcitriol directly up-regulates the adipose tissue RAS and that calcitriol stimulation of inflammatory cytokine expression is partially mediated by activating the adipocyte RAS.

Methods

Cell Culture and Differentiation of 3T3-L1

3T3-L1 preadipocytes were obtained from American Type Culture Collection (Manassas, VA, USA). Preadipocytes were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (PS) and incubated at 37°C with 5% CO₂ in air. Confluent preadipocytes were induced to differentiate with standard differentiation cocktail containing: DMEM, 10% FBS, 1% PS, 1 µM dexamethasone, 0.5 mM isobutyl-1-methyl-xanthine (IBMX). Preadipocytes remained in differentiation medium for three days and then transferred to adipocyte medium; cells were re-fed every two days until 90% of cells were fully differentiated and lipid-filled.

RNA Extraction

RNA extraction from 3T3-L1 cells was performed using Total Cellular RNA Isolation Kit (Ambion Inc., Austin, TX), according to manufacturer's guidelines. The RNA concentration was determined by measuring the optical density (OD) at 260. All samples were diluted to 20 ng total RNA/µL in diethylpyrocarbonate (DEPC) water.

Quantitative Real Time Polymerase Chain Reaction

18S, IL6, NOX4, MCP-1 and adiponectin mRNA levels were quantitatively determined using a Smart Cycler Real-Time Polymerase Chain Reaction (PCR) System (Cepheid, Sunnyvale, CA, USA) with a TaqMan 1000 Core Reagent Kit (Applied Biosystems, Branchburg,

NJ, USA). The primer and probe sets were supplied by Applied Biosystems TaqMan Assays-on-Demand Gene Expression primer and probe set collection. 3T3-L1 adipocyte total RNA was serial-diluted in the range of 1.5625-25 ng and used to construct a linear standard curve. Total RNAs for samples were also diluted in this range and then calculated for quantification of mRNA of unknown samples from the standard curve. This method evaluates the cycle threshold changes for each target gene and reports the quantification of each target in arbitrary units. Accurate quantification of the arbitrary units of the target gene of interest are normalized as ratios to 18S arbitrary units. Quantitative real-time PCR for standards and unknown samples were performed in accordance to the instructions of the Smart Cycler System and TaqMan Real Time PCR Core Kit supplied by Applied Biosystems.

Gene Silencing with Small Interfering RNA

Small interfering RNA (siRNA) targeted against Angiotensin II type 1 receptor (AT1R) mRNA was purchased from Applied Biosystems (Foster City, CA USA). The two sequences CCUCGAUGGUAUAAAUGUtt (sense) and ACAUUUAUUACCAUCGAtt (antisense) were simultaneously transfected into differentiated 3T3-L1 cells according to manufacturer's instructions (catalog # AM16708). Non-targeting siRNA provided by the manufacturer was used as a negative control to test non-specific effects on gene expression (catalog # AM4611). Differentiated 3T3-L1 cells were transfected using siPORT NeoFX transfection agent for 48 hours in 6 well plates with 5 nmole/well/siRNA for a total siRNA concentration of 10 nM siRNA/well. At 48 hours post-transfection cells were treated with angiotensin II (1 nM), angiotensin II (100 nM), or calcitriol (10 nM) for an additional 48 hours. RNA analysis and Real-Time PCR were used to measure knockdown of the AT1R.

Chemicals

Angiotensin II, calcitriol, P5749 S-(+)-PD 123177 (PD), telmisartan, dexamethasone, PS, FBS, and IBMX were all obtained from Sigma (St. Louis, MO USA). The pre-designed siRNA AT1R, negative control, and siPORT NeoFX transfection agent was supplied by Applied Biosystems.

Statistical Analysis

Data were evaluated for statistical significance by analysis of variance to determine if there were pair wise differences between means of cytokine ratio. Significantly different group means were then separated by the least significant difference test by using SPSS (SPSS Inc., Chicago, IL). The alpha level of 0.05 was used to determine statistical significance. All data presented are expressed as mean $\pm$ standard deviation (SD).

Results

Effects of Calcitriol

Treatment with calcitriol for 48 hours significantly up-regulated the expression of the inflammatory cytokines IL-6, MCP-1, and NOX4 (Figure 1A, 1B, 1C) compared to control group. This effect was significantly inhibited by the addition of the Angiotensin II type 2 receptor (AT2R) inhibitor (PD) for all three cytokines compared to Calcitriol group. The addition of the angiotensin converting enzyme (ACE) inhibitor (enalapril) significantly attenuated the effects of calcitriol on MCP-1 and NOX4 expression. The addition of the Angiotensin II type 1 receptor (AT1R) inhibitor (telmisartan) significantly blocked the expression of NOX4 compared to calcitriol group. Calcitriol suppressed the expression of the anti-inflammatory cytokine adiponectin compared to control. Adiponectin expression was recovered by the addition of AT2R inhibitor PD and the ACE inhibitor enalapril compared to calcitriol group.

Effect of RAS

ANGII significantly up-regulated the expression of IL-6 and MCP-1 (Figure 2A and 2B). This effect was reversed by the AT2R inhibitor PD. The addition of the AT1R inhibitor telmisartan also significantly suppressed the expression of IL-6 compared to ANGII group (Figure 2A). Adiponectin expression was significantly up-regulated by the addition of both the AT1R and AT2R inhibitors compared to ANGII treated cells, while the AT2R antagonist also significantly increased the expression of adiponectin compared to control group (Figure 2C).

Effect of AT1R Knockdown

Treatment with ANGII increased expression of AT1R (Figure 3A). AT1R siRNA treatment resulted in AT1R knockdown of 68% and resulted in significant inhibition of MCP-1 and NOX4 expression (Figure 3B and 3C). AT1R knockdown significantly decreased expression of AT1R, MCP-1, and NOX4 (Figure 4A, 4B, 4C), and attenuated calcitriol stimulation of MCP-1 and NOX4 expression (Figure 4B and 4C).

Discussion

Data from this study demonstrates that calcitriol mediated oxidative and inflammatory stress results, in part, from calcitriol modulation of the adipocyte RAS. Human adipose tissue contains all the functional components of an active RAS, and ANGII stimulates oxidative and inflammatory stress^{37, 213, 443, 453-459}. Our data confirm that ANGII up-regulates expression of IL-6 and MCP-1, while antagonism of either subtype 1 or subtype 2 ANGII receptor attenuated suppressed IL-6 expression. Antagonism of the AT2R with PD suppressed MCP-1 expression. These findings are congruent with other data demonstrating that ANGII directly affects adipocytokine expression, resulting in an up-regulation in the inflammatory cytokines MCP-1 and IL-6^{328, 400}. ANGII also stimulates production of ROS by up-regulating expression of NADPH oxidase cytosolic proteins required for the activation of NOX4⁴⁶⁰⁻⁴⁶². In addition, ANGII has been demonstrated to affect adipose tissue metabolism by up-regulating expression of key lipogenic enzymes in 3T3-L1 and human adipocytes while suppressing lipolysis^{206, 463}. An inverse relationship has been reported between the expression of adipose tissue derived ANGII converting enzymes and degree of insulin sensitivity and inhibition ameliorates RAS induced oxidative stress^{208, 431, 464-467}.

We also have demonstrated that calcitriol alone stimulates a similar pattern of up-regulation of the inflammatory cytokines IL-6, MCP-1, and NOX4 and suppresses expression of the anti-inflammatory cytokine adiponectin. These effects were partially reversed by the AT2R inhibitor, PD, while the addition of the AT1R inhibitor, telmisartan, had no effect. Adipose tissue oxidative stress suppresses adiponectin levels, and decreased adiponectin levels have been reported in obese and diabetic subjects^{306, 468-470}. A possible explanation for this hypoadiponectinemia may be a consequence of local oxidative stress inhibiting adiponectin gene transcription and rapidly degrading adiponectin mRNA, resulting in reduced adiponectin levels

⁴⁶⁸. Previous work from our laboratory has shown calcitriol to induce oxidative stress and increase lipid accumulation in adipocytes mediated by modulating calcium signaling as well as mitochondrial uncoupling ^{80, 86}.

Our incongruent results between AT1R and AT2R inhibitors on cytokine expression are puzzling, as AT1R has been reported to remain stable during adipogenesis while the AT2R was reported to be suppressed and eventually undetectable in mature adipocytes ⁴⁷¹. However, other results demonstrate expression of AT2R in mature adipocytes ^{206, 472-475}. Prostacyclin has been shown to stimulate maturation of adipocytes mediated by the AT2R, confirming the presence of this receptor in late differentiated adipocytes ^{475, 476}. This finding was affirmed by data showing that ANGII increased production of prostacyclin 4-6 fold higher in mature differentiated adipocytes compared to preadipocytes ⁴⁷⁵. The effect of prostacyclin was abolished by the addition of the AT2R antagonist, PD, while the AT1R antagonist, losartan had no effect ⁴⁷⁵. In addition, radioligand binding studies also demonstrated that the high affinity ANGII binding sites in mature adipocytes were of the AT2R subtype ²⁰⁶. Both ANGII and the AT2R antagonist, PD were reported to compete for radiolabeled ANGII binding while the AT1R antagonist (losartan) had no effect ²⁰⁶.

A key factor influencing the effects of ANGII is the intracellular location of the ANGII receptors ⁴⁷¹. Intracellular distribution studies using green fluorescent fusion protein for AT1R revealed nuclear localization of the AT1R directly after treatment of cells with ANGII ⁴⁷⁷. This internalization of the ANGII bound receptors down-regulates the number of ANGII receptors available for binding at the plasma membrane affecting the quantity of receptors at the plasma membrane ⁴⁷⁸. The type of ANGII receptor and quantity of receptor subtype present at the plasma membrane appears to be a critical factor in determining the effect on adipose tissue ^{206, 477, 479, 480}.

Increased ANGII levels have been positively correlated with increased adiposity and in expression of inflammatory cytokines^{206, 212, 213, 328, 474}. These findings in accordance with our ANGII results provide support for the role of ANGII in modulating cytokine expression and increasing inflammatory stress in adipose tissue. However, our findings with the ANGII pharmacological antagonists were inconclusive with regard to which ANGII receptor specificity. These incongruent results may be attributed to the non-specific actions of the chemical ANGII receptor antagonists, as these have been indicated to produce non-ANGII receptor related events in various cell types^{206, 312, 480-482}. There appears to be a small population of non-angiotensin receptor related binding sites that bind with high affinity for the chemical ANGII receptor antagonists^{483, 484}. The binding of the chemical antagonists to these non-angiotensin receptor binding sites may modify the true pharmacophore effects of the chemical receptor antagonists for the ANGII subtype receptors.

It is well recognized that the inflammatory effects of ANGII are mediated by the pleiotropic activation of the transcription factor, NFκB, stimulating expression of inflammatory gene products in various cell types⁴⁸⁵⁻⁴⁸⁸. In human preadipocytes ANGII has been demonstrated to degrade IκB an inhibitor of NFκB, phosphorylating the p65 subunit of NF-κB with translocation to the nucleus mediated by AT1R⁴⁰⁰. These effects of ANGII were reversed by the addition of a NFκB inhibitor (BAY 117082)⁴⁰⁰. In addition, an antagonist for AT1R abolished NFκB activity, demonstrating that ANGII is a stimulator of NFκB signaling in adipocytes^{268, 485}. These findings indicate that in adipocytes, the inflammatory effects of ANGII are mediated, in part, by stimulation of NFκB signaling pathway^{217, 400}. In newly differentiated adipocytes, the majority of ANGII effects are mediated by the AT1R; however the AT2R is also expressed in adipocytes^{400, 479}. The exact functional role of AT2R is unclear, as some reports indicate that AT2R antagonizes the effects of AT1R^{485, 489, 490}. However, other reports suggest that AT2R

participates in inflammatory events directly mediated by activating NFκB signaling pathway³⁹⁹,
491-495

It has been proposed that chronic activation of RAS may result in altering the function of AT2R in favor of mediating inflammation and oxidative stress^{496, 497}. These findings provide framework for potential overlap in common signaling pathways shared by the two receptor subtypes resulting in modulation in the effects of ANGII⁴⁰⁰. ANGII induces oxidative stress by activating NADPH oxidase resulting in stimulation of the redox sensitive NFκB pathway⁴⁹. The ANGII induced activity of NADPH oxidase has been demonstrated to be down-regulated by the addition of both chemical antagonists for AT1R and AT2R and with the antioxidant tempol⁴⁹⁸.

Since chemical antagonism of the ANGII receptor resulted in inconclusive results on cytokine expression we used small interfering RNA to specifically knock down expression of the AT1R and then re-examine adipocytokine expression mediated by ANGII and calcitriol. The AT1R knockdown markedly inhibited expression of MCP-1 and NOX4 stimulated by treatment with ANGII. This finding is consistent with our earlier observation that Telmisartan inhibited the effects of NOX4 and MCP-1, and demonstrate that the AT1R is responsible for mediating some of the inflammatory effects of calcitriol.

Conclusions

Based on these findings RAS via AT1R has been demonstrated to mediate the expression of some of the inflammatory cytokines induced by calcitriol. Although chemical antagonism of the AT1R led to inconclusive results, the siRNA knockdown of AT1R did provide evidence for supporting the role of AT1R in mediating some of the inflammatory effects of calcitriol.

Accordingly, strategies designed to down-regulate adipose tissue levels of calcitriol by consuming a diet high in calcium could play a role in attenuating adipose tissue inflammation associated with obesity.

Future Research

In the present study, ANGII via AT1R was demonstrated to mediate the mRNA expression of some inflammatory cytokines induced by calcitriol. Future research is needed to investigate if the protein levels of these inflammatory cytokines are also up-regulated. If so, this would provide a complete framework demonstrating that AT1R mediates an inflammatory response induced by calcitriol stimulation at each level of gene transcription. In addition, the co- treatment of adipocytes with ARBs and also with siRNA of AT1R needs to examine simultaneous dual antagonism of the AT1R by pharmacological antagonism and direct knockdown of AT1R and the effects on inflammatory cytokine expression.

List of References

1. Ahima RS. Adipose tissue as an endocrine organ. *Obesity* (Silver Spring) 2006;14 Suppl 5:242S-49S.
2. Witkos M, Uttaburanont M, Lang CD, Arora R. Costs of and reasons for obesity. *J Cardiometab Syndr* 2008;3(3):173-6.
3. Hossain P, Kavar B, El Nahas M. Obesity and diabetes in the developing world--a growing challenge. *N Engl J Med* 2007;356(3):213-5.
4. Qi L, Cho YA. Gene-environment interaction and obesity. *Nutr Rev* 2008;66(12):684-94.
5. Caballero B. The global epidemic of obesity: an overview. *Epidemiol Rev* 2007;29:1-5.
6. Rissanen AM, Heliovaara M, Knekt P, Reunanen A, Aromaa A. Determinants of weight gain and overweight in adult Finns. *Eur J Clin Nutr* 1991;45(9):419-30.
7. Burdette HL, Whitaker RC. A national study of neighborhood safety, outdoor play, television viewing, and obesity in preschool children. *Pediatrics* 2005;116(3):657-62.
8. Schoeller DA. Balancing energy expenditure and body weight. *Am J Clin Nutr* 1998;68(4):956S-61S.
9. Williamson DF, Madans J, Pamuk E, Flegal KM, Kendrick JS, Serdula MK. A prospective study of childbearing and 10-year weight gain in US white women 25 to 45 years of age. *Int J Obes Relat Metab Disord* 1994;18(8):561-9.
10. Arora S, Anubhuti. Role of neuropeptides in appetite regulation and obesity--a review. *Neuropeptides* 2006;40(6):375-401.
11. Engeli S, Schling P, Gorzelniak K, Boschmann M, Janke J, Ailhaud G, et al. The adipose-tissue renin-angiotensin-aldosterone system: role in the metabolic syndrome? *Int J Biochem Cell Biol* 2003;35(6):807-25.
12. Prins JB. Adipose tissue as an endocrine organ. *Best Pract Res Clin Endocrinol Metab* 2002;16(4):639-51.

13. Massiera F, Bloch-Faure M, Ceiler D, Murakami K, Fukamizu A, Gasc JM, et al. Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *Faseb J* 2001;15(14):2727-9.
14. Kopecky J, Hodny Z, Rossmeisl M, Syrový I, Kozak LP. Reduction of dietary obesity in aP2-Ucp transgenic mice: physiology and adipose tissue distribution. *Am J Physiol* 1996;270(5 Pt 1):E768-75.
15. Wozniak SE, Gee LL, Wachtel MS, Frezza EE. Adipose Tissue: The New Endocrine Organ? A Review Article. *Dig Dis Sci* 2008.
16. Goossens GH. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. *Physiol Behav* 2008;94(2):206-18.
17. Hajer GR, van Haeften TW, Visseren FL. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. *Eur Heart J* 2008.
18. Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, et al. Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* 1999;48(5):1113-9.
19. Frayn KN. Adipose tissue and the insulin resistance syndrome. *Proc Nutr Soc* 2001;60(3):375-80.
20. Trayhurn P, Wang B, Wood IS. Hypoxia and the endocrine and signalling role of white adipose tissue. *Arch Physiol Biochem* 2008;114(4):267-76.
21. Lundgren M, Svensson M, Lindmark S, Renstrom F, Ruge T, Eriksson JW. Fat cell enlargement is an independent marker of insulin resistance and 'hyperleptinaemia'. *Diabetologia* 2007;50(3):625-33.
22. Ducharme NA, Bickel PE. Lipid droplets in lipogenesis and lipolysis. *Endocrinology* 2008;149(3):942-9.
23. Nozaki M, Fukuhara A, Segawa K, Okuno Y, Abe M, Hosogai N, et al. Nitric oxide dysregulates adipocytokine expression in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 2007;364(1):33-9.
24. Chehab FF. Obesity and lipodystrophy--where do the circles intersect? *Endocrinology* 2008;149(3):925-34.

25. Bullo M, Casas-Agustench P, Amigo-Correig P, Aranceta J, Salas-Salvado J. Inflammation, obesity and comorbidities: the role of diet. *Public Health Nutr* 2007;10(10A):1164-72.
26. Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell* 2008;134(6):933-44.
27. Despres JP, Arsenault BJ, Cote M, Cartier A, Lemieux I. Abdominal obesity: the cholesterol of the 21st century? *Can J Cardiol* 2008;24 Suppl D:7D-12D.
28. Montague CT, O'Rahilly S. The perils of portliness: causes and consequences of visceral adiposity. *Diabetes* 2000;49(6):883-8.
29. Sarrafzadegan N, Kelishadi R, Baghaei A, Hussein Sadri G, Malekafzali H, Mohammadifard N, et al. Metabolic syndrome: an emerging public health problem in Iranian Women: Isfahan Healthy Heart Program. *Int J Cardiol* 2008;131(1):90-6.
30. Vague J. The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. *Am J Clin Nutr* 1956;4(1):20-34.
31. Krotkiewski M, Bjorntorp P, Sjostrom L, Smith U. Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. *J Clin Invest* 1983;72(3):1150-62.
32. Ohlson LO, Larsson B, Svardsudd K, Welin L, Eriksson H, Wilhelmsen L, et al. The influence of body fat distribution on the incidence of diabetes mellitus. 13.5 years of follow-up of the participants in the study of men born in 1913. *Diabetes* 1985;34(10):1055-8.
33. Lapidus L, Bengtsson C, Larsson B, Pennert K, Rybo E, Sjostrom L. Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. *Br Med J (Clin Res Ed)* 1984;289(6454):1257-61.
34. Kissebah AH, Vydelingum N, Murray R, Evans DJ, Hartz AJ, Kalkhoff RK, et al. Relation of body fat distribution to metabolic complications of obesity. *J Clin Endocrinol Metab* 1982;54(2):254-60.

35. Gillum RF. The association of body fat distribution with hypertension, hypertensive heart disease, coronary heart disease, diabetes and cardiovascular risk factors in men and women aged 18-79 years. *J Chronic Dis* 1987;40(5):421-8.
36. Kannel WB, Cupples LA, Ramaswami R, Stokes J, 3rd, Kreger BE, Higgins M. Regional obesity and risk of cardiovascular disease; the Framingham Study. *J Clin Epidemiol* 1991;44(2):183-90.
37. Goossens GH, Blaak EE, van Baak MA. Possible involvement of the adipose tissue renin-angiotensin system in the pathophysiology of obesity and obesity-related disorders. *Obes Rev* 2003;4(1):43-55.
38. Chudek J, Wiecek A. Adipose tissue, inflammation and endothelial dysfunction. *Pharmacol Rep* 2006;58 Suppl:81-8.
39. van Herpen NA, Schrauwen-Hinderling VB. Lipid accumulation in non-adipose tissue and lipotoxicity. *Physiol Behav* 2008;94(2):231-41.
40. Kopecky J, Flachs P, Bardova K, Brauner P, Prazak T, Sponarova J. Modulation of lipid metabolism by energy status of adipocytes: implications for insulin sensitivity. *Ann N Y Acad Sci* 2002;967:88-101.
41. Kopecky J, Rossmeisl M, Flachs P, Brauner P, Sponarova J, Matejkova O, et al. Energy metabolism of adipose tissue--physiological aspects and target in obesity treatment. *Physiol Res* 2004;53 Suppl 1:S225-32.
42. Ganda OP. Lipoatrophy, lipodystrophy, and insulin resistance. *Ann Intern Med* 2000;133(4):304-6.
43. Gavrilova O, Marcus-Samuels B, Graham D, Kim JK, Shulman GI, Castle AL, et al. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J Clin Invest* 2000;105(3):271-8.
44. Campbell PJ, Carlson MG, Nurjhan N. Fat metabolism in human obesity. *Am J Physiol* 1994;266(4 Pt 1):E600-5.
45. Lupi R, Dotta F, Marselli L, Del Guerra S, Masini M, Santangelo C, et al. Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. *Diabetes* 2002;51(5):1437-42.

46. Frayn KN, Williams CM, Arner P. Are increased plasma non-esterified fatty acid concentrations a risk marker for coronary heart disease and other chronic diseases? *Clin Sci (Lond)* 1996;90(4):243-53.
47. Chavey C, Mari B, Monthouel MN, Bonnafous S, Anglard P, Van Obberghen E, et al. Matrix metalloproteinases are differentially expressed in adipose tissue during obesity and modulate adipocyte differentiation. *J Biol Chem* 2003;278(14):11888-96.
48. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006;444(7121):860-7.
49. Takahashi M, Suzuki E, Takeda R, Oba S, Nishimatsu H, Kimura K, et al. Angiotensin II and tumor necrosis factor- α synergistically promote monocyte chemoattractant protein-1 expression: roles of NF- κ B, p38, and reactive oxygen species. *Am J Physiol Heart Circ Physiol* 2008;294(6):H2879-88.
50. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005;115(5):1111-9.
51. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- α : direct role in obesity-linked insulin resistance. *Science* 1993;259(5091):87-91.
52. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. *Gastroenterology* 2007;132(6):2169-80.
53. Suganami T, Nishida J, Ogawa Y. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor α . *Arterioscler Thromb Vasc Biol* 2005;25(10):2062-8.
54. Fain JN. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. *Vitam Horm* 2006;74:443-77.
55. Fantuzzi G. Adiponectin and inflammation: consensus and controversy. *J Allergy Clin Immunol* 2008;121(2):326-30.
56. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003;112(12):1796-808.

57. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr* 2008;88(2):491S-99S.
58. DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 2004;80(6 Suppl):1689S-96S.
59. Lips P. Relative value of 25(OH)D and 1,25(OH)₂D measurements. *J Bone Miner Res* 2007;22(11):1668-71.
60. Sun X, Zemel MB. Role of uncoupling protein 2 (UCP2) expression and 1 α , 25-dihydroxyvitamin D₃ in modulating adipocyte apoptosis. *Faseb J* 2004;18(12):1430-2.
61. Fukugawa M, Kurokawa K. Calcium homeostasis and imbalance. *Nephron* 2002;92 Suppl 1:41-5.
62. Black BL, Jarett L, McDonald JM. The regulation of endoplasmic reticulum calcium uptake of adipocytes by cytoplasmic calcium. *J Biol Chem* 1981;256(1):322-9.
63. Shi H, Halvorsen YD, Ellis PN, Wilkison WO, Zemel MB. Role of intracellular calcium in human adipocyte differentiation. *Physiol Genomics* 2000;3(2):75-82.
64. Sutton AL, MacDonald PN. Vitamin D: more than a "bone-a-fide" hormone. *Mol Endocrinol* 2003;17(5):777-91.
65. Norman AW. Minireview: vitamin D receptor: new assignments for an already busy receptor. *Endocrinology* 2006;147(12):5542-8.
66. Holick MF. The vitamin D deficiency pandemic and consequences for nonskeletal health: Mechanisms of action. *Mol Aspects Med* 2008.
67. Norman AW, Song X, Zanello L, Bula C, Okamura WH. Rapid and genomic biological responses are mediated by different shapes of the agonist steroid hormone, 1 α ,25(OH)₂vitamin D₃. *Steroids* 1999;64(1-2):120-8.
68. Nemere I, Yoshimoto Y, Norman AW. Calcium transport in perfused duodena from normal chicks: enhancement within fourteen minutes of exposure to 1,25-dihydroxyvitamin D₃. *Endocrinology* 1984;115(4):1476-83.

69. Le Mellay V, Grosse B, Lieberherr M. Phospholipase C beta and membrane action of calcitriol and estradiol. *J Biol Chem* 1997;272(18):11902-7.
70. Vazquez G, de Boland AR, Boland RL. 1alpha,25-dihydroxy-vitamin-D3-induced store-operated Ca²⁺ influx in skeletal muscle cells. Modulation by phospholipase c, protein kinase c, and tyrosine kinases. *J Biol Chem* 1998;273(51):33954-60.
71. Morelli S, Buitrago C, Vazquez G, De Boland AR, Boland R. Involvement of tyrosine kinase activity in 1alpha,25(OH)₂-vitamin D₃ signal transduction in skeletal muscle cells. *J Biol Chem* 2000;275(46):36021-8.
72. Nemere I, Ray R, McManus W. Immunochemical studies on the putative plasmalemmal receptor for 1, 25(OH)₂D₃. I. Chick intestine. *Am J Physiol Endocrinol Metab* 2000;278(6):E1104-14.
73. Rohe B, Safford SE, Nemere I, Farach-Carson MC. Identification and characterization of 1,25D₃-membrane-associated rapid response, steroid (1,25D₃-MARRS)-binding protein in rat IEC-6 cells. *Steroids* 2005;70(5-7):458-63.
74. Fleet JC. Rapid, membrane-initiated actions of 1,25 dihydroxyvitamin D: what are they and what do they mean? *J Nutr* 2004;134(12):3215-8.
75. Zanello LP, Norman AW. Rapid modulation of osteoblast ion channel responses by 1alpha,25(OH)₂-vitamin D₃ requires the presence of a functional vitamin D nuclear receptor. *Proc Natl Acad Sci U S A* 2004;101(6):1589-94.
76. Krishnan AV, Moreno J, Nonn L, Swami S, Peehl DM, Feldman D. Calcitriol as a chemopreventive and therapeutic agent in prostate cancer: role of anti-inflammatory activity. *J Bone Miner Res* 2007;22 Suppl 2:V74-80.
77. DeLuca HF, Zierold C. Mechanisms and functions of vitamin D. *Nutr Rev* 1998;56(2 Pt 2):S4-10; discussion S 54-75.
78. Myrthue A, Rademacher BL, Pittsenbarger J, Kutyba-Brooks B, Gantner M, Qian DZ, et al. The iroquois homeobox gene 5 is regulated by 1,25-dihydroxyvitamin D₃ in human prostate cancer and regulates apoptosis and the cell cycle in LNCaP prostate cancer cells. *Clin Cancer Res* 2008;14(11):3562-70.
79. Ponsonby AL, Pezic A, Ellis J, Morley R, Cameron F, Carlin J, et al. Variation in associations between allelic variants of the vitamin D receptor gene and onset of type 1

diabetes mellitus by ambient winter ultraviolet radiation levels: a meta-regression analysis. *Am J Epidemiol* 2008;168(4):358-65.

80. Shi H, Norman AW, Okamura WH, Sen A, Zemel MB. 1 α ,25-dihydroxyvitamin D₃ inhibits uncoupling protein 2 expression in human adipocytes. *Faseb J* 2002;16(13):1808-10.
81. Sun X, Zemel MB. Calcitriol and calcium regulate cytokine production and adipocyte-macrophage cross-talk. *J Nutr Biochem* 2008;19(6):392-9.
82. Sun X, Zemel MB. Calcium and 1,25-dihydroxyvitamin D₃ regulation of adipokine expression. *Obesity (Silver Spring)* 2007;15(2):340-8.
83. Sun X, Zemel MB. 1 α ,25-dihydroxyvitamin D₃ modulation of adipocyte reactive oxygen species production. *Obesity (Silver Spring)* 2007;15(8):1944-53.
84. Sun X, Zemel MB. Dietary calcium regulates ROS production in aP2-agouti transgenic mice on high-fat/high-sucrose diets. *Int J Obes (Lond)* 2006;30(9):1341-6.
85. Sun X, Zemel MB. Effects of mitochondrial uncoupling on adipocyte intracellular Ca(2+) and lipid metabolism. *J Nutr Biochem* 2003;14(4):219-26.
86. Shi H, Norman AW, Okamura WH, Sen A, Zemel MB. 1 α ,25-Dihydroxyvitamin D₃ modulates human adipocyte metabolism via nongenomic action. *Faseb J* 2001;15(14):2751-3.
87. Shi H, Dirienzo D, Zemel MB. Effects of dietary calcium on adipocyte lipid metabolism and body weight regulation in energy-restricted aP2-agouti transgenic mice. *Faseb J* 2001;15(2):291-3.
88. Sun X, Zemel MB. 1 α , 25-dihydroxyvitamin D and corticosteroid regulate adipocyte nuclear vitamin D receptor. *Int J Obes (Lond)* 2008;32(8):1305-11.
89. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC. Regulation of adiposity by dietary calcium. *Faseb J* 2000;14(9):1132-8.
90. Zemel MB. Calcium modulation of hypertension and obesity: mechanisms and implications. *J Am Coll Nutr* 2001;20(5 Suppl):428S-35S; discussion 40S-42S.

91. Foss YJ. Vitamin D deficiency is the cause of common obesity. *Med Hypotheses* 2009;72(3):314-21.
92. Bell NH, Epstein S, Greene A, Shary J, Oexmann MJ, Shaw S. Evidence for alteration of the vitamin D-endocrine system in obese subjects. *J Clin Invest* 1985;76(1):370-3.
93. Stein MS, Flicker L, Scherer SC, Paton LM, O'Brien ML, Walton SC, et al. Relationships with serum parathyroid hormone in old institutionalized subjects. *Clin Endocrinol (Oxf)* 2001;54(5):583-92.
94. Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J, et al. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J Clin Endocrinol Metab* 2004;89(3):1196-9.
95. Kamycheva E, Sundsfjord J, Jorde R. Serum parathyroid hormone level is associated with body mass index. The 5th Tromso study. *Eur J Endocrinol* 2004;151(2):167-72.
96. Reinehr T, de Sousa G, Alexy U, Kersting M, Andler W. Vitamin D status and parathyroid hormone in obese children before and after weight loss. *Eur J Endocrinol* 2007;157(2):225-32.
97. Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, et al. Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *J Clin Endocrinol Metab* 2005;90(7):4119-23.
98. Rajakumar K, Fernstrom JD, Holick MF, Janosky JE, Greenspan SL. Vitamin D status and response to Vitamin D(3) in obese vs. non-obese African American children. *Obesity (Silver Spring)* 2008;16(1):90-5.
99. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000;72(3):690-3.
100. Hey H, Stokholm KH, Lund B, Sorensen OH. Vitamin D deficiency in obese patients and changes in circulating vitamin D metabolites following jejunoileal bypass. *Int J Obes* 1982;6(5):473-9.
101. Zamboni G, Soffiati M, Giavarina D, Tato L. Mineral metabolism in obese children. *Acta Paediatr Scand* 1988;77(5):741-6.

102. Compston JE, Vedi S, Ledger JE, Webb A, Gazet JC, Pilkington TR. Vitamin D status and bone histomorphometry in gross obesity. *Am J Clin Nutr* 1981;34(11):2359-63.
103. Zemel MB. Regulation of adiposity and obesity risk by dietary calcium: mechanisms and implications. *J Am Coll Nutr* 2002;21(2):146S-51S.
104. Xue B, Moustaid N, Wilkison WO, Zemel MB. The agouti gene product inhibits lipolysis in human adipocytes via a Ca^{2+} -dependent mechanism. *Faseb J* 1998;12(13):1391-6.
105. Xue B, Greenberg AG, Kraemer FB, Zemel MB. Mechanism of intracellular calcium ($[\text{Ca}^{2+}]_i$) inhibition of lipolysis in human adipocytes. *Faseb J* 2001;15(13):2527-9.
106. Jones BH, Kim JH, Zemel MB, Woychik RP, Michaud EJ, Wilkison WO, et al. Upregulation of adipocyte metabolism by agouti protein: possible paracrine actions in yellow mouse obesity. *Am J Physiol* 1996;270(1 Pt 1):E192-6.
107. Xue B, Zemel MB. Relationship between human adipose tissue agouti and fatty acid synthase (FAS). *J Nutr* 2000;130(10):2478-81.
108. Ni Z, Smogorzewski M, Massry SG. Effects of parathyroid hormone on cytosolic calcium of rat adipocytes. *Endocrinology* 1994;135(5):1837-44.
109. Wu Z, Rosen ED, Brun R, Hauser S, Adelmant G, Troy AE, et al. Cross-regulation of C/EBP alpha and PPAR gamma controls the transcriptional pathway of adipogenesis and insulin sensitivity. *Mol Cell* 1999;3(2):151-8.
110. Pessin JE, Saltiel AR. Signaling pathways in insulin action: molecular targets of insulin resistance. *J Clin Invest* 2000;106(2):165-9.
111. Holman GD, Kasuga M. From receptor to transporter: insulin signalling to glucose transport. *Diabetologia* 1997;40(9):991-1003.
112. Begum N, Leitner W, Reusch JE, Sussman KE, Draznin B. GLUT-4 phosphorylation and its intrinsic activity. Mechanism of Ca^{2+} -induced inhibition of insulin-stimulated glucose transport. *J Biol Chem* 1993;268(5):3352-6.
113. Begum N, Sussman KE, Draznin B. Calcium-induced inhibition of phosphoserine phosphatase in insulin target cells is mediated by the phosphorylation and activation of inhibitor 1. *J Biol Chem* 1992;267(9):5959-63.

114. Draznin B. Cytosolic calcium and insulin resistance. *Am J Kidney Dis* 1993;21(6 Suppl 3):32-8.
115. Zemel MB. Mechanisms of dairy modulation of adiposity. *J Nutr* 2003;133(1):252S-56S.
116. Zemel MB. Role of dietary calcium and dairy products in modulating adiposity. *Lipids* 2003;38(2):139-46.
117. Zemel MB, Sun X. Dietary calcium and dairy products modulate oxidative and inflammatory stress in mice and humans. *J Nutr* 2008;138(6):1047-52.
118. Rosen ED, Spiegelman BM. Molecular regulation of adipogenesis. *Annu Rev Cell Dev Biol* 2000;16:145-71.
119. Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. *J Cell Physiol* 1999;181(1):67-73.
120. Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. *Genes Dev* 2000;14(11):1293-307.
121. Wagatsuma A. Adipogenic potential can be activated during muscle regeneration. *Mol Cell Biochem* 2007;304(1-2):25-33.
122. Student AK, Hsu RY, Lane MD. Induction of fatty acid synthetase synthesis in differentiating 3T3-L1 preadipocytes. *J Biol Chem* 1980;255(10):4745-50.
123. Brandes R, Arad R, Bar-Tana J. Inducers of adipose conversion activate transcription promoted by a peroxisome proliferators response element in 3T3-L1 cells. *Biochem Pharmacol* 1995;50(11):1949-51.
124. Otto TC, Lane MD. Adipose development: from stem cell to adipocyte. *Crit Rev Biochem Mol Biol* 2005;40(4):229-42.
125. Lane MD, Tang QQ, Jiang MS. Role of the CCAAT enhancer binding proteins (C/EBPs) in adipocyte differentiation. *Biochem Biophys Res Commun* 1999;266(3):677-83.
126. Zhang JW, Klemm DJ, Vinson C, Lane MD. Role of CREB in transcriptional regulation of CCAAT/enhancer-binding protein beta gene during adipogenesis. *J Biol Chem* 2004;279(6):4471-8.

127. Tang QQ, Gronborg M, Huang H, Kim JW, Otto TC, Pandey A, et al. Sequential phosphorylation of CCAAT enhancer-binding protein beta by MAPK and glycogen synthase kinase 3beta is required for adipogenesis. *Proc Natl Acad Sci U S A* 2005;102(28):9766-71.
128. Blumberg JM, Tzamelis I, Astapova I, Lam FS, Flier JS, Hollenberg AN. Complex role of the vitamin D receptor and its ligand in adipogenesis in 3T3-L1 cells. *J Biol Chem* 2006;281(16):11205-13.
129. Kong J, Li YC. Molecular mechanism of 1,25-dihydroxyvitamin D3 inhibition of adipogenesis in 3T3-L1 cells. *Am J Physiol Endocrinol Metab* 2006;290(5):E916-24.
130. Dace A, Martin-el Yazidi C, Bonne J, Planells R, Torresani J. Calcitriol is a positive effector of adipose differentiation in the OB 17 cell line: relationship with the adipogenic action of triiodothyronine. *Biochem Biophys Res Commun* 1997;232(3):771-6.
131. Narvaez CJ, Matthews D, Broun E, Chan M, Welsh J. Lean phenotype and resistance to diet-induced obesity in VDR knockout mice correlates with induction of uncoupling protein-1 in white adipose tissue. *Endocrinology* 2008.
132. Kawada T, Kamei Y, Sugimoto E. The possibility of active form of vitamins A and D as suppressors on adipocyte development via ligand-dependent transcriptional regulators. *Int J Obes Relat Metab Disord* 1996;20 Suppl 3:S52-7.
133. Sato M, Hiragun A. Demonstration of 1 alpha,25-dihydroxyvitamin D3 receptor-like molecule in ST 13 and 3T3 L1 preadipocytes and its inhibitory effects on preadipocyte differentiation. *J Cell Physiol* 1988;135(3):545-50.
134. Hida Y, Kawada T, Kayahashi S, Ishihara T, Fushiki T. Counteraction of retinoic acid and 1,25-dihydroxyvitamin D3 on up-regulation of adipocyte differentiation with PPARgamma ligand, an antidiabetic thiazolidinedione, in 3T3-L1 cells. *Life Sci* 1998;62(14):PL205-11.
135. Atmani H, Chappard D, Basle MF. Proliferation and differentiation of osteoblasts and adipocytes in rat bone marrow stromal cell cultures: effects of dexamethasone and calcitriol. *J Cell Biochem* 2003;89(2):364-72.
136. Vu D, Ong JM, Clemens TL, Kern PA. 1,25-Dihydroxyvitamin D induces lipoprotein lipase expression in 3T3-L1 cells in association with adipocyte differentiation. *Endocrinology* 1996;137(5):1540-4.

137. Bellows CG, Heersche JN. The frequency of common progenitors for adipocytes and osteoblasts and of committed and restricted adipocyte and osteoblast progenitors in fetal rat calvaria cell populations. *J Bone Miner Res* 2001;16(11):1983-93.
138. Bellows CG, Wang YH, Heersche JN, Aubin JE. 1,25-dihydroxyvitamin D3 stimulates adipocyte differentiation in cultures of fetal rat calvaria cells: comparison with the effects of dexamethasone. *Endocrinology* 1994;134(5):2221-9.
139. Zinser GM, Welsh J. Vitamin D receptor status alters mammary gland morphology and tumorigenesis in MMTV-neu mice. *Carcinogenesis* 2004;25(12):2361-72.
140. Costford S, Gowing A, Harper ME. Mitochondrial uncoupling as a target in the treatment of obesity. *Curr Opin Clin Nutr Metab Care* 2007;10(6):671-8.
141. Rossmeisl M, Barbatelli G, Flachs P, Brauner P, Zingaretti MC, Marelli M, et al. Expression of the uncoupling protein 1 from the aP2 gene promoter stimulates mitochondrial biogenesis in unilocular adipocytes in vivo. *Eur J Biochem* 2002;269(1):19-28.
142. Ricquier D. Respiration uncoupling and metabolism in the control of energy expenditure. *Proc Nutr Soc* 2005;64(1):47-52.
143. Rossmeisl M, Flachs P, Brauner P, Sponarova J, Matejkova O, Prazak T, et al. Role of energy charge and AMP-activated protein kinase in adipocytes in the control of body fat stores. *Int J Obes Relat Metab Disord* 2004;28 Suppl 4:S38-44.
144. Harper ME, Green K, Brand MD. The efficiency of cellular energy transduction and its implications for obesity. *Annu Rev Nutr* 2008;28:13-33.
145. Baumruk F, Flachs P, Horakova M, Floryk D, Kopecky J. Transgenic UCP1 in white adipocytes modulates mitochondrial membrane potential. *FEBS Lett* 1999;444(2-3):206-10.
146. Affourtit C, Crichton PG, Parker N, Brand MD. Novel uncoupling proteins. *Novartis Found Symp* 2007;287:70-80; discussion 80-91.
147. Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, et al. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat Genet* 1997;15(3):269-72.

148. Boss O, Samec S, Paoloni-Giacobino A, Rossier C, Dulloo A, Seydoux J, et al. Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett* 1997;408(1):39-42.
149. Brand MD, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. *Cell Metab* 2005;2(2):85-93.
150. Sluse FE, Jarmuszkiewicz W, Navet R, Douette P, Mathy G, Sluse-Goffart CM. Mitochondrial UCPs: new insights into regulation and impact. *Biochim Biophys Acta* 2006;1757(5-6):480-5.
151. Rossmeisl M, Kovar J, Syrový I, Flachs P, Bobkova D, Kolar F, et al. Triglyceride-lowering effect of respiratory uncoupling in white adipose tissue. *Obes Res* 2005;13(5):835-44.
152. Orci L, Cook WS, Ravazzola M, Wang MY, Park BH, Montesano R, et al. Rapid transformation of white adipocytes into fat-oxidizing machines. *Proc Natl Acad Sci U S A* 2004;101(7):2058-63.
153. Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, et al. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 1997;387(6628):90-4.
154. Himms-Hagen J, Harper ME. Physiological role of UCP3 may be export of fatty acids from mitochondria when fatty acid oxidation predominates: an hypothesis. *Exp Biol Med* (Maywood) 2001;226(2):78-84.
155. Beck V, Jaburek M, Demina T, Rupprecht A, Porter RK, Jezek P, et al. Polyunsaturated fatty acids activate human uncoupling proteins 1 and 2 in planar lipid bilayers. *Faseb J* 2007;21(4):1137-44.
156. Alberici LC, Oliveira HC, Bighetti EJ, de Faria EC, Degaspari GR, Souza CT, et al. Hypertriglyceridemia increases mitochondrial resting respiration and susceptibility to permeability transition. *J Bioenerg Biomembr* 2003;35(5):451-7.
157. Zemel MB. The role of dairy foods in weight management. *J Am Coll Nutr* 2005;24(6 Suppl):537S-46S.
158. Zemel MB, Miller SL. Dietary calcium and dairy modulation of adiposity and obesity risk. *Nutr Rev* 2004;62(4):125-31.

159. Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, et al. A transgenic model of visceral obesity and the metabolic syndrome. *Science* 2001;294(5549):2166-70.
160. Mayo-Smith W, Hayes CW, Biller BM, Klibanski A, Rosenthal H, Rosenthal DI. Body fat distribution measured with CT: correlations in healthy subjects, patients with anorexia nervosa, and patients with Cushing syndrome. *Radiology* 1989;170(2):515-8.
161. Hauner H, Schmid P, Pfeiffer EF. Glucocorticoids and insulin promote the differentiation of human adipocyte precursor cells into fat cells. *J Clin Endocrinol Metab* 1987;64(4):832-5.
162. Eckel RH. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med* 1989;320(16):1060-8.
163. Ottosson M, Lonnroth P, Bjorntorp P, Eden S. Effects of cortisol and growth hormone on lipolysis in human adipose tissue. *J Clin Endocrinol Metab* 2000;85(2):799-803.
164. Bronnegard M, Arner P, Hellstrom L, Akner G, Gustafsson JA. Glucocorticoid receptor messenger ribonucleic acid in different regions of human adipose tissue. *Endocrinology* 1990;127(4):1689-96.
165. Tchkonina T, Lenburg M, Thomou T, Giorgadze N, Frampton G, Pirtskhalava T, et al. Identification of depot-specific human fat cell progenitors through distinct expression profiles and developmental gene patterns. *Am J Physiol Endocrinol Metab* 2007;292(1):E298-307.
166. Engfeldt P, Arner P. Lipolysis in human adipocytes, effects of cell size, age and of regional differences. *Horm Metab Res Suppl* 1988;19:26-9.
167. Leclercq IA, Da Silva Morais A, Schroyen B, Van Hul N, Geerts A. Insulin resistance in hepatocytes and sinusoidal liver cells: mechanisms and consequences. *J Hepatol* 2007;47(1):142-56.
168. McCarty MF. Modulation of adipocyte lipoprotein lipase expression as a strategy for preventing or treating visceral obesity. *Med Hypotheses* 2001;57(2):192-200.
169. Rebuffe-Scrive M, Bronnegard M, Nilsson A, Eldh J, Gustafsson JA, Bjorntorp P. Steroid hormone receptors in human adipose tissues. *J Clin Endocrinol Metab* 1990;71(5):1215-9.

170. Bujalska IJ, Kumar S, Stewart PM. Does central obesity reflect "Cushing's disease of the omentum"? *Lancet* 1997;349(9060):1210-3.
171. Tomlinson JW, Stewart PM. The functional consequences of 11 β -hydroxysteroid dehydrogenase expression in adipose tissue. *Horm Metab Res* 2002;34(11-12):746-51.
172. Tomlinson JW, Moore J, Cooper MS, Bujalska I, Shahmanesh M, Burt C, et al. Regulation of expression of 11 β -hydroxysteroid dehydrogenase type 1 in adipose tissue: tissue-specific induction by cytokines. *Endocrinology* 2001;142(5):1982-9.
173. Moore JS, Monson JP, Kaltsas G, Putignano P, Wood PJ, Sheppard MC, et al. Modulation of 11 β -hydroxysteroid dehydrogenase isozymes by growth hormone and insulin-like growth factor: in vivo and in vitro studies. *J Clin Endocrinol Metab* 1999;84(11):4172-7.
174. Miller KK, Daly PA, Sentocnik D, Doweiko J, Samore M, Basgoz NO, et al. Pseudo-Cushing's syndrome in human immunodeficiency virus-infected patients. *Clin Infect Dis* 1998;27(1):68-72.
175. Mori Y, Murakawa Y, Okada K, Horikoshi H, Yokoyama J, Tajima N, et al. Effect of troglitazone on body fat distribution in type 2 diabetic patients. *Diabetes Care* 1999;22(6):908-12.
176. Kawai T, Takei I, Oguma Y, Ohashi N, Tokui M, Oguchi S, et al. Effects of troglitazone on fat distribution in the treatment of male type 2 diabetes. *Metabolism* 1999;48(9):1102-7.
177. Akazawa S, Sun F, Ito M, Kawasaki E, Eguchi K. Efficacy of troglitazone on body fat distribution in type 2 diabetes. *Diabetes Care* 2000;23(8):1067-71.
178. Samaras K, Wand H, Law M, Emery S, Cooper D, Carr A. Prevalence of metabolic syndrome in HIV-infected patients receiving highly active antiretroviral therapy using International Diabetes Foundation and Adult Treatment Panel III criteria: associations with insulin resistance, disturbed body fat compartmentalization, elevated C-reactive protein, and [corrected] hypoadiponectinemia. *Diabetes Care* 2007;30(1):113-9.
179. Reynolds RM, Chapman KE, Seckl JR, Walker BR, McKeigue PM, Lithell HO. Skeletal muscle glucocorticoid receptor density and insulin resistance. *JAMA* 2002;287(19):2505-6.

180. Rask E, Olsson T, Soderberg S, Andrew R, Livingstone DE, Johnson O, et al. Tissue-specific dysregulation of cortisol metabolism in human obesity. *J Clin Endocrinol Metab* 2001;86(3):1418-21.
181. Rask E, Walker BR, Soderberg S, Livingstone DE, Eliasson M, Johnson O, et al. Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11beta-hydroxysteroid dehydrogenase type 1 activity. *J Clin Endocrinol Metab* 2002;87(7):3330-6.
182. Whorwood CB, Donovan SJ, Flanagan D, Phillips DI, Byrne CD. Increased glucocorticoid receptor expression in human skeletal muscle cells may contribute to the pathogenesis of the metabolic syndrome. *Diabetes* 2002;51(4):1066-75.
183. Abdallah BM, Beck-Nielsen H, Gaster M. Increased expression of 11beta-hydroxysteroid dehydrogenase type 1 in type 2 diabetic myotubes. *Eur J Clin Invest* 2005;35(10):627-34.
184. Livingstone DE, Jones GC, Smith K, Jamieson PM, Andrew R, Kenyon CJ, et al. Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. *Endocrinology* 2000;141(2):560-3.
185. Livingstone DE, Kenyon CJ, Walker BR. Mechanisms of dysregulation of 11 beta-hydroxysteroid dehydrogenase type 1 in obese Zucker rats. *J Endocrinol* 2000;167(3):533-9.
186. Masuzaki H, Yamamoto H, Kenyon CJ, Elmquist JK, Morton NM, Paterson JM, et al. Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice. *J Clin Invest* 2003;112(1):83-90.
187. Kotelevtsev Y, Holmes MC, Burchell A, Houston PM, Schmolli D, Jamieson P, et al. 11beta-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proc Natl Acad Sci U S A* 1997;94(26):14924-9.
188. Morton NM, Holmes MC, Fievet C, Staels B, Tailleux A, Mullins JJ, et al. Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11beta-hydroxysteroid dehydrogenase type 1 null mice. *J Biol Chem* 2001;276(44):41293-300.
189. Morton NM, Ramage L, Seckl JR. Down-regulation of adipose 11beta-hydroxysteroid dehydrogenase type 1 by high-fat feeding in mice: a potential adaptive mechanism counteracting metabolic disease. *Endocrinology* 2004;145(6):2707-12.

190. Morris KL, Zemel MB. 1,25-dihydroxyvitamin D3 modulation of adipocyte glucocorticoid function. *Obes Res* 2005;13(4):670-7.
191. Bujalska IJ, Kumar S, Hewison M, Stewart PM. Differentiation of adipose stromal cells: the roles of glucocorticoids and 11 β -hydroxysteroid dehydrogenase. *Endocrinology* 1999;140(7):3188-96.
192. Bujalska IJ, Walker EA, Tomlinson JW, Hewison M, Stewart PM. 11 β -hydroxysteroid dehydrogenase type 1 in differentiating omental human preadipocytes: from de-activation to generation of cortisol. *Endocr Res* 2002;28(4):449-61.
193. Pausova Z. From big fat cells to high blood pressure: a pathway to obesity-associated hypertension. *Curr Opin Nephrol Hypertens* 2006;15(2):173-8.
194. Daou GB, Srivastava AK. Reactive oxygen species mediate Endothelin-1-induced activation of ERK1/2, PKB, and Pyk2 signaling, as well as protein synthesis, in vascular smooth muscle cells. *Free Radic Biol Med* 2004;37(2):208-15.
195. Schling P, Mallow H, Trindl A, Loffler G. Evidence for a local renin angiotensin system in primary cultured human preadipocytes. *Int J Obes Relat Metab Disord* 1999;23(4):336-41.
196. Campbell DJ, Kladis A. Simultaneous radioimmunoassay of six angiotensin peptides in arterial and venous plasma of man. *J Hypertens* 1990;8(2):165-72.
197. Nishiyama A, Seth DM, Navar LG. Renal interstitial fluid concentrations of angiotensins I and II in anesthetized rats. *Hypertension* 2002;39(1):129-34.
198. Unger T, Gohlke P, Paul M, Rettig R. Tissue renin-angiotensin systems: fact or fiction? *J Cardiovasc Pharmacol* 1991;18 Suppl 2:S20-5.
199. Dzau VJ, Brody T, Ellison KE, Pratt RE, Ingelfinger JR. Tissue-specific regulation of renin expression in the mouse. *Hypertension* 1987;9(6 Pt 2):III36-41.
200. Dzau VJ, Ellison KE, Brody T, Ingelfinger J, Pratt RE. A comparative study of the distributions of renin and angiotensinogen messenger ribonucleic acids in rat and mouse tissues. *Endocrinology* 1987;120(6):2334-8.
201. Zimmerman BG, Dunham EW. Tissue renin-angiotensin system: a site of drug action? *Annu Rev Pharmacol Toxicol* 1997;37:53-69.

202. Cassis LA, Police SB, Yiannikouris F, Thatcher SE. Local adipose tissue renin-angiotensin system. *Curr Hypertens Rep* 2008;10(2):93-8.
203. Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM. Mature adipocytes inhibit in vitro differentiation of human preadipocytes via angiotensin type 1 receptors. *Diabetes* 2002;51(6):1699-707.
204. Fruhbeck G. The adipose tissue as a source of vasoactive factors. *Curr Med Chem Cardiovasc Hematol Agents* 2004;2(3):197-208.
205. Saint-Marc P, Kozak LP, Ailhaud G, Darimont C, Negrel R. Angiotensin II as a trophic factor of white adipose tissue: stimulation of adipose cell formation. *Endocrinology* 2001;142(1):487-92.
206. Jones BH, Standridge MK, Moustaid N. Angiotensin II increases lipogenesis in 3T3-L1 and human adipose cells. *Endocrinology* 1997;138(4):1512-9.
207. Schling P, Loffler G. Effects of angiotensin II on adipose conversion and expression of genes of the renin-angiotensin system in human preadipocytes. *Horm Metab Res* 2001;33(4):189-95.
208. Gorzelniak K, Engeli S, Janke J, Luft FC, Sharma AM. Hormonal regulation of the human adipose-tissue renin-angiotensin system: relationship to obesity and hypertension. *J Hypertens* 2002;20(5):965-73.
209. Lyle RE, Habener JF, McGehee RE, Jr. Antisense oligonucleotides to differentiation-specific element binding protein (DSEB) mRNA inhibit adipocyte differentiation. *Biochem Biophys Res Commun* 1996;228(3):709-15.
210. Brucher R, Cifuentes M, Acuna MJ, Albala C, Rojas CV. Larger anti-adipogenic effect of angiotensin II on omental preadipose cells of obese humans. *Obesity (Silver Spring)* 2007;15(7):1643-6.
211. Van Harmelen V, Ariapart P, Hoffstedt J, Lundkvist I, Bringman S, Arner P. Increased adipose angiotensinogen gene expression in human obesity. *Obes Res* 2000;8(4):337-41.
212. Rahmouni K, Mark AL, Haynes WG, Sigmund CD. Adipose depot-specific modulation of angiotensinogen gene expression in diet-induced obesity. *Am J Physiol Endocrinol Metab* 2004;286(6):E891-5.

213. Giacchetti G, Faloia E, Mariniello B, Sardu C, Gatti C, Camilloni MA, et al. Overexpression of the renin-angiotensin system in human visceral adipose tissue in normal and overweight subjects. *Am J Hypertens* 2002;15(5):381-8.
214. Li J, Brasier AR. Angiotensinogen gene activation by angiotensin II is mediated by the rel A (nuclear factor-kappaB p65) transcription factor: one mechanism for the renin angiotensin system positive feedback loop in hepatocytes. *Mol Endocrinol* 1996;10(3):252-64.
215. Brasier AR, Li J, Copland A. Transcription factors modulating angiotensinogen gene expression in hepatocytes. *Kidney Int* 1994;46(6):1564-6.
216. Aubert J, Darimont C, Safonova I, Ailhaud G, Negrel R. Regulation by glucocorticoids of angiotensinogen gene expression and secretion in adipose cells. *Biochem J* 1997;328 (Pt 2):701-6.
217. Tsuchiya K, Yoshimoto T, Hirono Y, Tateno T, Sugiyama T, Hirata Y. Angiotensin II induces monocyte chemoattractant protein-1 expression via a nuclear factor-kappaB-dependent pathway in rat preadipocytes. *Am J Physiol Endocrinol Metab* 2006;291(4):E771-8.
218. Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J Clin Invest* 2000;105(11):1605-12.
219. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 2003;112(12):1821-30.
220. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw* 2006;17(1):4-12.
221. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004;114(12):1752-61.
222. Chabrashvili T, Kitiyakara C, Blau J, Karber A, Aslam S, Welch WJ, et al. Effects of ANG II type 1 and 2 receptors on oxidative stress, renal NADPH oxidase, and SOD expression. *Am J Physiol Regul Integr Comp Physiol* 2003;285(1):R117-24.

223. Higuchi S, Ohtsu H, Suzuki H, Shirai H, Frank GD, Eguchi S. Angiotensin II signal transduction through the AT1 receptor: novel insights into mechanisms and pathophysiology. *Clin Sci (Lond)* 2007;112(8):417-28.
224. Hunyady L, Catt KJ. Pleiotropic AT1 receptor signaling pathways mediating physiological and pathogenic actions of angiotensin II. *Mol Endocrinol* 2006;20(5):953-70.
225. Violin JD, Dewire SM, Barnes WG, Lefkowitz RJ. G protein-coupled receptor kinase and beta-arrestin-mediated desensitization of the angiotensin II type 1A receptor elucidated by diacylglycerol dynamics. *J Biol Chem* 2006;281(47):36411-9.
226. Hjorth SA, Schambye HT, Greenlee WJ, Schwartz TW. Identification of peptide binding residues in the extracellular domains of the AT1 receptor. *J Biol Chem* 1994;269(49):30953-9.
227. Schambye HT, von Wijk B, Hjorth SA, Wienen W, Entzeroth M, Bergsma DJ, et al. Mutations in transmembrane segment VII of the AT1 receptor differentiate between closely related insurmountable and competitive angiotensin antagonists. *Br J Pharmacol* 1994;113(2):331-3.
228. Schwartz TW, Frimurer TM, Holst B, Rosenkilde MM, Eling CE. Molecular mechanism of 7TM receptor activation--a global toggle switch model. *Annu Rev Pharmacol Toxicol* 2006;46:481-519.
229. Hubbell WL, Altenbach C, Hubbell CM, Khorana HG. Rhodopsin structure, dynamics, and activation: a perspective from crystallography, site-directed spin labeling, sulfhydryl reactivity, and disulfide cross-linking. *Adv Protein Chem* 2003;63:243-90.
230. Farrens DL, Altenbach C, Yang K, Hubbell WL, Khorana HG. Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. *Science* 1996;274(5288):768-70.
231. Gether U, Lin S, Ghanouni P, Ballesteros JA, Weinstein H, Kobilka BK. Agonists induce conformational changes in transmembrane domains III and VI of the beta2 adrenoceptor. *EMBO J* 1997;16(22):6737-47.
232. Martin SS, Holleran BJ, Escher E, Guillemette G, Leduc R. Activation of the angiotensin II type 1 receptor leads to movement of the sixth transmembrane domain: analysis by the substituted cysteine accessibility method. *Mol Pharmacol* 2007;72(1):182-90.

233. Miura S, Saku K, Karnik SS. Molecular analysis of the structure and function of the angiotensin II type 1 receptor. *Hypertens Res* 2003;26(12):937-43.
234. Pitcher JA, Freedman NJ, Lefkowitz RJ. G protein-coupled receptor kinases. *Annu Rev Biochem* 1998;67:653-92.
235. Aplin M, Christensen GL, Hansen JL. Pharmacologic perspectives of functional selectivity by the angiotensin II type 1 receptor. *Trends Cardiovasc Med* 2008;18(8):305-12.
236. Wei H, Ahn S, Shenoy SK, Karnik SS, Hunyady L, Luttrell LM, et al. Independent beta-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *Proc Natl Acad Sci U S A* 2003;100(19):10782-7.
237. Shenoy SK, Lefkowitz RJ. Angiotensin II-stimulated signaling through G proteins and beta-arrestin. *Sci STKE* 2005;2005(311):cm14.
238. Noma T, Lemaire A, Naga Prasad SV, Barki-Harrington L, Tilley DG, Chen J, et al. Beta-arrestin-mediated beta1-adrenergic receptor transactivation of the EGFR confers cardioprotection. *J Clin Invest* 2007;117(9):2445-58.
239. Tohgo A, Pierce KL, Choy EW, Lefkowitz RJ, Luttrell LM. beta-Arrestin scaffolding of the ERK cascade enhances cytosolic ERK activity but inhibits ERK-mediated transcription following angiotensin AT1a receptor stimulation. *J Biol Chem* 2002;277(11):9429-36.
240. Vilardaga JP, Frank M, Krasel C, Dees C, Nissenson RA, Lohse MJ. Differential conformational requirements for activation of G proteins and the regulatory proteins arrestin and G protein-coupled receptor kinase in the G protein-coupled receptor for parathyroid hormone (PTH)/PTH-related protein. *J Biol Chem* 2001;276(36):33435-43.
241. Sheikh SP, Vilardaga JP, Baranski TJ, Lichtarge O, Iiri T, Meng EC, et al. Similar structures and shared switch mechanisms of the beta2-adrenoceptor and the parathyroid hormone receptor. Zn(II) bridges between helices III and VI block activation. *J Biol Chem* 1999;274(24):17033-41.
242. Kim J, Ahn S, Ren XR, Whalen EJ, Reiter E, Wei H, et al. Functional antagonism of different G protein-coupled receptor kinases for beta-arrestin-mediated angiotensin II receptor signaling. *Proc Natl Acad Sci U S A* 2005;102(5):1442-7.

243. Maudsley S, Martin B, Luttrell LM. G protein-coupled receptor signaling complexity in neuronal tissue: implications for novel therapeutics. *Curr Alzheimer Res* 2007;4(1):3-19.
244. Hansen JL, Aplin M, Hansen JT, Christensen GL, Bonde MM, Schneider M, et al. The human angiotensin AT(1) receptor supports G protein-independent extracellular signal-regulated kinase 1/2 activation and cellular proliferation. *Eur J Pharmacol* 2008;590(1-3):255-63.
245. Ahn S, Shenoy SK, Wei H, Lefkowitz RJ. Differential kinetic and spatial patterns of beta-arrestin and G protein-mediated ERK activation by the angiotensin II receptor. *J Biol Chem* 2004;279(34):35518-25.
246. Matsuzawa-Nagata N, Takamura T, Ando H, Nakamura S, Kurita S, Misu H, et al. Increased oxidative stress precedes the onset of high-fat diet-induced insulin resistance and obesity. *Metabolism* 2008;57(8):1071-7.
247. Gordeeva AV, Zvyagilskaya RA, Labas YA. Cross-talk between reactive oxygen species and calcium in living cells. *Biochemistry (Mosc)* 2003;68(10):1077-80.
248. Koren R, Hadari-Naor I, Zuck E, Rotem C, Liberman UA, Ravid A. Vitamin D is a prooxidant in breast cancer cells. *Cancer Res* 2001;61(4):1439-44.
249. Nozoe M, Hirooka Y, Koga Y, Araki S, Konno S, Kishi T, et al. Mitochondria-derived reactive oxygen species mediate sympathoexcitation induced by angiotensin II in the rostral ventrolateral medulla. *J Hypertens* 2008;26(11):2176-84.
250. Ma LQ, Zhang LL, Zhang YP, Wang LJ, Li ZB, Cao TB, et al. [Renin-angiotensin system in mesenteric adipose tissues in rats with metabolic syndrome]. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 2006;28(6):770-5.
251. Burdon RH. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radic Biol Med* 1995;18(4):775-94.
252. Miwa S, Brand MD. Mitochondrial matrix reactive oxygen species production is very sensitive to mild uncoupling. *Biochem Soc Trans* 2003;31(Pt 6):1300-1.
253. Turpaev KT. [Role of transcription factor AP-1 in integration of cellular signalling systems]. *Mol Biol (Mosk)* 2006;40(6):945-61.

254. Chang L, Karin M. Mammalian MAP kinase signalling cascades. *Nature* 2001;410(6824):37-40.
255. Chen A, Davis BH, Bissonnette M, Scaglione-Sewell B, Brasitus TA. 1,25-Dihydroxyvitamin D(3) stimulates activator protein-1-dependent Caco-2 cell differentiation. *J Biol Chem* 1999;274(50):35505-13.
256. Schmitz U, Berk BC. Angiotensin II signal transduction: Stimulation of multiple mitogen-activated protein kinase pathways. *Trends Endocrinol Metab* 1997;8(7):261-6.
257. Zwirska-Korczala K, Adameczyk-Sowa M, Sowa P, Pilc K, Suchanek R, Pierzchala K, et al. Role of leptin, ghrelin, angiotensin II and orexins in 3T3 L1 preadipocyte cells proliferation and oxidative metabolism. *J Physiol Pharmacol* 2007;58 Suppl 1:53-64.
258. Mates JM, Segura JA, Alonso FJ, Marquez J. Intracellular redox status and oxidative stress: implications for cell proliferation, apoptosis, and carcinogenesis. *Arch Toxicol* 2008;82(5):273-99.
259. Black PH. The inflammatory consequences of psychologic stress: relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II. *Med Hypotheses* 2006;67(4):879-91.
260. Gordon S. The role of the macrophage in immune regulation. *Res Immunol* 1998;149(7-8):685-8.
261. Odegaard JI, Chawla A. Mechanisms of macrophage activation in obesity-induced insulin resistance. *Nat Clin Pract Endocrinol Metab* 2008.
262. Heilbronn LK, Campbell LV. Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. *Curr Pharm Des* 2008;14(12):1225-30.
263. Zeyda M, Stulnig TM. Adipose tissue macrophages. *Immunol Lett* 2007;112(2):61-7.
264. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* 2003;3(1):23-35.
265. Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. *Nat Rev Immunol* 2005;5(8):641-54.

266. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* 2007;117(1):175-84.
267. Lee JY, Sohn KH, Rhee SH, Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. *J Biol Chem* 2001;276(20):16683-9.
268. Takahashi K, Mizuarai S, Araki H, Mashiko S, Ishihara A, Kanatani A, et al. Adiposity elevates plasma MCP-1 levels leading to the increased CD11b-positive monocytes in mice. *J Biol Chem* 2003;278(47):46654-60.
269. Lumeng CN, Delproposto JB, Westcott DJ, Saltiel AR. Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. *Diabetes* 2008.
270. Nguyen MT, Favelyukis S, Nguyen AK, Reichart D, Scott PA, Jenn A, et al. A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. *J Biol Chem* 2007;282(48):35279-92.
271. Toyoda T, Kamei Y, Kato H, Sugita S, Takeya M, Suganami T, et al. Effect of peroxisome proliferator-activated receptor-alpha ligands in the interaction between adipocytes and macrophages in obese adipose tissue. *Obesity (Silver Spring)* 2008;16(6):1199-207.
272. Charriere G, Cousin B, Arnaud E, Andre M, Bacou F, Penicaud L, et al. Preadipocyte conversion to macrophage. Evidence of plasticity. *J Biol Chem* 2003;278(11):9850-5.
273. Abuissa H, Jones PG, Marso SP, O'Keefe JH, Jr. Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers for prevention of type 2 diabetes: a meta-analysis of randomized clinical trials. *J Am Coll Cardiol* 2005;46(5):821-6.
274. Andraws R, Brown DL. Effect of inhibition of the renin-angiotensin system on development of type 2 diabetes mellitus (meta-analysis of randomized trials). *Am J Cardiol* 2007;99(7):1006-12.
275. Boschmann M, Kreuzberg U, Engeli S, Adams F, Franke G, Klaua S, et al. The effect of oral glucose loads on tissue metabolism during angiotensin II receptor and beta-receptor blockade in obese hypertensive subjects. *Horm Metab Res* 2006;38(5):323-9.

276. Gillespie EL, White CM, Kardas M, Lindberg M, Coleman CI. The impact of ACE inhibitors or angiotensin II type 1 receptor blockers on the development of new-onset type 2 diabetes. *Diabetes Care* 2005;28(9):2261-6.
277. Califf RM, Boolell M, Haffner SM, Bethel MA, McMurray J, Duggal A, et al. Prevention of diabetes and cardiovascular disease in patients with impaired glucose tolerance: rationale and design of the Nateglinide And Valsartan in Impaired Glucose Tolerance Outcomes Research (NAVIGATOR) Trial. *Am Heart J* 2008;156(4):623-32.
278. Suzuki K, Nakagawa O, Aizawa Y. Improved early-phase insulin response after candesartan treatment in hypertensive patients with impaired glucose tolerance. *Clin Exp Hypertens* 2008;30(5):309-14.
279. Derosa G, Cicero AF, D'Angelo A, Ragonesi PD, Ciccarelli L, Piccinni MN, et al. Telmisartan and irbesartan therapy in type 2 diabetic patients treated with rosiglitazone: effects on insulin-resistance, leptin and tumor necrosis factor-alpha. *Hypertens Res* 2006;29(11):849-56.
280. Koh KK, Quon MJ, Han SH, Chung WJ, Lee Y, Shin EK. Anti-inflammatory and metabolic effects of candesartan in hypertensive patients. *Int J Cardiol* 2006;108(1):96-100.
281. Shargorodsky M, Hass E, Boaz M, Gavish D, Zimlichman R. High dose treatment with angiotensin II receptor blocker in patients with hypertension: differential effect of tissue protection versus blood pressure lowering. *Atherosclerosis* 2008;197(1):303-10.
282. Ichikawa Y. Comparative effects of telmisartan and valsartan on insulin resistance in hypertensive patients with metabolic syndrome. *Intern Med* 2007;46(17):1331-6.
283. Vitale C, Mercurio G, Castiglioni C, Cornoldi A, Tulli A, Fini M, et al. Metabolic effect of telmisartan and losartan in hypertensive patients with metabolic syndrome. *Cardiovasc Diabetol* 2005;4:6.
284. Boustany CM, Bharadwaj K, Daugherty A, Brown DR, Randall DC, Cassis LA. Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension. *Am J Physiol Regul Integr Comp Physiol* 2004;287(4):R943-9.
285. Benson SC, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, Pravenec M, et al. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. *Hypertension* 2004;43(5):993-1002.

286. Tomono Y, Iwai M, Inaba S, Mogi M, Horiuchi M. Blockade of AT1 receptor improves adipocyte differentiation in atherosclerotic and diabetic models. *Am J Hypertens* 2008;21(2):206-12.
287. Shiuchi T, Iwai M, Li HS, Wu L, Min LJ, Li JM, et al. Angiotensin II type-1 receptor blocker valsartan enhances insulin sensitivity in skeletal muscles of diabetic mice. *Hypertension* 2004;43(5):1003-10.
288. Henriksen EJ, Jacob S, Kinnick TR, Teachey MK, Krekler M. Selective angiotensin II receptor antagonist reduces insulin resistance in obese Zucker rats. *Hypertension* 2001;38(4):884-90.
289. Leung PS, de Gasparo M. Involvement of the pancreatic renin-angiotensin system in insulin resistance and the metabolic syndrome. *J Cardimetab Syndr* 2006;1(3):197-203.
290. Chan P, Liu IM, Tzeng TF, Yang TL, Cheng JT. Mechanism for blockade of angiotensin subtype 1 receptors to lower plasma glucose in streptozotocin-induced diabetic rats. *Diabetes Obes Metab* 2007;9(1):39-49.
291. Folli F, Kahn CR, Hansen H, Bouchie JL, Feener EP. Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. *J Clin Invest* 1997;100(9):2158-69.
292. Koh KK, Quon MJ, Han SH, Chung WJ, Ahn JY, Seo YH, et al. Additive beneficial effects of losartan combined with simvastatin in the treatment of hypercholesterolemic, hypertensive patients. *Circulation* 2004;110(24):3687-92.
293. Furuhashi M, Ura N, Higashiura K, Murakami H, Tanaka M, Moniwa N, et al. Blockade of the renin-angiotensin system increases adiponectin concentrations in patients with essential hypertension. *Hypertension* 2003;42(1):76-81.
294. Scheen AJ. Prevention of type 2 diabetes mellitus through inhibition of the Renin-Angiotensin system. *Drugs* 2004;64(22):2537-65.
295. Anan F, Takahashi N, Ooie T, Hara M, Yoshimatsu H, Saikawa T. Candesartan, an angiotensin II receptor blocker, improves left ventricular hypertrophy and insulin resistance. *Metabolism* 2004;53(6):777-81.
296. Clasen R, Schupp M, Foryst-Ludwig A, Sprang C, Clemenz M, Krikov M, et al. PPARgamma-activating angiotensin type-1 receptor blockers induce adiponectin. *Hypertension* 2005;46(1):137-43.

297. Schupp M, Clemenz M, Gineste R, Witt H, Janke J, Helleboid S, et al. Molecular characterization of new selective peroxisome proliferator-activated receptor gamma modulators with angiotensin receptor blocking activity. *Diabetes* 2005;54(12):3442-52.
298. Xu Y, Mirmalek-Sani SH, Yang X, Zhang J, Oreffo RO. The use of small interfering RNAs to inhibit adipocyte differentiation in human preadipocytes and fetal-femur-derived mesenchymal cells. *Exp Cell Res* 2006;312(10):1856-64.
299. Perera RJ, Marcusson EG, Koo S, Kang X, Kim Y, White N, et al. Identification of novel PPARgamma target genes in primary human adipocytes. *Gene* 2006;369:90-9.
300. Schupp M, Janke J, Clasen R, Unger T, Kintscher U. Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor-gamma activity. *Circulation* 2004;109(17):2054-7.
301. Lindholm LH, Ibsen H, Borch-Johnsen K, Olsen MH, Wachtell K, Dahlof B, et al. Risk of new-onset diabetes in the Losartan Intervention For Endpoint reduction in hypertension study. *J Hypertens* 2002;20(9):1879-86.
302. Julius S, Kjeldsen SE, Weber M, Brunner HR, Ekman S, Hansson L, et al. Outcomes in hypertensive patients at high cardiovascular risk treated with regimens based on valsartan or amlodipine: the VALUE randomised trial. *Lancet* 2004;363(9426):2022-31.
303. Negro R, Formoso G, Hassan H. The effects of irbesartan and telmisartan on metabolic parameters and blood pressure in obese, insulin resistant, hypertensive patients. *J Endocrinol Invest* 2006;29(11):957-61.
304. Murakami H, Ura N, Furuhashi M, Higashiura K, Miura T, Shimamoto K. Role of adiponectin in insulin-resistant hypertension and atherosclerosis. *Hypertens Res* 2003;26(9):705-10.
305. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999;257(1):79-83.
306. Dibello JR, Baylin A, Viali S, Tuitele J, Bausserman L, McGarvey ST. Adiponectin and type 2 diabetes in Samoan adults. *Am J Hum Biol* 2008.
307. Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, et al. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes* 2003;52(7):1655-63.

308. Moriuchi A, Yamasaki H, Shimamura M, Kita A, Kuwahara H, Fujishima K, et al. Induction of human adiponectin gene transcription by telmisartan, angiotensin receptor blocker, independently on PPAR-gamma activation. *Biochem Biophys Res Commun* 2007;356(4):1024-30.
309. Kakuta H, Sudoh K, Sasamata M, Yamagishi S. Telmisartan has the strongest binding affinity to angiotensin II type 1 receptor: comparison with other angiotensin II type 1 receptor blockers. *Int J Clin Pharmacol Res* 2005;25(1):41-6.
310. Kurtz TW. Treating the metabolic syndrome: telmisartan as a peroxisome proliferator-activated receptor-gamma activator. *Acta Diabetol* 2005;42 Suppl 1:S9-16.
311. Tagami T, Yamamoto H, Moriyama K, Sawai K, Usui T, Shimatsu A, et al. A selective peroxisome proliferator-activated receptor-gamma modulator, telmisartan, binds to the receptor in a different fashion from thiazolidinediones. *Endocrinology* 2009;150(2):862-70.
312. Berellini G, Cruciani G, Mannhold R. Pharmacophore, drug metabolism, and pharmacokinetics models on non-peptide AT1, AT2, and AT1/AT2 angiotensin II receptor antagonists. *J Med Chem* 2005;48(13):4389-99.
313. Sugimoto K, Qi NR, Kazdova L, Pravenec M, Ogihara T, Kurtz TW. Telmisartan but not valsartan increases caloric expenditure and protects against weight gain and hepatic steatosis. *Hypertension* 2006;47(5):1003-9.
314. Shao J, Nangaku M, Inagi R, Kato H, Miyata T, Matsusaka T, et al. Receptor-independent intracellular radical scavenging activity of an angiotensin II receptor blocker. *J Hypertens* 2007;25(8):1643-9.
315. Patny A, Desai PV, Avery MA. Ligand-supported homology modeling of the human angiotensin II type 1 (AT(1)) receptor: insights into the molecular determinants of telmisartan binding. *Proteins* 2006;65(4):824-42.
316. Oberfield JL, Collins JL, Holmes CP, Goreham DM, Cooper JP, Cobb JE, et al. A peroxisome proliferator-activated receptor gamma ligand inhibits adipocyte differentiation. *Proc Natl Acad Sci U S A* 1999;96(11):6102-6.
317. Abu-Elheiga L, Matzuk MM, Abo-Hashema KA, Wakil SJ. Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. *Science* 2001;291(5513):2613-6.

318. Berger JP, Petro AE, Macnaul KL, Kelly LJ, Zhang BB, Richards K, et al. Distinct properties and advantages of a novel peroxisome proliferator-activated protein [gamma] selective modulator. *Mol Endocrinol* 2003;17(4):662-76.
319. Chang CH, McNamara LA, Wu MS, Muise ES, Tan Y, Wood HB, et al. A novel selective peroxisome proliferator-activator receptor-gamma modulator-SPPARgammaM5 improves insulin sensitivity with diminished adverse cardiovascular effects. *Eur J Pharmacol* 2008;584(1):192-201.
320. Pickart CM, Cohen RE. Proteasomes and their kin: proteases in the machine age. *Nat Rev Mol Cell Biol* 2004;5(3):177-87.
321. de Souza CJ, Eckhardt M, Gagen K, Dong M, Chen W, Laurent D, et al. Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. *Diabetes* 2001;50(8):1863-71.
322. Zanchi A, Dulloo AG, Perregaux C, Montani JP, Burnier M. Telmisartan prevents the glitazone-induced weight gain without interfering with its insulin-sensitizing properties. *Am J Physiol Endocrinol Metab* 2007;293(1):E91-5.
323. Enjoji M, Kotoh K, Kato M, Higuchi N, Kohjima M, Nakashima M, et al. Therapeutic effect of ARBs on insulin resistance and liver injury in patients with NAFLD and chronic hepatitis C: a pilot study. *Int J Mol Med* 2008;22(4):521-7.
324. Araki K, Masaki T, Katsuragi I, Tanaka K, Kakuma T, Yoshimatsu H. Telmisartan prevents obesity and increases the expression of uncoupling protein 1 in diet-induced obese mice. *Hypertension* 2006;48(1):51-7.
325. Lee MH, Song HK, Ko GJ, Kang YS, Han SY, Han KH, et al. Angiotensin receptor blockers improve insulin resistance in type 2 diabetic rats by modulating adipose tissue. *Kidney Int* 2008;74(7):890-900.
326. Kurata A, Nishizawa H, Kihara S, Maeda N, Sonoda M, Okada T, et al. Blockade of Angiotensin II type-1 receptor reduces oxidative stress in adipose tissue and ameliorates adipocytokine dysregulation. *Kidney Int* 2006;70(10):1717-24.
327. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002;8(11):1288-95.

328. Kouyama R, Suganami T, Nishida J, Tanaka M, Toyoda T, Kiso M, et al. Attenuation of diet-induced weight gain and adiposity through increased energy expenditure in mice lacking angiotensin II type 1a receptor. *Endocrinology* 2005;146(8):3481-9.
329. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, et al. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes* 2007;56(4):901-11.
330. Brook CG, Lloyd JK, Wolf OH. Relation between age of onset of obesity and size and number of adipose cells. *Br Med J* 1972;2(5804):25-7.
331. Helmlinger G, Yuan F, Dellian M, Jain RK. Interstitial pH and pO₂ gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. *Nat Med* 1997;3(2):177-82.
332. Lolmede K, Durand de Saint Front V, Galitzky J, Lafontan M, Bouloumie A. Effects of hypoxia on the expression of proangiogenic factors in differentiated 3T3-F442A adipocytes. *Int J Obes Relat Metab Disord* 2003;27(10):1187-95.
333. Wang B, Wood IS, Trayhurn P. Hypoxia induces leptin gene expression and secretion in human preadipocytes: differential effects of hypoxia on adipokine expression by preadipocytes. *J Endocrinol* 2008;198(1):127-34.
334. Wood IS, Wang B, Lorente-Cebrian S, Trayhurn P. Hypoxia increases expression of selective facilitative glucose transporters (GLUT) and 2-deoxy-D-glucose uptake in human adipocytes. *Biochem Biophys Res Commun* 2007;361(2):468-73.
335. Wang B, Wood IS, Trayhurn P. Dysregulation of the expression and secretion of inflammation-related adipokines by hypoxia in human adipocytes. *Pflugers Arch* 2007;455(3):479-92.
336. Regazzetti C, Peraldi P, Gremeaux T, Najem-Lendom R, Ben-Sahra I, Cormont M, et al. Hypoxia decreases insulin signaling pathways in adipocytes. *Diabetes* 2009;58(1):95-103.
337. Rausch ME, Weisberg S, Vardhana P, Tortoriello DV. Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. *Int J Obes (Lond)* 2008;32(3):451-63.
338. Cao Y. Angiogenesis modulates adipogenesis and obesity. *J Clin Invest* 2007;117(9):2362-8.

339. Araki-Taguchi M, Nomura S, Ino K, Sumigama S, Yamamoto E, Kotani-Ito T, et al. Angiotensin II mimics the hypoxic effect on regulating trophoblast proliferation and differentiation in human placental explant cultures. *Life Sci* 2008;82(1-2):59-67.
340. Shi RZ, Wang JC, Huang SH, Wang XJ, Li QP. Angiotensin II induces vascular endothelial growth factor synthesis in mesenchymal stem cells. *Exp Cell Res* 2009;315(1):10-5.
341. Rakusan K, Chvojková Z, Oliviero P, Ostadalova I, Kolar F, Chassagne C, et al. ANG II type 1 receptor antagonist irbesartan inhibits coronary angiogenesis stimulated by chronic intermittent hypoxia in neonatal rats. *Am J Physiol Heart Circ Physiol* 2007;292(3):H1237-44.
342. Rupnick MA, Panigrahy D, Zhang CY, Dallabrida SM, Lowell BB, Langer R, et al. Adipose tissue mass can be regulated through the vasculature. *Proc Natl Acad Sci U S A* 2002;99(16):10730-5.
343. Brakenhielm E, Cao R, Gao B, Angelin B, Cannon B, Parini P, et al. Angiogenesis inhibitor, TNP-470, prevents diet-induced and genetic obesity in mice. *Circ Res* 2004;94(12):1579-88.
344. Miura S, Saku K. Regulation of angiogenesis and angiogenic factors by cardiovascular medications. *Curr Pharm Des* 2007;13(20):2113-7.
345. Inamoto S, Hayashi T, Tazawa N, Mori T, Yamashita C, Nakano D, et al. Angiotensin-II receptor blocker exerts cardioprotection in diabetic rats exposed to hypoxia. *Circ J* 2006;70(6):787-92.
346. Aldhahi W, Hamdy O. Adipokines, inflammation, and the endothelium in diabetes. *Curr Diab Rep* 2003;3(4):293-8.
347. Nickenig G, Harrison DG. The AT(1)-type angiotensin receptor in oxidative stress and atherogenesis: Part II: AT(1) receptor regulation. *Circulation* 2002;105(4):530-6.
348. Nickenig G, Harrison DG. The AT(1)-type angiotensin receptor in oxidative stress and atherogenesis: part I: oxidative stress and atherogenesis. *Circulation* 2002;105(3):393-6.
349. Bendall JK, Rinze R, Adlam D, Tatham AL, de Bono J, Wilson N, et al. Endothelial Nox2 overexpression potentiates vascular oxidative stress and hemodynamic response to angiotensin II: studies in endothelial-targeted Nox2 transgenic mice. *Circ Res* 2007;100(7):1016-25.

350. Rueckschloss U, Quinn MT, Holtz J, Morawietz H. Dose-dependent regulation of NAD(P)H oxidase expression by angiotensin II in human endothelial cells: protective effect of angiotensin II type 1 receptor blockade in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 2002;22(11):1845-51.
351. Fujita K, Nishizawa H, Funahashi T, Shimomura I, Shimabukuro M. Systemic oxidative stress is associated with visceral fat accumulation and the metabolic syndrome. *Circ J* 2006;70(11):1437-42.
352. de Cavanagh EM, Inserra F, Toblli J, Stella I, Fraga CG, Ferder L. Enalapril attenuates oxidative stress in diabetic rats. *Hypertension* 2001;38(5):1130-6.
353. Dandona P, Kumar V, Aljada A, Ghanim H, Syed T, Hofmayer D, et al. Angiotensin II receptor blocker valsartan suppresses reactive oxygen species generation in leukocytes, nuclear factor-kappa B, in mononuclear cells of normal subjects: evidence of an antiinflammatory action. *J Clin Endocrinol Metab* 2003;88(9):4496-501.
354. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 1994;74(6):1141-8.
355. Berry C, Hamilton CA, Brosnan MJ, Magill FG, Berg GA, McMurray JJ, et al. Investigation into the sources of superoxide in human blood vessels: angiotensin II increases superoxide production in human internal mammary arteries. *Circulation* 2000;101(18):2206-12.
356. Dikalov SI, Dikalova AE, Bikineyeva AT, Schmidt HH, Harrison DG, Griendling KK. Distinct roles of Nox1 and Nox4 in basal and angiotensin II-stimulated superoxide and hydrogen peroxide production. *Free Radic Biol Med* 2008;45(9):1340-51.
357. Harrison DG, Cai H, Landmesser U, Griendling KK. Interactions of angiotensin II with NAD(P)H oxidase, oxidant stress and cardiovascular disease. *J Renin Angiotensin Aldosterone Syst* 2003;4(2):51-61.
358. Yang HY, Kao PF, Chen TH, Tomlinson B, Ko WC, Chan P. Effects of the angiotensin II type 1 receptor antagonist valsartan on the expression of superoxide dismutase in hypertensive patients. *J Clin Pharmacol* 2007;47(3):397-403.
359. Seeger H, Mueck AO, Lippert TH. Effects of valsartan and 17 beta-estradiol on the oxidation of low-density lipoprotein in vitro. *Coron Artery Dis* 2000;11(4):347-9.

360. Chan P, Cheng JT, Tsao CW, Niu CS, Hong CY. The in vitro antioxidant activity of trilinolein and other lipid-related natural substances as measured by enhanced chemiluminescence. *Life Sci* 1996;59(24):2067-73.
361. Koh KK, Ahn JY, Han SH, Kim DS, Jin DK, Kim HS, et al. Pleiotropic effects of angiotensin II receptor blocker in hypertensive patients. *J Am Coll Cardiol* 2003;42(5):905-10.
362. Sola S, Mir MQ, Cheema FA, Khan-Merchant N, Menon RG, Parthasarathy S, et al. Irbesartan and lipoic acid improve endothelial function and reduce markers of inflammation in the metabolic syndrome: results of the Irbesartan and Lipoic Acid in Endothelial Dysfunction (ISLAND) study. *Circulation* 2005;111(3):343-8.
363. Rosei EA, Rizzoni D, Muiesan ML, Sleiman I, Salvetti M, Monteduro C, et al. Effects of candesartan cilexetil and enalapril on inflammatory markers of atherosclerosis in hypertensive patients with non-insulin-dependent diabetes mellitus. *J Hypertens* 2005;23(2):435-44.
364. Navalkar S, Parthasarathy S, Santanam N, Khan BV. Irbesartan, an angiotensin type 1 receptor inhibitor, regulates markers of inflammation in patients with premature atherosclerosis. *J Am Coll Cardiol* 2001;37(2):440-4.
365. Khan BV, Navalkar S, Khan QA, Rahman ST, Parthasarathy S. Irbesartan, an angiotensin type 1 receptor inhibitor, regulates the vascular oxidative state in patients with coronary artery disease. *J Am Coll Cardiol* 2001;38(6):1662-7.
366. Monacelli F, Poggi A, Storace D, Durante A, Traverso N, Viviani GL, et al. Effects of valsartan therapy on protein glycoxidation. *Metabolism* 2006;55(12):1619-24.
367. Izuhara Y, Nangaku M, Inagi R, Tominaga N, Aizawa T, Kurokawa K, et al. Renoprotective properties of angiotensin receptor blockers beyond blood pressure lowering. *J Am Soc Nephrol* 2005;16(12):3631-41.
368. Izuhara Y, Nangaku M, Takizawa S, Takahashi S, Shao J, Oishi H, et al. A novel class of advanced glycation inhibitors ameliorates renal and cardiovascular damage in experimental rat models. *Nephrol Dial Transplant* 2008;23(2):497-509.
369. Miyata T, van Ypersele de Strihou C, Ueda Y, Ichimori K, Inagi R, Onogi H, et al. Angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors lower in vitro the formation of advanced glycation end products: biochemical mechanisms. *J Am Soc Nephrol* 2002;13(10):2478-87.

370. Mehta R, Shangari N, O'Brien PJ. Preventing cell death induced by carbonyl stress, oxidative stress or mitochondrial toxins with vitamin B anti-AGE agents. *Mol Nutr Food Res* 2008;52(3):379-85.
371. Negre-Salvayre A, Coatrieux C, Ingueneau C, Salvayre R. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. *Br J Pharmacol* 2008;153(1):6-20.
372. Monnier VM. Transition metals redox: reviving an old plot for diabetic vascular disease. *J Clin Invest* 2001;107(7):799-801.
373. Ishizaka N, Aizawa T, Mori I, Taguchi J, Yazaki Y, Nagai R, et al. Heme oxygenase-1 is upregulated in the rat heart in response to chronic administration of angiotensin II. *Am J Physiol Heart Circ Physiol* 2000;279(2):H672-8.
374. Ishizaka N, Saito K, Mitani H, Yamazaki I, Sata M, Usui S, et al. Iron overload augments angiotensin II-induced cardiac fibrosis and promotes neointima formation. *Circulation* 2002;106(14):1840-6.
375. Ferris CD, Jaffrey SR, Sawa A, Takahashi M, Brady SD, Barrow RK, et al. Haem oxygenase-1 prevents cell death by regulating cellular iron. *Nat Cell Biol* 1999;1(3):152-7.
376. Chan YC, Leung PS. AT1 receptor antagonism ameliorates acute pancreatitis-associated pulmonary injury. *Regul Pept* 2006;134(1):46-53.
377. Tikellis C, Wookey PJ, Candido R, Andrikopoulos S, Thomas MC, Cooper ME. Improved islet morphology after blockade of the renin- angiotensin system in the ZDF rat. *Diabetes* 2004;53(4):989-97.
378. Chu KY, Lau T, Carlsson PO, Leung PS. Angiotensin II type 1 receptor blockade improves beta-cell function and glucose tolerance in a mouse model of type 2 diabetes. *Diabetes* 2006;55(2):367-74.
379. Henriksen EJ. Improvement of insulin sensitivity by antagonism of the renin-angiotensin system. *Am J Physiol Regul Integr Comp Physiol* 2007;293(3):R974-80.
380. Ko SH, Hong OK, Kim JW, Ahn YB, Song KH, Cha BY, et al. High glucose increases extracellular matrix production in pancreatic stellate cells by activating the renin-angiotensin system. *J Cell Biochem* 2006;98(2):343-55.

381. Lau T, Carlsson PO, Leung PS. Evidence for a local angiotensin-generating system and dose-dependent inhibition of glucose-stimulated insulin release by angiotensin II in isolated pancreatic islets. *Diabetologia* 2004;47(2):240-8.
382. Tsang SW, Ip SP, Leung PS. Prophylactic and therapeutic treatments with AT 1 and AT 2 receptor antagonists and their effects on changes in the severity of pancreatitis. *Int J Biochem Cell Biol* 2004;36(2):330-9.
383. Gerich JE. Redefining the clinical management of type 2 diabetes: matching therapy to pathophysiology. *Eur J Clin Invest* 2002;32 Suppl 3:46-53.
384. Leung PS. Mechanisms of protective effects induced by blockade of the renin-angiotensin system: novel role of the pancreatic islet angiotensin-generating system in Type 2 diabetes. *Diabet Med* 2007;24(2):110-6.
385. Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab* 2007;92(3):1023-33.
386. Strissel KJ, Stancheva Z, Miyoshi H, Perfield JW, 2nd, DeFuria J, Jick Z, et al. Adipocyte death, adipose tissue remodeling, and obesity complications. *Diabetes* 2007;56(12):2910-8.
387. Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE. Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. *Diabetologia* 2000;43(12):1498-506.
388. Wueest S, Rapold RA, Rytka JM, Schoenle EJ, Konrad D. Basal lipolysis, not the degree of insulin resistance, differentiates large from small isolated adipocytes in high-fat fed mice. *Diabetologia* 2009;52(3):541-6.
389. Okada K, Hirano T, Ran J, Adachi M. Olmesartan medoxomil, an angiotensin II receptor blocker ameliorates insulin resistance and decreases triglyceride production in fructose-fed rats. *Hypertens Res* 2004;27(4):293-9.
390. Higashiura K, Ura N, Takada T, Li Y, Torii T, Togashi N, et al. The effects of an angiotensin-converting enzyme inhibitor and an angiotensin II receptor antagonist on insulin resistance in fructose-fed rats. *Am J Hypertens* 2000;13(3):290-7.
391. Higashiura K, Ura N, Miyazaki Y, Shimamoto K. Effect of an angiotensin II receptor antagonist, candesartan, on insulin resistance and pressor mechanisms in essential hypertension. *J Hum Hypertens* 1999;13 Suppl 1:S71-4.

392. Townsend RR, DiPette DJ. Pressor doses of angiotensin II increase insulin-mediated glucose uptake in normotensive men. *Am J Physiol* 1993;265(3 Pt 1):E362-6.
393. Togashi N, Ura N, Higashiura K, Murakami H, Shimamoto K. Effect of TNF- α -converting enzyme inhibitor on insulin resistance in fructose-fed rats. *Hypertension* 2002;39(2 Pt 2):578-80.
394. Wei Y, Whaley-Connell AT, Chen K, Habibi J, Uptergrove GM, Clark SE, et al. NADPH oxidase contributes to vascular inflammation, insulin resistance, and remodeling in the transgenic (mRen2) rat. *Hypertension* 2007;50(2):384-91.
395. Saitoh S. [Insulin resistance and renin-angiotensin-aldosterone system]. *Nippon Rinsho* 2009;67(4):729-34.
396. Kranzhofer R, Browatzki M, Schmidt J, Kubler W. Angiotensin II activates the proinflammatory transcription factor nuclear factor-kappaB in human monocytes. *Biochem Biophys Res Commun* 1999;257(3):826-8.
397. Zahradka P, Werner JP, Buhay S, Litchie B, Helwer G, Thomas S. NF-kappaB activation is essential for angiotensin II-dependent proliferation and migration of vascular smooth muscle cells. *J Mol Cell Cardiol* 2002;34(12):1609-21.
398. Pueyo ME, Gonzalez W, Nicoletti A, Savoie F, Arnal JF, Michel JB. Angiotensin II stimulates endothelial vascular cell adhesion molecule-1 via nuclear factor-kappaB activation induced by intracellular oxidative stress. *Arterioscler Thromb Vasc Biol* 2000;20(3):645-51.
399. Ruiz-Ortega M, Lorenzo O, Ruperez M, Konig S, Wittig B, Egido J. Angiotensin II activates nuclear transcription factor kappaB through AT(1) and AT(2) in vascular smooth muscle cells: molecular mechanisms. *Circ Res* 2000;86(12):1266-72.
400. Skurk T, van Harmelen V, Hauner H. Angiotensin II stimulates the release of interleukin-6 and interleukin-8 from cultured human adipocytes by activation of NF-kappaB. *Arterioscler Thromb Vasc Biol* 2004;24(7):1199-203.
401. Barnes PJ, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997;336(15):1066-71.
402. Phillips MI, Kagiya S. Angiotensin II as a pro-inflammatory mediator. *Curr Opin Investig Drugs* 2002;3(4):569-77.

403. Ghosh S, Hayden MS. New regulators of NF-kappaB in inflammation. *Nat Rev Immunol* 2008;8(11):837-48.
404. Hacker H, Karin M. Regulation and function of IKK and IKK-related kinases. *Sci STKE* 2006;2006(357):re13.
405. Gilmore TD. Introduction to NF-kappaB: players, pathways, perspectives. *Oncogene* 2006;25(51):6680-4.
406. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. *Cell* 2008;132(3):344-62.
407. Ozawa Y, Kobori H. Crucial role of Rho-nuclear factor-kappaB axis in angiotensin II-induced renal injury. *Am J Physiol Renal Physiol* 2007;293(1):F100-9.
408. Zhuo JL. Monocyte chemoattractant protein-1: a key mediator of angiotensin II-induced target organ damage in hypertensive heart disease? *J Hypertens* 2004;22(3):451-4.
409. Ruiz-Ortega M, Ruperez M, Lorenzo O, Esteban V, Blanco J, Mezzano S, et al. Angiotensin II regulates the synthesis of proinflammatory cytokines and chemokines in the kidney. *Kidney Int Suppl* 2002(82):S12-22.
410. Ozawa Y, Kobori H, Suzaki Y, Navar LG. Sustained renal interstitial macrophage infiltration following chronic angiotensin II infusions. *Am J Physiol Renal Physiol* 2007;292(1):F330-9.
411. Skurk T, Lee YM, Hauner H. Angiotensin II and its metabolites stimulate PAI-1 protein release from human adipocytes in primary culture. *Hypertension* 2001;37(5):1336-40.
412. Chen S, Ge Y, Si J, Rifai A, Dworkin LD, Gong R. Candesartan suppresses chronic renal inflammation by a novel antioxidant action independent of AT1R blockade. *Kidney Int* 2008;74(9):1128-38.
413. Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proc Natl Acad Sci U S A* 2003;100(12):7265-70.
414. Delhalle S, Blasius R, Dicato M, Diederich M. A beginner's guide to NF-kappaB signaling pathways. *Ann N Y Acad Sci* 2004;1030:1-13.

415. Egan LJ, Toruner M. NF-kappaB signaling: pros and cons of altering NF-kappaB as a therapeutic approach. *Ann N Y Acad Sci* 2006;1072:114-22.
416. Luo JL, Kamata H, Karin M. IKK/NF-kappaB signaling: balancing life and death--a new approach to cancer therapy. *J Clin Invest* 2005;115(10):2625-32.
417. Neumann M, Naumann M. Beyond IkappaBs: alternative regulation of NF-kappaB activity. *Faseb J* 2007;21(11):2642-54.
418. Zhuo JL, Carretero OA, Li XC. Effects of AT1 receptor-mediated endocytosis of extracellular Ang II on activation of nuclear factor-kappa B in proximal tubule cells. *Ann N Y Acad Sci* 2006;1091:336-45.
419. Saito Y, Berk BC. Transactivation: a novel signaling pathway from angiotensin II to tyrosine kinase receptors. *J Mol Cell Cardiol* 2001;33(1):3-7.
420. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International union of pharmacology. XXIII. The angiotensin II receptors. *Pharmacol Rev* 2000;52(3):415-72.
421. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol* 2007;292(1):C82-97.
422. Zhang L, Ma Y, Zhang J, Cheng J, Du J. A new cellular signaling mechanism for angiotensin II activation of NF-kappaB: An IkappaB-independent, RSK-mediated phosphorylation of p65. *Arterioscler Thromb Vasc Biol* 2005;25(6):1148-53.
423. Douillette A, Bibeau-Poirier A, Gravel SP, Clement JF, Chenard V, Moreau P, et al. The proinflammatory actions of angiotensin II are dependent on p65 phosphorylation by the IkappaB kinase complex. *J Biol Chem* 2006;281(19):13275-84.
424. Cui R, Tieu B, Recinos A, Tilton RG, Brasier AR. RhoA mediates angiotensin II-induced phospho-Ser536 nuclear factor kappaB/RelA subunit exchange on the interleukin-6 promoter in VSMCs. *Circ Res* 2006;99(7):723-30.
425. Zhang L, Cheng J, Ma Y, Thomas W, Zhang J, Du J. Dual pathways for nuclear factor kappaB activation by angiotensin II in vascular smooth muscle: phosphorylation of p65 by IkappaB kinase and ribosomal kinase. *Circ Res* 2005;97(10):975-82.

426. Kralisch S, Sommer G, Stangl V, Kohler U, Kratzsch J, Stepan H, et al. Secretory products from human adipocytes impair endothelial function via nuclear factor kappaB. *Atherosclerosis* 2008;196(2):523-31.
427. Fantuzzi G, Mazzone T. Adipose tissue and atherosclerosis: exploring the connection. *Arterioscler Thromb Vasc Biol* 2007;27(5):996-1003.
428. Shimano H. [Obesity and atherosclerosis]. *Nippon Rinsho* 2009;67(2):333-7.
429. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* 2006;116(6):1494-505.
430. Guo C, Yuan L, Liu X, Du A, Huang Y, Zhang L. Effect of ARB on expression of CD68 and MCP-1 in adipose tissue of rats on long-term high fat diet. *J Huazhong Univ Sci Technolog Med Sci* 2008;28(3):257-60.
431. Hasegawa G, Fukui M, Hosoda H, Asano M, Harusato I, Tanaka M, et al. Telmisartan, an angiotensin II type 1 receptor blocker, prevents the development of diabetes in male Spontaneously Diabetic Torii rats. *Eur J Pharmacol* 2009.
432. Clement K, Langin D. Regulation of inflammation-related genes in human adipose tissue. *J Intern Med* 2007;262(4):422-30.
433. Urakawa H, Katsuki A, Sumida Y, Gabazza EC, Murashima S, Morioka K, et al. Oxidative stress is associated with adiposity and insulin resistance in men. *J Clin Endocrinol Metab* 2003;88(10):4673-6.
434. Lyon CJ, Law RE, Hsueh WA. Minireview: adiposity, inflammation, and atherogenesis. *Endocrinology* 2003;144(6):2195-200.
435. Wisse BE. The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. *J Am Soc Nephrol* 2004;15(11):2792-800.
436. Pantsulaia I, Trofimova S, Kobylansky E, Livshits G. Relationship between obesity, adipocytokines, and blood pressure: possible common genetic and environmental factors. *Am J Hum Biol* 2009;21(1):84-90.

437. Soares AF, Guichardant M, Cozzone D, Bernoud-Hubac N, Bouzaidi-Tiali N, Lagarde M, et al. Effects of oxidative stress on adiponectin secretion and lactate production in 3T3-L1 adipocytes. *Free Radic Biol Med* 2005;38(7):882-9.
438. Quinn LS, Strait-Bodey L, Anderson BG, Argiles JM, Havel PJ. Interleukin-15 stimulates adiponectin secretion by 3T3-L1 adipocytes: evidence for a skeletal muscle-to-fat signaling pathway. *Cell Biol Int* 2005;29(6):449-57.
439. Kobayashi K. Adipokines: therapeutic targets for metabolic syndrome. *Curr Drug Targets* 2005;6(4):525-9.
440. Weiland F, Verspohl EJ. Variety of Angiotensin Receptors in 3T3-L1 Preadipose Cells and Differentiated Adipocytes. *Horm Metab Res* 2008;40(11):760-6.
441. Phillips MI, Speakman EA, Kimura B. Levels of angiotensin and molecular biology of the tissue renin angiotensin systems. *Regul Pept* 1993;43(1-2):1-20.
442. Lohmeier TE, Cowley AW, Jr., Trippodo NC, Hall JE, Guyton AC. Effects of endogenous angiotensin II on renal sodium excretion and renal hemodynamics. *Am J Physiol* 1977;233(5):F388-95.
443. Karlsson C, Lindell K, Ottosson M, Sjostrom L, Carlsson B, Carlsson LM. Human adipose tissue expresses angiotensinogen and enzymes required for its conversion to angiotensin II. *J Clin Endocrinol Metab* 1998;83(11):3925-9.
444. Rasouli N, Kern PA. Adipocytokines and the metabolic complications of obesity. *J Clin Endocrinol Metab* 2008;93(11 Suppl 1):S64-73.
445. Jiao P, Chen Q, Shah S, Du J, Tao B, Tzameli I, et al. Obesity-related upregulation of monocyte chemotactic factors in adipocytes: involvement of nuclear factor-kappaB and c-Jun NH2-terminal kinase pathways. *Diabetes* 2009;58(1):104-15.
446. Fasshauer M, Klein J, Kralisch S, Klier M, Lossner U, Bluher M, et al. Monocyte chemoattractant protein 1 expression is stimulated by growth hormone and interleukin-6 in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 2004;317(2):598-604.
447. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab* 2001;280(5):E745-51.

448. Ghanim H, Aljada A, Daoud N, Deopurkar R, Chaudhuri A, Dandona P. Role of inflammatory mediators in the suppression of insulin receptor phosphorylation in circulating mononuclear cells of obese subjects. *Diabetologia* 2007;50(2):278-85.
449. Xu ZG, Lanting L, Vaziri ND, Li Z, Sepassi L, Rodriguez-Iturbe B, et al. Upregulation of angiotensin II type 1 receptor, inflammatory mediators, and enzymes of arachidonate metabolism in obese Zucker rat kidney: reversal by angiotensin II type 1 receptor blockade. *Circulation* 2005;111(15):1962-9.
450. Kim CH, Vaziri ND, Rodriguez-Iturbe B. Integrin expression and H₂O₂ production in circulating and splenic leukocytes of obese rats. *Obesity (Silver Spring)* 2007;15(9):2209-16.
451. Boschmann M, Jordan J, Schmidt S, Adams F, Luft FC, Klaus S. Gender-specific response to interstitial angiotensin II in human white adipose tissue. *Horm Metab Res* 2002;34(11-12):726-30.
452. Zemel MB. Nutritional and endocrine modulation of intracellular calcium: implications in obesity, insulin resistance and hypertension. *Mol Cell Biochem* 1998;188(1-2):129-36.
453. Engeli S, Gorzelniak K, Kreutz R, Runkel N, Distler A, Sharma AM. Co-expression of renin-angiotensin system genes in human adipose tissue. *J Hypertens* 1999;17(4):555-60.
454. Laursen JB, Rajagopalan S, Galis Z, Tarpey M, Freeman BA, Harrison DG. Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation* 1997;95(3):588-93.
455. Goossens GH, Blaak EE, Arner P, Saris WH, van Baak MA. Angiotensin II: a hormone that affects lipid metabolism in adipose tissue. *Int J Obes (Lond)* 2007;31(2):382-4.
456. Engeli S, Negrel R, Sharma AM. Physiology and pathophysiology of the adipose tissue renin-angiotensin system. *Hypertension* 2000;35(6):1270-7.
457. Giacchetti G, Faloia E, Sardu C, Camilloni MA, Mariniello B, Gatti C, et al. Gene expression of angiotensinogen in adipose tissue of obese patients. *Int J Obes Relat Metab Disord* 2000;24 Suppl 2:S142-3.
458. Jonsson JR, Game PA, Head RJ, Frewin DB. The expression and localisation of the angiotensin-converting enzyme mRNA in human adipose tissue. *Blood Press* 1994;3(1-2):72-5.

459. Crandall DL, Armellino DC, Busler DE, McHendry-Rinde B, Kral JG. Angiotensin II receptors in human preadipocytes: role in cell cycle regulation. *Endocrinology* 1999;140(1):154-8.
460. Wolf G. Free radical production and angiotensin. *Curr Hypertens Rep* 2000;2(2):167-73.
461. Mahadev K, Motoshima H, Wu X, Ruddy JM, Arnold RS, Cheng G, et al. The NAD(P)H oxidase homolog Nox4 modulates insulin-stimulated generation of H₂O₂ and plays an integral role in insulin signal transduction. *Mol Cell Biol* 2004;24(5):1844-54.
462. Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griending KK, et al. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest* 1996;97(8):1916-23.
463. Kim S, Dugail I, Standridge M, Claycombe K, Chun J, Moustaid-Moussa N. Angiotensin II-responsive element is the insulin-responsive element in the adipocyte fatty acid synthase gene: role of adipocyte determination and differentiation factor 1/sterol-regulatory-element-binding protein 1c. *Biochem J* 2001;357(Pt 3):899-904.
464. Storka A, Vojtassakova E, Mueller M, Kapiotis S, Haider DG, Jungbauer A, et al. Angiotensin inhibition stimulates PPARgamma and the release of visfatin. *Eur J Clin Invest* 2008;38(11):820-6.
465. Moan A, Hoiegggen A, Nordby G, Eide IK, Kjeldsen SE. Effects of losartan on insulin sensitivity in severe hypertension: connections through sympathetic nervous system activity? *J Hum Hypertens* 1995;9 Suppl 5:S45-50.
466. Paolisso G, Tagliamonte MR, Gambardella A, Manzella D, Gualdiero P, Varricchio G, et al. Losartan mediated improvement in insulin action is mainly due to an increase in non-oxidative glucose metabolism and blood flow in insulin-resistant hypertensive patients. *J Hum Hypertens* 1997;11(5):307-12.
467. Shimamoto K, Ura N, Nakagawa M, Higashiura K, Takizawa H, Miyazaki Y, et al. The mechanisms of the improvement of insulin sensitivity by angiotensin converting enzyme inhibitor. *Clin Exp Hypertens* 1996;18(2):257-66.
468. Hattori Y, Akimoto K, Gross SS, Hattori S, Kasai K. Angiotensin-II-induced oxidative stress elicits hypoadiponectinaemia in rats. *Diabetologia* 2005;48(6):1066-74.

469. Yan WJ, Wu J, Mo J, Huang CW, Peng LW, Xu L. [Plasma levels of adiponectin and tumor necrosis factor- α in children with obesity.]. *Zhongguo Dang Dai Er Ke Za Zhi* 2009;11(1):47-50.
470. Iwasaki E, Suzuki H, Sugino Y, Iida T, Nishizawa T, Masaoka T, et al. Decreased levels of adiponectin in obese patients with gastroesophageal reflux evaluated by videosophagography: possible relationship between gastroesophageal reflux and metabolic syndrome. *J Gastroenterol Hepatol* 2008;23 Suppl 2:S216-21.
471. Mallow H, Trindl A, Loffler G. Production of angiotensin II receptors type one (AT1) and type two (AT2) during the differentiation of 3T3-L1 preadipocytes. *Horm Metab Res* 2000;32(11-12):500-3.
472. Schling P. Expression of angiotensin II receptors type 1 and type 2 in human preadipose cells during differentiation. *Horm Metab Res* 2002;34(11-12):709-15.
473. Matsushita K, Wu Y, Okamoto Y, Pratt RE, Dzau VJ. Local renin angiotensin expression regulates human mesenchymal stem cell differentiation to adipocytes. *Hypertension* 2006;48(6):1095-102.
474. Yvan-Charvet L, Even P, Bloch-Faure M, Guerre-Millo M, Moustaid-Moussa N, Ferre P, et al. Deletion of the angiotensin type 2 receptor (AT2R) reduces adipose cell size and protects from diet-induced obesity and insulin resistance. *Diabetes* 2005;54(4):991-9.
475. Darimont C, Vassaux G, Ailhaud G, Negrel R. Differentiation of preadipose cells: paracrine role of prostacyclin upon stimulation of adipose cells by angiotensin-II. *Endocrinology* 1994;135(5):2030-6.
476. Aubert J, Ailhaud G, Negrel R. Evidence for a novel regulatory pathway activated by (carba)prostacyclin in preadipose and adipose cells. *FEBS Lett* 1996;397(1):117-21.
477. Chen R, Mukhin YV, Garnovskaya MN, Thielen TE, Iijima Y, Huang C, et al. A functional angiotensin II receptor-GFP fusion protein: evidence for agonist-dependent nuclear translocation. *Am J Physiol Renal Physiol* 2000;279(3):F440-8.
478. Conchon S, Monnot C, Teutsch B, Corvol P, Clauser E. Internalization of the rat AT1a and AT1b receptors: pharmacological and functional requirements. *FEBS Lett* 1994;349(3):365-70.
479. Crandall DL, Herzlinger HE, Saunders BD, Armellino DC, Kral JG. Distribution of angiotensin II receptors in rat and human adipocytes. *J Lipid Res* 1994;35(8):1378-85.

480. Ciuffo GM, Saavedra JM. Selective peptide and nonpeptide ligands differentially bind to angiotensin II AT2 receptor and a non-angiotensin II CGP42112 binding site. *J Pharmacol Exp Ther* 1995;274(3):1129-34.
481. Speth RC, Thompson SM, Johns SJ. Angiotensin II receptors. Structural and functional considerations. *Adv Exp Med Biol* 1995;377:169-92.
482. Tuccinardi T, Calderone V, Rapposelli S, Martinelli A. Proposal of a new binding orientation for non-peptide AT1 antagonists: homology modeling, docking and three-dimensional quantitative structure-activity relationship analysis. *J Med Chem* 2006;49(14):4305-16.
483. Widdowson PS, Renouard A, Vilaine JP. Binding of [3H]angiotensin II and [3H]DuP 753 (Losartan) to rat liver homogenates reveals multiple sites. Relationship to AT1a- and AT1b-type angiotensin receptors and novel nonangiotensin binding sites. *Peptides* 1993;14(4):829-37.
484. Grove KL, Speth RC. Angiotensin II and non-angiotensin II displaceable binding sites for [3H]losartan in the rat liver. *Biochem Pharmacol* 1993;46(9):1653-60.
485. Ruiz-Ortega M, Lorenzo O, Ruperez M, Suzuki Y, Egido J. Angiotensin II activates nuclear transcription factor-kappaB in aorta of normal rats and in vascular smooth muscle cells of AT1 knockout mice. *Nephrol Dial Transplant* 2001;16 Suppl 1:27-33.
486. Brasier AR, Jamaluddin M, Han Y, Patterson C, Runge MS. Angiotensin II induces gene transcription through cell-type-dependent effects on the nuclear factor-kappaB (NF-kappaB) transcription factor. *Mol Cell Biochem* 2000;212(1-2):155-69.
487. Jamaluddin M, Meng T, Sun J, Boldogh I, Han Y, Brasier AR. Angiotensin II induces nuclear factor (NF)-kappaB1 isoforms to bind the angiotensinogen gene acute-phase response element: a stimulus-specific pathway for NF-kappaB activation. *Mol Endocrinol* 2000;14(1):99-113.
488. Han Y, Runge MS, Brasier AR. Angiotensin II induces interleukin-6 transcription in vascular smooth muscle cells through pleiotropic activation of nuclear factor-kappa B transcription factors. *Circ Res* 1999;84(6):695-703.
489. Ruiz-Ortega M, Lorenzo O, Suzuki Y, Ruperez M, Egido J. Proinflammatory actions of angiotensins. *Curr Opin Nephrol Hypertens* 2001;10(3):321-9.

490. Gallinat S, Busche S, Raizada MK, Sumners C. The angiotensin II type 2 receptor: an enigma with multiple variations. *Am J Physiol Endocrinol Metab* 2000;278(3):E357-74.
491. Cao Z, Dean R, Wu L, Casley D, Cooper ME. Role of angiotensin receptor subtypes in mesenteric vascular proliferation and hypertrophy. *Hypertension* 1999;34(3):408-14.
492. Booz GW, Baker KM. Role of type 1 and type 2 angiotensin receptors in angiotensin II-induced cardiomyocyte hypertrophy. *Hypertension* 1996;28(4):635-40.
493. Lopez JJ, Lorell BH, Ingelfinger JR, Weinberg EO, Schunkert H, Diamant D, et al. Distribution and function of cardiac angiotensin AT1- and AT2-receptor subtypes in hypertrophied rat hearts. *Am J Physiol* 1994;267(2 Pt 2):H844-52.
494. Otsuka S, Sugano M, Makino N, Sawada S, Hata T, Niho Y. Interaction of mRNAs for angiotensin II type 1 and type 2 receptors to vascular remodeling in spontaneously hypertensive rats. *Hypertension* 1998;32(3):467-72.
495. Tsutsumi Y, Matsubara H, Ohkubo N, Mori Y, Nozawa Y, Murasawa S, et al. Angiotensin II type 2 receptor is upregulated in human heart with interstitial fibrosis, and cardiac fibroblasts are the major cell type for its expression. *Circ Res* 1998;83(10):1035-46.
496. Siragy HM, de Gasparo M, Carey RM. Angiotensin type 2 receptor mediates valsartan-induced hypotension in conscious rats. *Hypertension* 2000;35(5):1074-7.
497. Pinaud F, Bocquet A, Dumont O, Retailleau K, Baufreton C, Andriantsitohaina R, et al. Paradoxical role of angiotensin II type 2 receptors in resistance arteries of old rats. *Hypertension* 2007;50(1):96-102.
498. Li JM, Shah AM. Mechanism of endothelial cell NADPH oxidase activation by angiotensin II. Role of the p47phox subunit. *J Biol Chem* 2003;278(14):12094-100.

Appendix

Figures

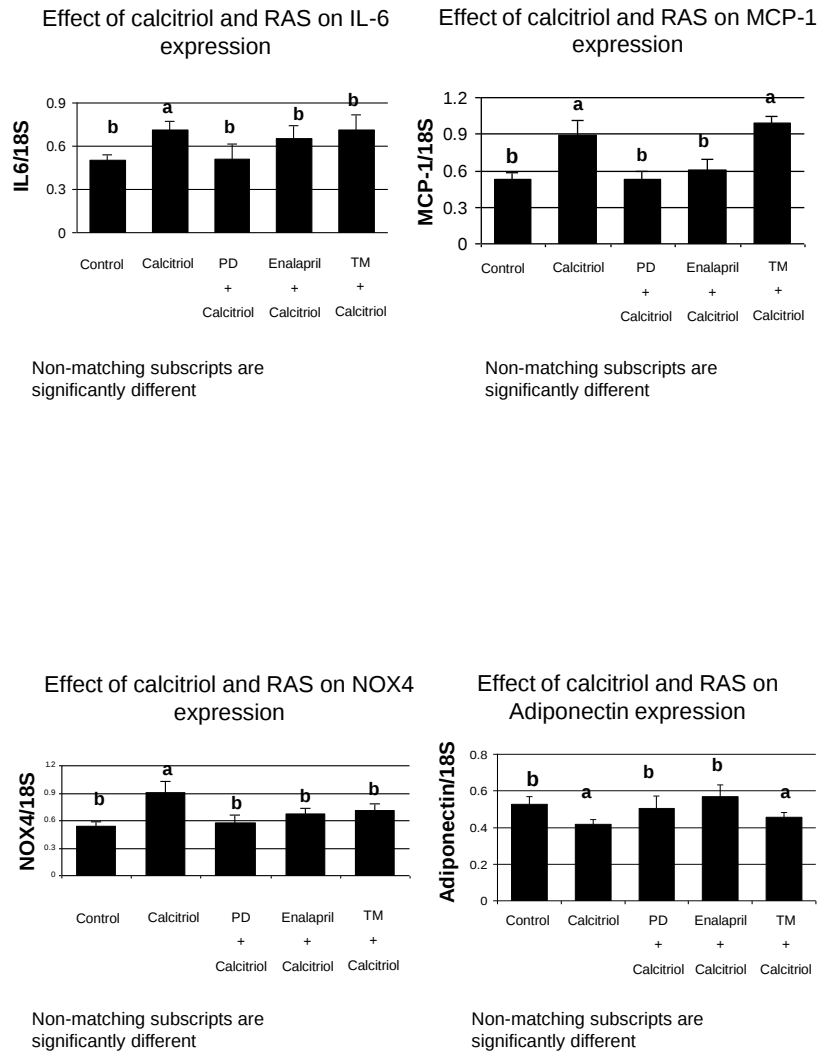


Figure 1. Effects of calcitriol and RAS on: IL-6 and 18S expression ratio (A), MCP-1 and 18S expression ratio (B), NOX4 and 18S expression ratio (C), Adiponectin and 18S expression ratio (D) with differentiated 3T3-L1 adipocytes.

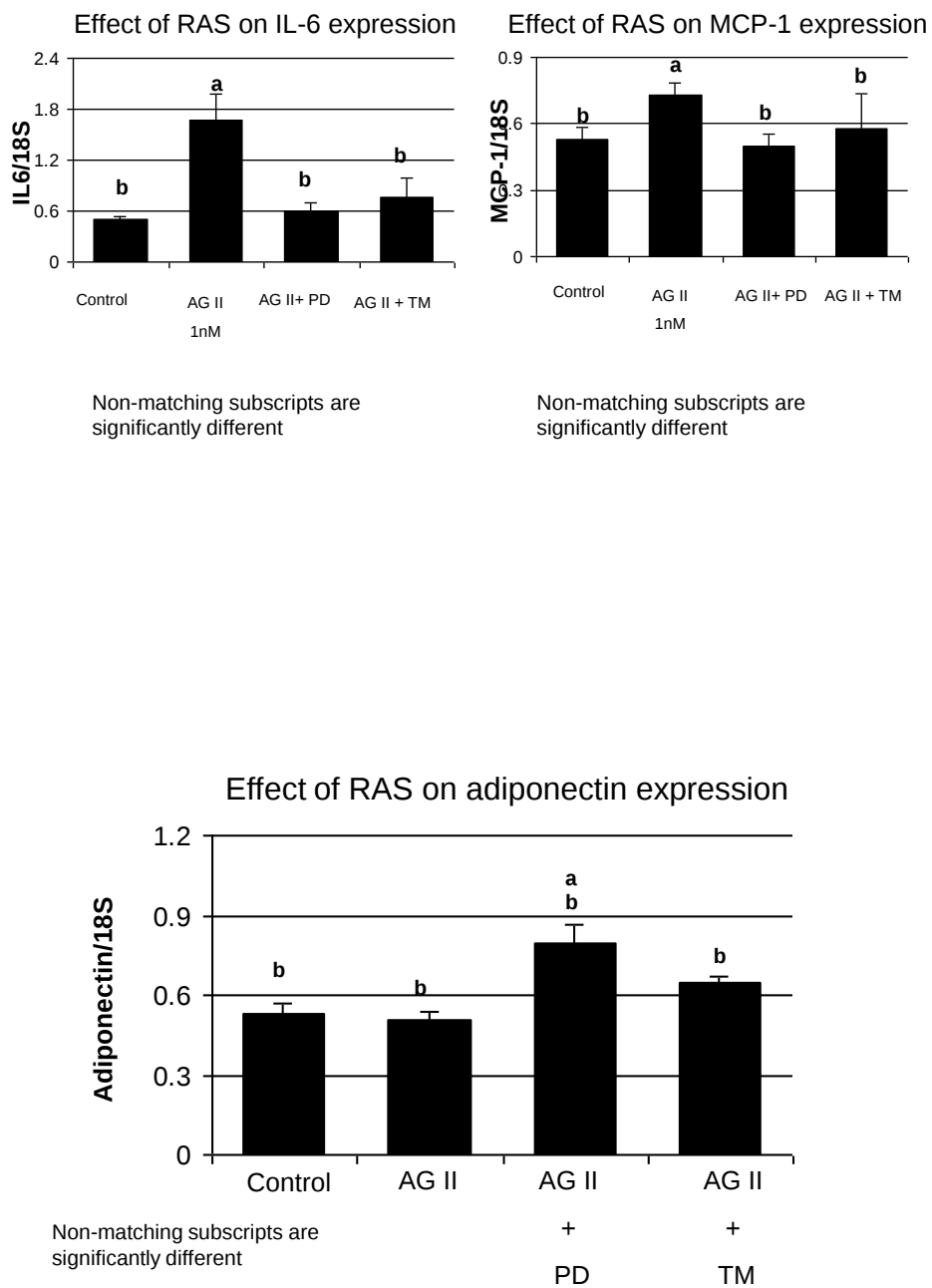


Figure 2. Effects of RAS on IL-6 and 18S expression ratio (A), MCP-1 and 18S expression ratio (B), Adiponectin and 18S expression ratio (C) in differentiated 3T3-L1 adipocytes.

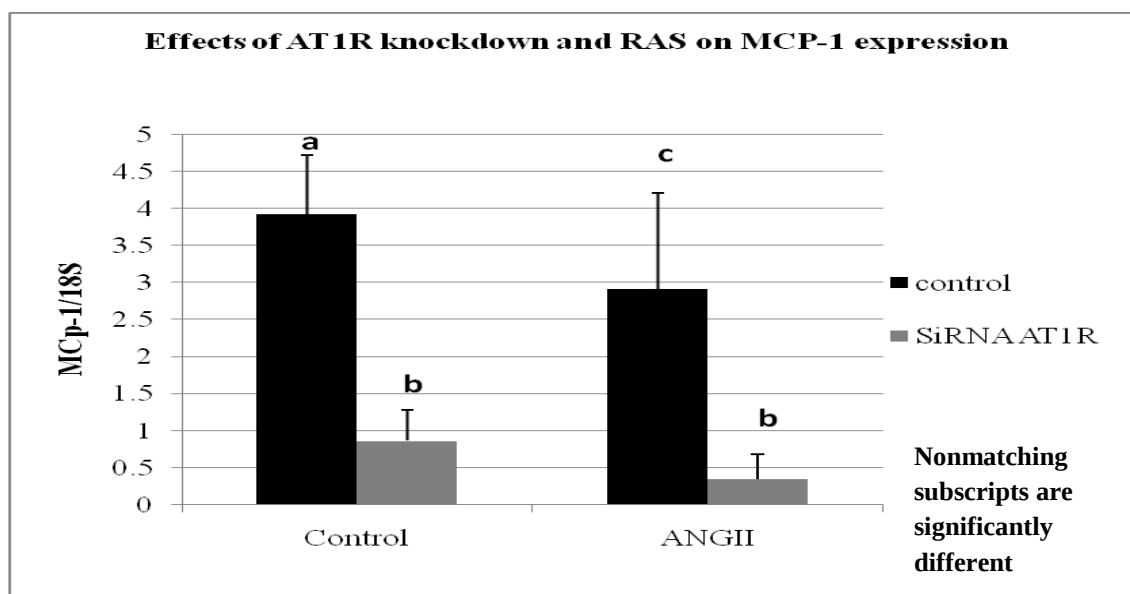
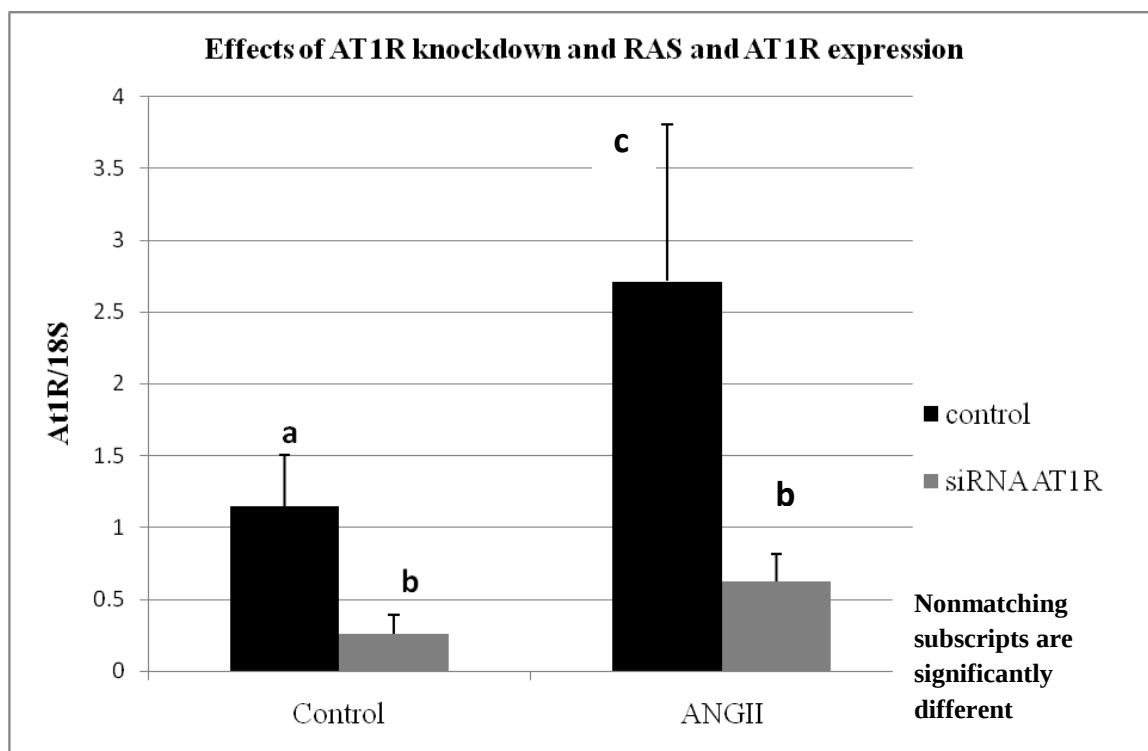


Figure 3(A and B). Effects of AT1R small interfering RNA knockdown and RAS on AT1R and 18S expression ratio (A) and MCP-1 and 18S expression ratio (B) in differentiated 3T3-L1 adipocytes.

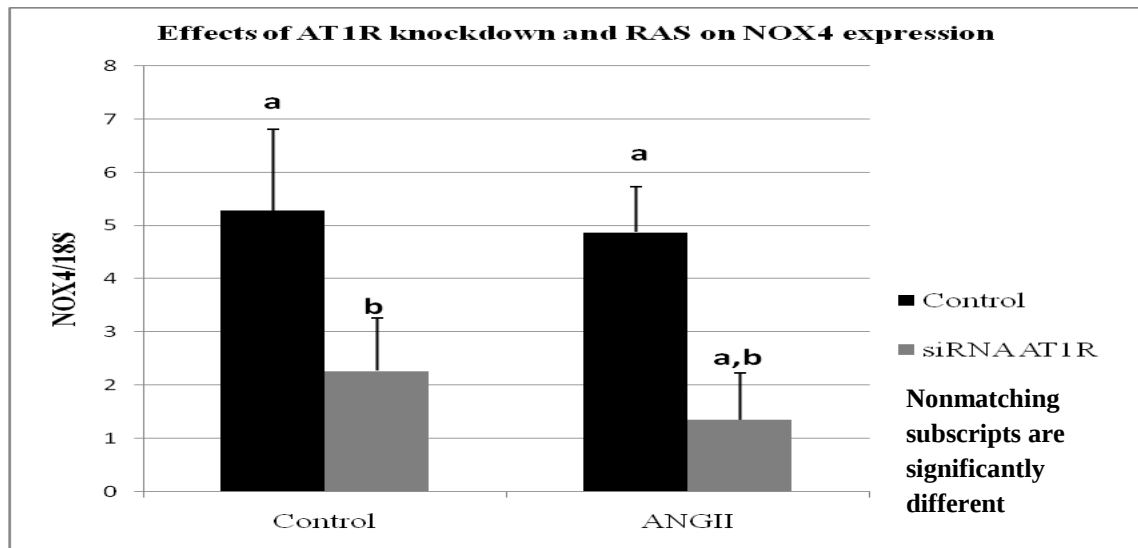


Figure 3C. Effects of AT1R small interfering RNA knockdown and RAS on NOX-4 and 18S expression ratio in differentiated 3T3-L1 adipocytes.

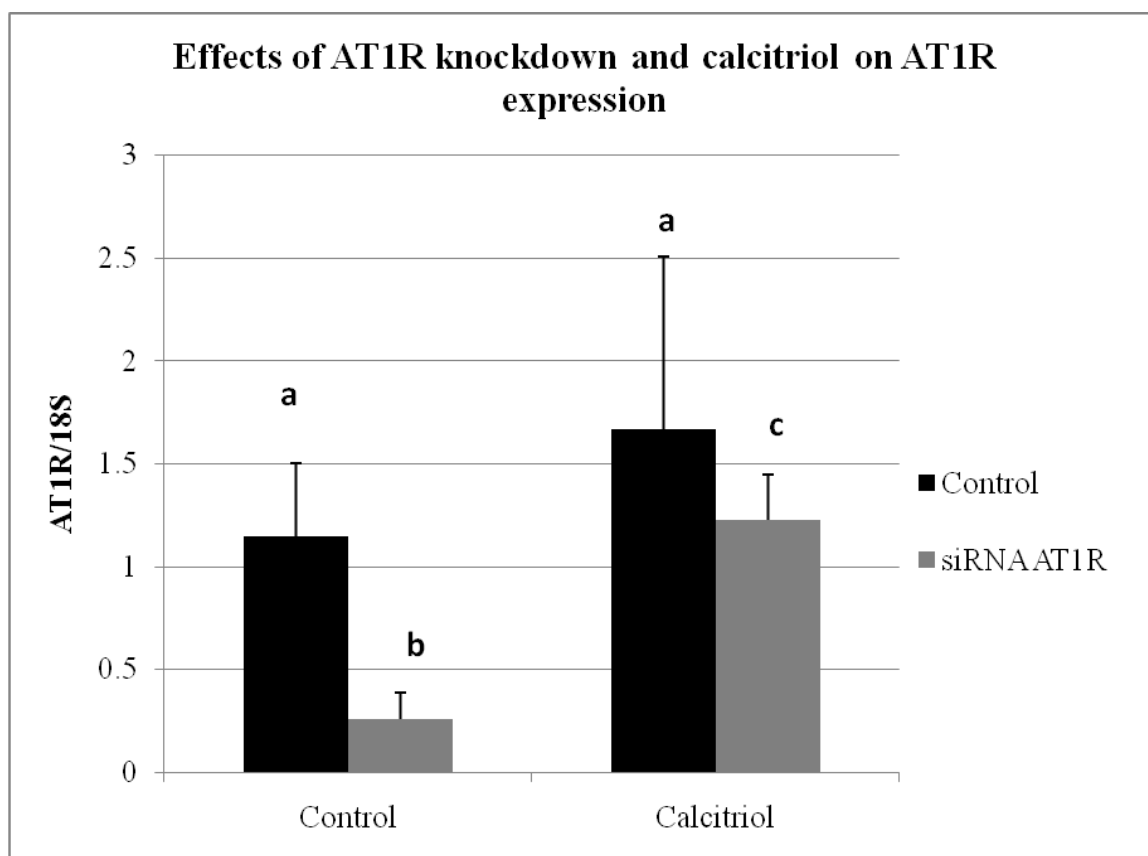


Figure 4A. Effects of AT1R small interfering RNA on AT1R and 18S expression ratio in differentiated 3T3-L1 adipocytes.

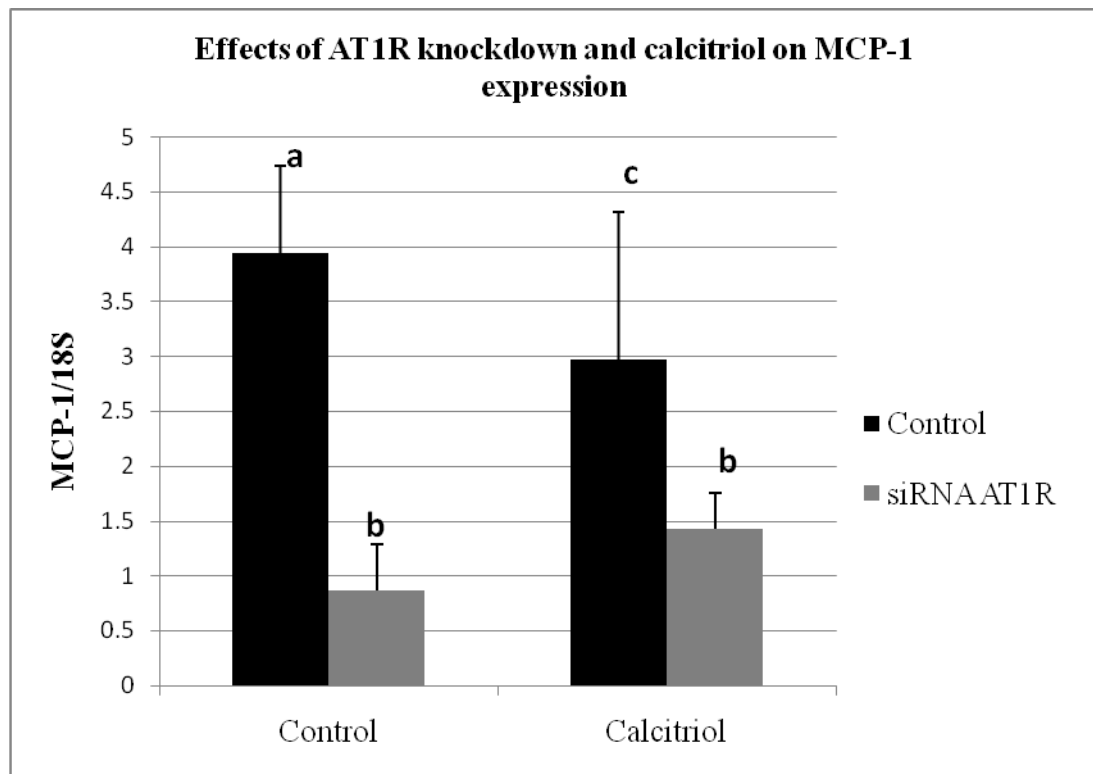


Figure 4B. Effects of AT1R small interfering RNA on MCP-1 and 18S expression ratio in differentiated 3T3-L1 adipocytes.

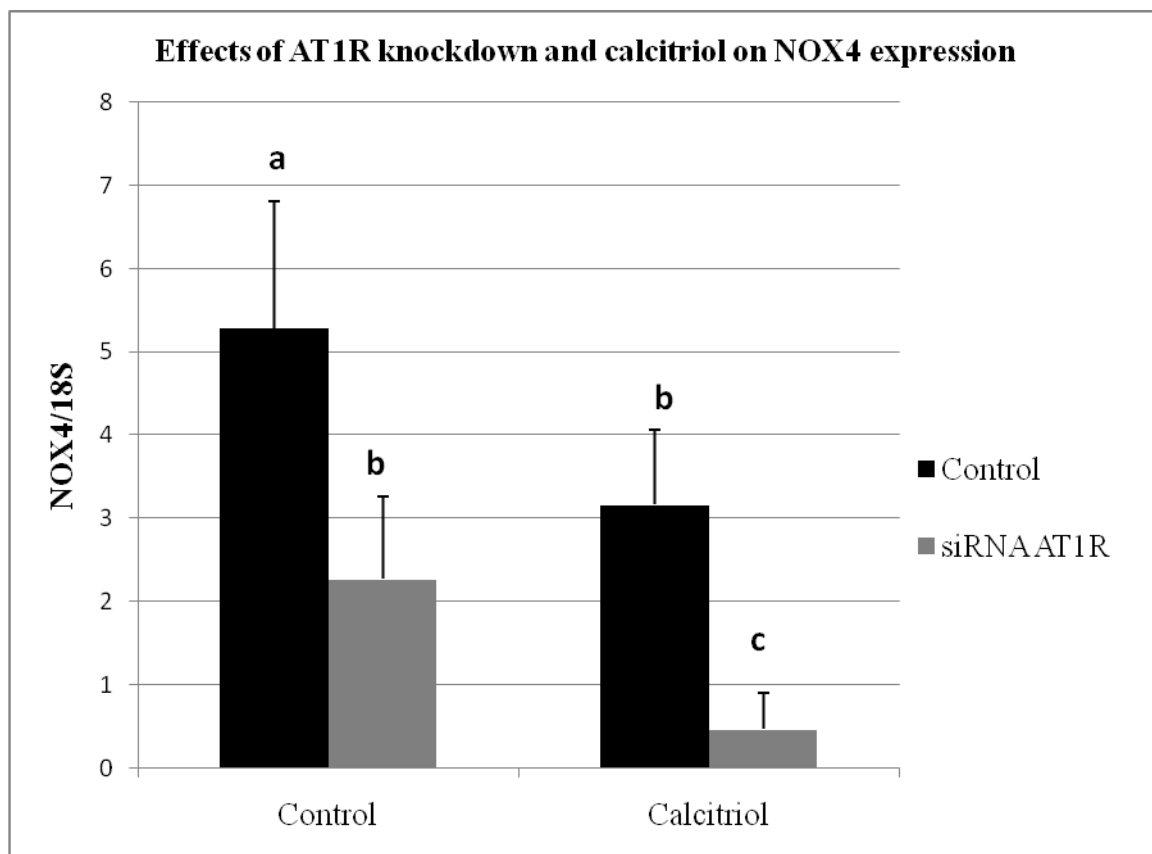


Figure 4C. Effects of AT1R small interfering RNA on NOX-4 and 18S expression ratio in differentiated 3T3-L1 adipocytes.

Vita

Christina Marie Caserio was born in Pittsburgh, PA on October 23, 1980. She was raised in Hendersonville, NC and went to grade school and junior high school at Immaculata in Hendersonville. She graduated from Hendersonville High School in 1999. From there, she went to the University of Tennessee, Knoxville and received a B.S. in psychology in 2005 and a M.S. in Nutrition in 2009. Christina is currently pursuing research in the field of biochemistry.