Western University

Scholarship@Western

2011

Effects of Neuropeptide Y on Adipocyte Metabolism

Raymond Li

Recommended Citation

Effects of Neuropeptide Y on Adipocyte Metabolism

(Thesis Format: Monograph)

by

Raymond Li

Graduate Program in Physiology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

THE UNIVERSITY OF WESTERN ONTARIO
SCHOOL OF GRADUATE AND POSTDOCTORAL STUDIES

CERTIFICATE OF EXAMINATION

Supervisor

_____
Dr. Kaiping Yang

Advisory Committee

_____
Dr. Dean Betts

_____
Dr. James Hammond

_____
Dr. Daniel Hardy

Examiners

_____
Dr. Timothy Regnault

_____
Dr. Nica Borradaile

_____
Dr. Edith Arany

The thesis by

Raymond Li

entitled:

Effects of Neuropeptide Y on Adipocyte Metabolism

is accepted in partial fulfillment of the
requirements for the degree of
Master of Science

Date_____   _____
                                    Chair of the Thesis Examination Board

ii

ABSTRACT

Recently, we have shown that neuropeptide Y (NPY) is produced and upregulated in visceral adipose tissue of an early-life programmed rat model of central obesity. Moreover, we have demonstrated that NPY contributes to the pathogenesis of obesity. However, the role of NPY in regulating adipocyte metabolism is poorly understood. The present study examined the effects of NPY on adipocyte metabolism using 3T3-L1 adipocytes. We found that NPY potentiated isoproterenol (β-adrenergic agonist) stimulated lipolysis. This potentiation occurred upstream of adenylyl cyclase, since NPY did not enhance forskolin (direct activator of adenylyl cyclase) stimulated lipolysis. The potentiation was mediated by increased phosphorylation of hormone sensitive lipase. In contrast, NPY did not alter the expression of several key lipolytic and lipogenic enzymes/proteins or glucose uptake. Our results revealed a novel cross talk between the NPY and β-adrenergic signaling pathways in regulating lipolysis and added a new dimension to the role NPY plays in regulating energy balance.

Keywords: neuropeptide y, adipocyte, lipolysis, lipogenesis, glucose uptake, potentiation, beta adrenergic receptor signalling, 3T3-L1, hormone sensitive lipase, isoproterenol

CO-AUTHORSHIP

This study was made possible with the knowledge and support of the following people:

Dr. K. Yang: Supervisor, provided support, advice and assistance with planning, critical thinking, presentations and writing

Dr. H. Guan: Lab Manager/Technician, provided support, technical advice, and assisted with laboratory techniques and troubleshooting

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

2-DOG	2-Deoxy-D-Glucose
α	alpha
ABHD5	α/β hydrolase domain-containing protein 5
AC	adenylyl cyclase
ATGL	adipose triglyceride lipase
ARC	arcuate nucleus
Asp	aspartate
β	beta
cAMP	cyclic adenosine monophosphate
CGI-58	comparative gene identification-58
CO_2	carbon dioxide
CoA	coenzyme-A
DMEM	Dulbecco's Modified Eagle's Medium
ERK	extracellular signal-regulated kinase
FAS	fatty acid synthase
FBS	fetal bovine serum
FSK	forskolin
g	grams
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GABA	gamma-Aminobutyric acid
GLUT	glucose transporter
^3H	tritium

His	histidine
HSL	hormone sensitive lipase
h	hour
ICV	intracerebroventricular
INS	insulin
ISO	isoproterenol
kg	kilogram
M	molar
MGL	monoglyceride lipase
min	minutes
ml	milliliter
mM	millimolar
MPR	maternal protein restriction
NADPH	nicotinamide adenine dinucleotide phosphate
NaOH	sodium hydroxide
NPY	neuropeptide Y
nM	nanomolar
PKA	protein kinase A
PP	phosphatase
PVDF	polyvinylidene fluoride
PVN	paraventricular nucleus
SCD1	stearoyl-CoA desaturase
Ser	Serine

SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	standard error of mean
TBS	Tris-buffered saline
TTBS	Tris-Tween buffered saline
U	units
μg	microgram
μl	microliter
μM	micromolar
VAT	visceral adipose tissue
Vol	volume
Wt	weight

Chapter 1

Introduction

1.1 Obesity Epidemic

Obesity is broadly defined as increased body weight caused by an excess accumulation of adipose tissue [Dixon, 2010]. Clinically, an individual with a body mass index of greater than 30 is considered obese. In recent years, the incidence and prevalence of obesity has increased at an alarming rate to epidemic levels in both developed and developing countries [James, 2008]. According to the 2004 Canadian Community Health Survey: Nutrition, 1 in 4 Canadians is obese and 59% of adult Canadians are overweight [CIHI, 2004]. Moreover, obesity rates are also rising among children [De Onis et al., 2010].

Obesity is a serious medical issue because it impairs quality of life and increases the risk for cardiovascular disease, metabolic syndrome, cancer and a host of other chronic illnesses [Dixon, 2010]. There are different types of obesity that have been defined based on the topography of fat deposition and studies *have shown that visceral or central obesity represents the greatest risk factor for the subsequent development of disease [Montague and O'Rahilly, 2000].* Currently, there is a lack of effective therapeutic interventions and thus, the primary focus is on prevention [Lang and Froelicher, 2006]. Altogether, there is a pressing need for more effective preventative and treatment options. However, a better understanding of the processes that underlie the development and maintenance of obesity are first required.

1.1.1 Etiology of Obesity

Obesity can result from an increase in cell size (hypertrophy), cell number (hyperplasia) or both [Jo et al., 2009]. Fundamentally, obesity is the result of a

chronic imbalance between energy storage and energy expenditure. However, it is a complex and multi-factorial disease because the interaction between genetic and environmental factors in numerous systems impacts energy balance [Hofbauer, 2002]. It is estimated that approximately 50% of the variation in body fat can be accounted for by genetic factors [Campfield and Smith, 1999] and a number of genes have been identified that are associated with the development of obesity including the genes that code for leptin and the fat mass and obesity-associated protein [Walley et al., 2009]. However, it is environmental and lifestyle factors that are responsible for the rapid increase in obesity worldwide [James, 2008].

1.2 Fetal Origins of Adult Disease

In recent years, one environmental influence that has gained increased interest is the *in utero* environment [Morley, 2006]. A number of studies have found a strong statistical association between poor fetal growth and the subsequent development of hypertension, insulin resistance, coronary heart disease and metabolic syndrome [Morley, 2006]. These observations were initially made by Barker and colleagues [Barker et al., 1993] . Since then, these finding has been replicated in a number of populations [Byrne and Phillips, 2000]. These studies have lead to the development of the fetal origins of adult disease hypothesis which postulates that a sub-optimal *in utero* environment leads to the programming of fetal tissues [Ozanne, 2001]. This programming leads to permanent alterations in metabolism and predisposes the individual to

developing disease later in life. Currently, the molecular mechanisms underpinning this programming are an area of intensive investigation.

A number of animal models have been developed to investigate the fetal origins of adult disease [Bertram and Hanson, 2001]. In these models, the effects of a variety of insults are explored including maternal malnutrition, maternal diabetes, hypoxia, maternal anaemia and overexposure to glucocorticoids. The maternal protein restriction (MPR) rat model is one of the most widely utilized models [Ozanne, 2001]. In this model, pregnant rat dams are fed an isocaloric low-protein diet consisting of 8% protein rather than the 20% protein found in the control diet [Holemans et al., 2003]. Dams are fed this diet throughout pregnancy and lactation and the resultant offspring are growth restricted. These MPR offspring go on to develop visceral obesity, hypertension, insulin resistance and metabolic syndrome. Overall, animal models provide strong experimental evidence supporting the epidemiological studies done in humans and will be valuable in uncovering the mechanisms linking insults to fetal development and adult disease [Bertram and Hanson, 2001].

1.2.1 Fetal Programming of Adipose Tissue

To date, a number of tissues have been shown to be structurally and functionally affected by *in utero* programming in humans and animal models [Byrne and Phillips, 2000; Bertram and Hanson, 2001]. These include alterations in the cardiovascular system, pancreas, muscle and liver. Given that MPR offspring develop visceral adiposity, our lab sought to investigate the underlying

molecular mechanisms [Guan et al., 2005]. To examine if adipose tissue plays a role in the fetal programming of obesity, our lab isolated visceral adipose tissue (VAT) from control and male MPR offspring and performed a microarray analysis. Analysis of the data revealed a distinct pattern of gene expression in the adipose tissue of MPR offspring [Guan et al., 2005]. The pattern of gene expression supported an increase in preadipocyte proliferation, adipocyte differentiation and lipogenesis. Furthermore, there was an upregulation in pro-angiogenic factors and angiogenesis is a prerequisite to adipose tissue expansion [Voros et al., 2005]. Taken together, the profile of gene expression supports an increase in adipogenesis and angiogenesis which explains the visceral adiposity of MPR offspring [Guan et al., 2005].

Further evidence that maternal protein restriction results in programming of adipose tissue comes from preadipocytes isolated from VAT of male MPR offspring [Zhang et al., 2007]. Our lab found that preadipocytes from MPR offspring displayed a two fold increase in the rate of proliferation. This increase seemed to be due to an inherent aberration because the increased proliferation persisted even in subculture. These findings along with our microarray data demonstrate that adipose tissue metabolism and development are permanently altered by maternal protein restriction [Guan et al., 2005; Zhang et al., 2007]. Therefore, programming of adipose tissue represents an important link between growth restriction and the development of obesity.

1.2.1.1 Candidate Gene Approach

To expand upon our findings, we took advantage of our previously published microarray data and used a candidate gene approach to select targets for further investigation. In our MPR rat model, male rats develop visceral obesity that is characterized by hyperplasia but not hypertrophy [Guan et al., 2005]. Accordingly, candidate genes were selected based on the criteria that they 1) are known to stimulate cell proliferation of nonadipose cells and 2) their expression is upregulated in VAT from our animal model. A candidate gene that was identified was neuropeptide Y (NPY) which stimulates proliferation in different cell types [Hansel et al., 2001; Pons et al., 2003] and is upregulated by 6-fold in our early life rat model of visceral obesity [Guan et al., 2005].

1.3 Neuropeptide Y

NPY was first isolated from porcine brain [Tatemoto et al., 1982] and belongs to a family of structurally related peptides, which includes peptide YY and pancreatic polypeptide [Silva et al., 2002]. The common structural feature of these peptides is a tertiary three-dimensional pancreatic polypeptide fold. NPY is synthesized as a prepropeptide that undergoes posttranslational processing which results in the active 36 amino acid peptide. Functions of NPY are mediated by five distinct receptor subtypes (known as Y_1, Y_2, Y_4, Y_5, and Y_6), which all belong to the G protein-coupled receptor superfamily [Yulyaningsih et al., 2011]. The NPY receptors are coupled to pertussis toxin sensitive G_i or G_0 heterotrimeric G-proteins. When activated, they induce a decrease in the

intracellular concentration of cAMP and/or an increase in intracellular calcium concentration depending on the cell type. There are a number of specific pharmacological agonists and antagonists that have been developed that have high affinities for the various receptor subtypes [Kamiji and Inui, 2007]. Additionally, mice in which NPY and specific NPY receptors are knocked out have been generated [Thorsell and Heilig, 2002]. NPY and receptor knockouts are grossly normal which suggests a redundancy of the system or the presence of compensatory mechanisms. However, the combination of knockout models and specific pharmacological tools has been valuable to study NPY's numerous physiological functions and elucidate the receptor subtypes involved [Yulyaningsih et al., 2011].

1.3.1 Central Effects of NPY

NPY is one of the most abundant neuropeptides in the brain and the highest levels are found in the hypothalamus, particularly in the arcuate nucleus (ARC) and paraventricular nucleus (PVN) [Higuchi et al., 1988]. It is also highly expressed in the cerebral cortex and moderate levels are found in the amygdala, hippocampus and basal ganglia [Thorsell and Heilig, 2002]. Corresponding to its widespread distribution, NPY has been implicated in regulating a number of physiological functions in the central nervous system [Silva et al., 2002; Pedrazzini et al., 2003]. Studies have found that NPY decreases anxiety in a number of animal models, reduces seizure activity, inhibits alcohol consumption and plays a role in memory retention.

One of the most well studied roles for NPY is in the central regulation of appetite [Kalra and Kalra, 2003]. NPY is the most potent appetite stimulating hormone in the brain and the drive to feed is mediated by the ARC and PVN. NPY expression in the PVN and ARC is increased during fasting and decreases after feeding [Kalra et al., 1991]. Intracerebroventricular (ICV) administration of NPY to mice and rats produces hyperphagia [Raposinho et al., 2001]. When administration is maintained, animals become obese and develop metabolic syndrome. Furthermore, hypothalamic NPY expression is increased in fatty Zucker rats and in the leptin-deficient ob/ob mouse model of obesity [Silva et al., 2002]. Knockout of NPY in ob/ob mice was found to attenuate the obese phenotype indicating that leptin's physiological action is facilitated in part by suppression of NPY expression [Erickson et al., 1996]. In addition to its direct orexigenic effects, NPY infused centrally was found to decrease the expression of anorexigenic signals that produce satiety [Raposinho et al., 2001]. Overall, hypothalamic NPY plays a critical role in the encoding of appetite and increased expression leads to the development of obesity and metabolic syndrome.

1.3.2 NPY and Adipose Tissue

1.3.2.1 Production of NPY by Adipose Tissue

Classically, NPY was thought to contribute to the pathogenesis of obesity mainly through its potent stimulation of appetite in the hypothalamus. Recently, this dogma has been challenged by the identification of adipose tissue as a novel

peripheral site of NPY biosynthesis by us [Yang et al., 2008] and others [Kos et al., 2007; Kuo et al., 2007]. In addition to being upregulated in VAT of our early-life model of visceral obesity, our lab found that NPY expression was upregulated in VAT of obese Zucker rats [Yang et al., 2008]. Furthermore, Kuo and colleagues reported a similar upregulation of NPY expression in adipose tissue of a diet and cold stress induced mouse model of obesity [Kuo et al., 2007]. Collectively, the upregulation of NPY expression in adipose tissue of several distinct rodent models of obesity underscores an important peripheral role for NPY in the pathogenesis of obesity.

1.3.2.2 NPY and Adipose Tissue Expansion

In support of a peripheral role for NPY in the development of obesity, an NPY implant in adipose tissue was found to increase both its weight and volume [Kuo et al., 2007]. In the same study, Kuo *et al.* found that NPY stimulated angiogenesis in adipose tissue and the formation of new blood vessels is a requirement for adipose tissue expansion. Furthermore, our lab found that NPY strongly stimulated 3T3-L1 preadipocyte proliferation through the Y_1 receptor and subsequent activation of the extracellular related kinase 1/2 pathway [Yang et al., 2008]. Since mature adipocytes do not multiply, the proliferation of adipocyte precursor cells is another critical pre-requisite to adipose tissue expansion [Otto and Lane, 2005]. Finally, several studies using isolated adipocytes from mice, rat, dog and humans have shown that NPY inhibits lipolysis [Valet et al., 1990; Castan et al., 1994; Serradeil-Le Gal et al., 2000]. However, the mechanisms of

action require further investigation and these systems commonly suffer from the drawback of effects due to cell breakage [Large et al., 2004]. Overall, the effects of NPY in adipose tissue and its roles in regulating adipocyte metabolism are still poorly understood and need to be explored.

1.4 Metabolic Functions of Adipocytes

1.4.1 3T3-L1 Cell Line

A number of immortalized cell lines have been used to study adipocyte differentiation and physiology [Gregoire et al., 1998]. 3T3-L1 cells are mouse embryonic fibroblasts that were clonally isolated from 3T3 cells derived from disaggregated embryos [Green and Kehinde, 1974]. These preadipocytes are committed to the adipocyte lineage and when stimulated appropriately undergo terminal differentiation into mature adipocytes. 3T3-L1 adipocytes have the biochemical and morphological properties of adipocytes [MacDougald and Lane, 1995]. They have been found to faithfully recapitulate the properties of adipocytes isolated from adipose tissue and are a well established *in vitro* model system to study adipocyte physiology and metabolism.

1.4.2 Lipolysis

Adipose tissue is the largest store of reserve energy in the body [Zechner et al., 2009]. During times of energy deficit, energy stored in adipocytes primarily in the form of triglycerides can be mobilized to meet energy requirements. Lipolysis is the process of energy mobilization and involves the hydrolysis of triglycerides releasing glycerol and free fatty acids for utilization by other tissues.

Adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL) and monoglyeride lipase (MGL) are responsible for the three hydrolytic reactions in the lipolysis reaction [Duncan et al., 2007]. In addition, a number of other proteins such as perilipin and adipocyte lipid binding protein are required in facilitating lipolysis.

1.4.2.1 Components of the Lipolytic Pathway

1.4.2.1.1 Adipose Triglyceride Lipase

ATGL is a recently discovered triglyceride hydrolase that is associated with lipid droplets [Zimmermann et al., 2004]. ATGL plays a crucial role in the lipolytic pathway since ATGL deficient mice become obese and have a 75% reduction in fat cell lipolysis [Haemmerle et al., 2006]. It has high substrate specificity for triacylglycerol and is responsible for catalyzing the first hydrolysis reaction producing diacylglycerol and a free fatty acid. To perform its lipolytic function, ATGL requires a co-activator known as α/β hydrolase domain-containing protein 5 (ABHD5), also known as comparative gene identification-58 (CGI-58) [Zechner et al., 2009]. The presence of ABHD5 increased mouse ATGL activity by approximately 20 fold and increased activation of human ATGL by 5 fold. When ABHD5 expression is silenced in human fat cells, the lipolytic action of ATGL is abolished. Moreover, it has been shown that ATGL activity is associated with its expression and overexpression of ATGL in a mouse fat cell line increased lipolysis while its knockdown decreased lipolysis [Lafontan and Langin, 2009]. Importantly, ATGL expression has been shown to be affected by

several hormones including insulin and tumor necrosis factor-alpha [Kralisch et al., 2005]. However, given its recent discovery, there is still much work to be done on investigating the regulation of ATGL activity.

1.4.2.1.2 Hormone Sensitive Lipase

HSL is expressed in a number of tissues including muscle, pancreatic β-cells, macrophages and white adipose tissue [Lafontan and Langin, 2009]. In adipose tissue, it plays a crucial role in lipid metabolism and is an important regulatory step in the lipolytic pathway [Holm et al., 2000b]. HSL possesses broad substrate specificity and is able to hydrolyze triacylglycerol, diacylglycerol, monoacylglycerol, steroid fatty acid esters and retinyl esters. In terms of triglyceride lipolysis, it has a much higher hydrolase activity against diacylglycerol compared to triacylglycerol [Schweiger et al., 2006]. The structure of HSL is composed of at least two domains [Holm et al., 2000b]. The N-terminal domain has been proposed to play a role in binding to lipid droplets and interaction with other protein partners. The C-terminal catalytic domain contains the catalytic site and also includes a regulatory module that has four phosphorylation sites.

1.4.2.1.2.1 Regulation of HSL Activity

HSL contains a regulatory module that has a number of serine residues that can be phosphorylated by different kinases [Lafontan and Langin, 2009]. Ser-563, Ser-659, and Ser-660 are targets of protein kinase A (PKA) and phosphorylation of these residues leads to an increase in HSL activity. In

contrast, Ser-565 is phosphorylated by AMP-activated protein kinase which sterically inhibits phosphorylation of Ser-563 and therefore decreases HSL activity [Carmen and Víctor, 2006]. To date, two phosphatases (PP) PP2A and PP1 are believed to play a role in the dephosphorylation of HSL [Lafontan and Langin, 2009]. Altogether, HSL enzyme activity can be controlled through its phosphorylation status.

In addition to phosphorylation, HSL function is also regulated by its cellular localization [Wang et al., 2009].Under basal conditions, HSL is primarily localized to the cytosol of adipocytes. However, upon beta-adrenergic stimulation and HSL phosphorylation, HSL translocates to the surface of lipid droplets. HSL subsequently binds to proteins at the surface of the lipid droplet which further activates HSL and facilitates its access to the lipids contained within the droplet. This translocation process is critical to the lipolytic function of HSL but is still poorly understood.

1.4.2.1.3 Monoglyceride Lipase

Monoglyceride lipase (MGL) is responsible for catalyzing the final hydrolysis reaction in the lipolytic process [Duncan et al., 2007]. It has high substrate specificity and only has catalytic activity against monoacylglycerols. The catalytic site of MGL including the catalytic triad has been identified and mutation of Ser^{122}, Asp^{239} or His^{269} abolishes lipase activity. However, MGL is expressed at high levels in adipocytes and is thought to not be rate limiting because of its abundance.

1.4.2.1.4 Perilipin

Perilipin is a protein that is abundantly expressed in adipocytes and coats the surface of lipid droplets [Martinez-Botas et al., 2000]. Perilipin has been found to be a critical regulator of lipolysis [Tansey et al., 2004]. Under basal conditions, perilipin restricts access of lipases to the triglycerides contained within the droplet. In addition, perilipin is bound to ABHD5 which sequesters it and keeps it from activating ATGL. Upon stimulation, perilipin is phosphorylated at multiple sites by PKA. Once perilipin is phosphorylated, the lipid droplet undergoes a structural remodelling and the central lipid droplet becomes fragmented into smaller micro droplets allowing for greater access of the lipases to their substrates [Brasaemle, 2007]. In addition, upon perilipin phosphorylation, ABHD5 is released and activates ATGL activity. Finally, perilipin facilitates the interaction between lipases and the lipid droplet by acting as a docking site [Tansey et al., 2004]. The study found that HSL translocation did not occur when PKA phosphorylation sites of perilipin were mutated. Thus, under basal conditions perilipin acts to inhibit lipolysis but under stimulated conditions, perilipin is critical for mediating lipolysis and is required to achieve maximal lipolytic rates.

1.4.2.2 Beta-adrenergic Stimulated Lipolysis

In terms of regulation, there are a number of hormones and signalling molecules that form a complex network and allows for the precise control of the lipolytic rate [Lafontan and Langin, 2009]. Catecholamines are the most

important lipolytic stimulus *in vivo* and stimulate lipolysis through beta-adrenergic receptors [Coleman and Mashek, 2011]. Murine adipose tissue expresses β1, β2 and β3 adrenergic receptors while human adipose tissue only possesses β1 and β2 receptors [Langin, 2006]. β-adrenergic receptors in adipose tissue are coupled to the Gs heterotrimeric G-protein. Figure 1.1 illustrates the stepwise hydrolysis of triglyceride and the signalling events that follow β-adrenergic receptor activation.

White adipose tissue is innervated by the sympathetic nervous system and the primary postganglionic neurotransmitter is norepinephrine [Bartness and Bamshad, 1998]. In isolated fat pads from rats, stimulation of these nerves promotes lipid mobilization, and denervation of fat pads has been found to decrease lipolysis. Besides sympathetic stimulation, adipose tissue is highly vascularized [Lijnen, 2008]. During fasting, the levels of circulating catecholamines increases, and stimulates energy mobilization from adipocytes.

Noradrenaline
Adrenaline

β₁/β₂-AR
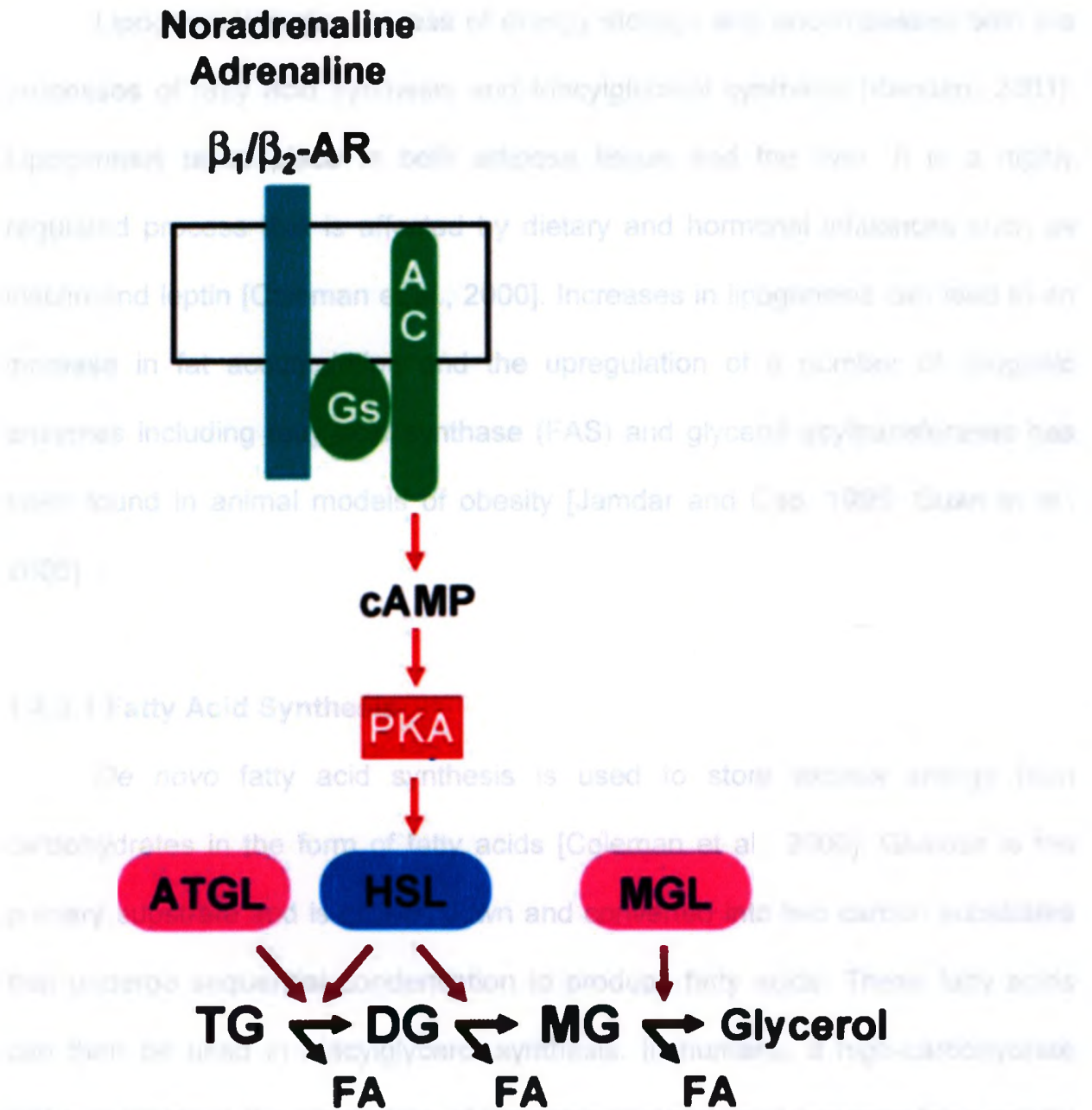
Figure 1.1 Overview of beta-adrenergic stimulated lipolysis.
Catecholamine binding to β-adrenergic receptors coupled to Gs heterotrimeric G-proteins activates adenylyl cyclase activity leading to an increase in intracellular cAMP and subsequent PKA activity. PKA phosphorylates a number of downstream targets including HSL. ATGL, HSL and MGL catalyze the complete hydrolysis of triglycerides leading to the release of free fatty acids and glycerol. (adapted from Langin, 2006)

1.4.3 Lipogenesis

Lipogenesis is the process of energy storage and encompasses both the processes of fatty acid synthesis and triacylglycerol synthesis [Kersten, 2001]. Lipogenesis takes place in both adipose tissue and the liver. It is a highly regulated process that is affected by dietary and hormonal influences such as insulin and leptin [Coleman et al., 2000]. Increases in lipogenesis can lead to an increase in fat accumulation and the upregulation of a number of lipogenic enzymes including fatty acid synthase (FAS) and glycerol acyltransferases has been found in animal models of obesity [Jamdar and Cao, 1995; Guan et al., 2005].

1.4.3.1 Fatty Acid Synthesis

De novo fatty acid synthesis is used to store excess energy from carbohydrates in the form of fatty acids [Coleman et al., 2000]. Glucose is the primary substrate and is broken down and converted into two carbon substrates that undergo sequential condensation to produce fatty acids. These fatty acids can then be used in triacylglycerol synthesis. In humans, a high-carbohydrate diet can increase the expression of lipogenic enzymes and increase their activity [Kersten, 2001]. This is important because the Western "cafeteria diet" is rich in both carbohydrates and fat.

1.4.3.1.1 Fatty Acid Synthase

Fatty acid synthase is the central enzyme responsible for the *de novo* synthesis of fatty acids [Smith et al., 2003]. It functions as a homodimer and is a

multifunctional enzyme that catalyzes all of the reactions necessary to produce fatty acids. The FAS gene encodes one long polypeptide chain that contains seven catalytic domains that are connected by linkers. The primary product of FAS is the 16-carbon fatty acid palmitate which is produced through the condensation of two carbon units provided by acetyl co-enzyme A (CoA) and malonyl-CoA, in the presence of NADPH. FAS expression is upregulated in genetically obese rats [Menendez et al., 2009] and one study found that lipogenesis and FAS activity were coordinately regulated [Wang et al., 2004]. In human visceral adipose tissue, expression of FAS was positively correlated with visceral fat area and inversely correlated with insulin sensitivity [Berndt et al., 2007]. Altogether, these findings suggest a possible role for FAS in the pathogenesis of obesity and its related metabolic abnormalities.

1.4.3.1.2 Stearoyl-CoA desaturase 1

Stearoyl-CoA desaturase (SCD1) is an endoplasmic reticulum enzyme that is highly expressed in adipose tissue [Dobrzyn and Ntambi, 2004]. It introduces a single double bond into fatty acyl-CoA substrates which is the rate limiting step in the synthesis of monounsaturated fatty acids. Monounsaturated fatty acids comprise the majority of fatty acids found in triglycerides and phospholipids [Jiang et al., 2005]. Knockout of SCD1 results in mice that are lean and resistant to diet induced obesity [Dobrzyn and Ntambi, 2004]. This resistance was attributed to an increase in metabolism and a decrease in lipogenesis. Overall, SCD1 plays an important role in regulating lipid metabolism and SCD1

inhibition is currently being explored as a treatment for obesity [Dobrzyn and Ntambi, 2005].

1.4.3.2 Triacylglycerol Synthesis

Triacylglycerol synthesis requires glycerol and fatty acid chains which are first converted to glycerol-3-phosphate and fatty acyl-CoA respectively [Coleman and Mashek, 2011]. Glycerol-3-phosphate can be produced from glucose through glycolysis or produced from glyceroneogenesis [Large et al., 2004]. Fatty acids are primarily derived from dietary sources but are also produced by *de novo* fatty acid synthesis. Triacylglycerol synthesis involves the sequential esterification of fatty acyl-CoA to glycerol-3-phosphate [Takeuchi and Reue, 2009]. These reactions are catalyzed by a number of acyltransferases. Various knockout mice that are deficient in acyltransferase enzymes have been generated which results in reduced adipose tissue stores [Coleman and Mashek, 2011].

1.4.4 Adipose Tissue and Glucose Uptake

Glucose uptake is an important function of adipose tissue and studies have shown that glucose is an important regulator of adipocyte metabolism [Rosen and Spiegelman, 2006]. Adipocytes express both glucose transporter (GLUT) 1 and GLUT4 glucose transporters [Pedersen et al., 1992]. GLUT1 is responsible for basal glucose uptake while GLUT4 is involved in insulin stimulated uptake. In adipocytes, glucose is utilized in the synthesis of the

glycerol-3-phosphate backbone which is required for triglyceride synthesis and fat deposition. In addition, glucose is also used in *de novo* fatty acid synthesis. Glucose transport is the rate limiting factor in glucose utilization and plays an important role in the development of obesity [Tozzo et al., 1995]. In a transgenic mouse model where GLUT4 was overexpressed in adipocytes, there was a significant upregulation in the activity of several major metabolic pathways. In particular, *de novo* fatty acid synthesis was preferentially increased [Shepherd et al., 1993]. These mice become obese and the increased adipose tissue mass was characterized by adipocyte hyperplasia. During the early development of obesity, adipocytes from fatty Zucker rats have a significant upregulation of both GLUT1 and GLUT4 expression and concomitant increases in glucose uptake [Pedersen et al., 1992]. Furthermore, there was also a significant increase in the conversion of glucose to total lipids. Altogether, an increase in glucose uptake can increase adipocyte metabolic activity and lipogenesis which leads to increased fat accumulation.

1.4.4.1 Adipose Tissue and Glucose Homeostasis

An increase in glucose uptake can contribute to the development of obesity. However, once an obese state is reached, insulin resistance can develop in part through the increased secretion of adipokines such as tumour necrosis factor alpha [Rosen and Spiegelman, 2006]. Muscle, liver and adipose tissue are the major insulin sensitive tissues in the body and they all play an important role in whole body glucose homeostasis. Although, adipose tissue

accounts for only 10-15% of insulin-stimulated glucose uptake, there is accumulating evidence that it plays an important role in the development of insulin resistance. Insulin resistance is the earliest defect in the development of type 2 diabetes [Cline et al., 1999]. In insulin-resistant states, there is often impairment in GLUT4 expression in adipose tissue but not muscle [Shepherd and Kahn, 1999]. Furthermore, adipocyte specific knockout of GLUT4 gene expression was found to induce insulin resistance to a similar degree as a muscle specific ablation of GLUT4 [Abel et al., 2001]. From these findings, it has been hypothesized that insulin resistance first develops in adipose tissue which then spreads to muscle and liver.

1.5 Rationale and Hypothesis

Adipose tissue has recently been identified as a novel source of NPY biosynthesis [Kos et al., 2007; Kuo et al., 2007; Yang et al., 2008]. Its expression was dramatically upregulated in our early life rat model of visceral obesity [Guan et al., 2005], in the obese Zucker rat [Yang et al., 2008] and in a diet and stress induced mouse model of obesity [Kuo et al., 2007]. Moreover, NPY was shown to directly stimulate adipose tissue expansion [Kuo et al., 2007]. It is evident that NPY plays an important peripheral role in the pathogenesis of obesity.

Obesity results from a chronic energy imbalance that can occur through a decrease in lipolysis and/or an increase in lipogenesis. Furthermore, an increase in adipocyte glucose uptake can also lead to obesity. Since the mechanisms underlying NPY's contribution to obesity remain poorly understood, this study

investigated the hypothesis that NPY promotes the development of obesity by inhibiting lipolysis, increasing lipogenesis and increasing glucose uptake. The overall objective of this study was to determine the role of NPY in regulating major metabolic pathways in adipocytes.

Chapter 2

Materials and Methods

Chapter 2

Materials and Methods

2.1 3T3-L1 Culture and Differentiation

The murine preadipocyte 3T3-L1 cell line was obtained from the American Type Culture Collection (Manassas, VA). 3T3-L1 cells were cultured and differentiated, as previously described [Yang et al., 2008]. Briefly, they were cultured in growth medium, consisting of DMEM (Sigma) supplemented with 50 U/ml penicillin, 50 µg/ml streptomycin (Invitrogen) and 10% fetal bovine serum (Sigma). Cultures were maintained in a humidified incubator at 5% CO_2 and 37° C. Medium was replaced every other day. At 2 days after confluence (day 0), cells were induced to differentiate by the addition of a standard differentiation cocktail containing 500 µM 3-isobutyl-1-methylxanthine (Sigma), 0.25 µM dexamethasone (Alpharma, Boucherville, Quebec, Canada), and 1 µg/ml insulin (Eli Lilly Canada Inc., Toronto, Ontario, Canada). At day 2, the medium was replaced with growth medium supplemented with 1 µg/ml insulin and then replaced at days 4 and 6 with growth medium. At day 8, differentiation was 90% or higher, as determined by Oil red O staining (Fig. 2.1). All treatments were carried out on differentiated 3T3-L1 adipocytes and preceded by a 2 hour starving period during which cells were washed and incubated in serum-free medium. Each treatment was performed in triplicate under serum-free conditions, and a total of four to six independent experiments were carried out. For each treatment condition, controls were included and treated for the same time with an identical volume of the vehicle, as described in detail in figure legends.

Control

Differentiation Cocktail

Figure 2.1 Oil red O staining of 3T3-L1 cells. 3T3-L1 preadipocytes were cultured in standard growth medium until confluent. At 2 days after confluence (day 0), cells were induced to differentiate by the addition of a standard differentiation cocktail containing 500 µM 3-isobutyl-1-methylxanthine , 0.25 µM dexamethasone, and 1 µg/ml insulin. At day 2, the medium was replaced with growth medium supplemented with 1 µg/ml insulin and then replaced at days 4 and 6 with growth medium. At day 8, differentiation is determined by Oil red O staining. Microphotographs 10 X magnification.
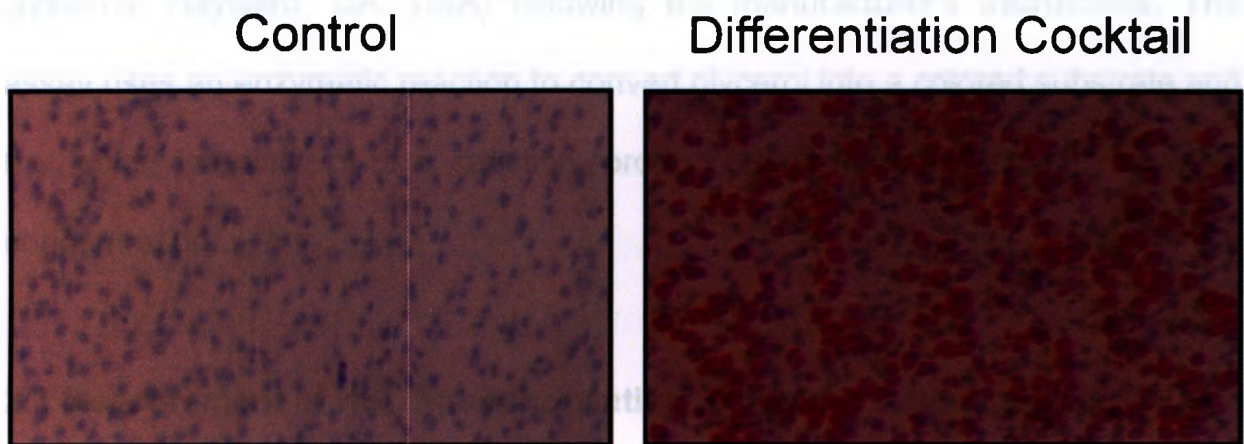
2.2 Assessment of Lipolysis

Differentiated 3T3-L1 adipocytes were pre-treated without or with 100 nM of NPY for 30 minutes. After 30 minutes, cells were treated without or with 10 nM of isoproterenol or with 50 nM of forskolin for 90 minutes. Medium was then collected and stored at -20°C. Glycerol released into the medium was assessed using a colorimetric assay (EnzyChrom™ Adipolysis Assay Kit, BioAssay Systems, Hayward, CA, USA) following the manufacturer's instructions. The assay uses an enzymatic reaction to convert glycerol into a colored substrate and the color intensity of the reaction product is proportional to the glycerol concentration.

2.3 Measurement of HSL Phosphorylation: Western Blotting

3T3-L1 adipocytes were pre-treated without or with 100 nM of NPY for 30 minutes. After 30 minutes, cells were treated without or with 10 nM of isoproterenol for 10 minutes. At the end of treatment, cells were lysed in SDS sample buffer (62.5 mM Tris-HCL, pH 6.8, 2% wt/vol SDS, 10% glycerol, 50mM dithiothreitol, and 0.01% wt/vol bromphenol blue) and stored at -80°C.

Western blotting was performed as described previously [Yang et al., 2008]. Briefly, equal volumes of cell lysate were subjected to a standard 10% SDS-PAGE. After electrophoresis, proteins were then transferred to a PVDF transfer membrane (Amersham Hybond™-P, GE Healthcare Canada, Baie D'Urfe, QC, Canada) using a Bio-Rad Mini Transfer Apparatus. The PVDF membrane was blocked for 1 h at room temperature with 5% milk in TTBS (0.05% Tween-20 in TBS) and then incubated with primary antibody in TTBS

overnight at 4°C. Phosphorylated HSL protein was detected using the Phospho-HSL (Ser563) Antibody (Cell Signaling; #4139; 1:1000 dilution). After three 10 min washes with TTBS, the membrane was incubated with Anti-Rabbit IgG-HRP (R&D Systems) secondary antibody (1:1000 dilution) and developed using chemiluminesence (Western Lightning™ Plus-ECL, PerkinElmer Life and Analytical Sciences). The membrane was then exposed to X-ray film (Eastman Kodak, Rochester, NY, USA). The membrane was stripped by incubation with stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, and 62.5 mM Tris-HCl pH 6.7) for 30 min at 55°C. After three 10 min washes in TTBS, the membrane was blocked as described above and re-probed to detect total HSL protein using the HSL Antibody (Cell signaling; #4107; 1:1000 dilution).

2.4 Measurement of Lipolytic and Lipogenic Proteins: Western Blotting

3T3-L1 adipocytes were treated with increasing concentrations of NPY for 12, 24 and 48 hours. At the end of treatment, cells were lysed in SDS sample buffer and stored at -80°C. Cell lysates were subjected to standard western blot analysis as described above. The primary antibodies were SCD1 antibody (Cell Signaling, Danvers, MA, USA; #2794), FAS antibody (Cell Signaling; #3819), perilipin antibody (Cell Signaling; #3470), HSL antibody (Cell Signaling; #4107), ATGL antibody (Cell Signaling; #2138), GAPDH antibody (Cell Signaling; #2118) and β-tubulin antibody (Imgenex Corp., San Diego, CA, USA; IMG-5810A). All primary antibodies were used at 1:1000 dilutions.

2.5 [1,2-^{3}H]2-Deoxy-D-Glucose (2-DOG) Uptake

Cells were washed twice with Krebs–Ringer-Hepes (KRH) buffer and incubated for 15 min without or with NPY (100 nM) and without or with Insulin (100 nM) in KRH buffer. Subsequently, 0.1 mM 2-DOG containing 0.5 µCi [1,2-^{3}H] 2-DOG (PerkinElmer Life and Analytical Sciences, Woodbridge, ON, Can) was added for 10 min. The reaction was terminated by addition of ice-cold phosphate buffered saline. Cells were washed three times with ice-cold phosphate buffered saline and then solubilized by the addition of 0.5M NaOH. The solubilized cell lysate was added to 4 ml of scintillation fluid, and the uptake of [1,2-^{3}H] 2-DOG was determined by liquid scintillation counting.

Chapter 3

Results

3.1 Effects of NPY on Lipolysis

Obesity results from a chronic imbalance between energy storage (i.e., lipogenesis) and energy expenditure (i.e., lipolysis) in adipose tissue. Since NPY expression is upregulated in visceral adipose tissue of our early-life programmed rat model of increased visceral adiposity [Yang et al., 2008], we hypothesized that NPY may act directly on adipocytes to inhibit lipolysis and/or stimulate lipogenesis thereby contributing to the pathogenesis of visceral obesity. As a first step in examining this hypothesis, we studied the effects of NPY on both basal and stimulated lipolysis. Since catecholamines are the major lipolytic stimuli *in vivo* and they signal through β-adrenergic receptors [Turtzo et al., 2001; Lafontan and Langin, 2009], we used isoproterenol, a well-known β-adrenergic agonist, to stimulate lipolysis. We showed that NPY had no effect on lipolysis under basal conditions. However, NPY potentiated isoproterenol-stimulated lipolysis by approximately 30% (Fig. 3.1A; $p < 0.05$).

The β-adrenergic receptors in adipocytes are coupled to the Gs heterotrimeric g-protein, which activates adenylyl cyclase resulting in an increase in intracellular levels of cAMP, leading to PKA activation, phosphorylation of downstream targets, and ultimately increased lipolysis [Duncan et al., 2007]. As a first step in deciphering the molecular mechanisms by which NPY potentiates β-adrenergic stimulation of lipolysis, we treated cells with forskolin, a direct activator of adenylyl cyclase. As shown in Fig. 3.1B, NPY treatment had no effect on forskolin stimulated lipolysis.
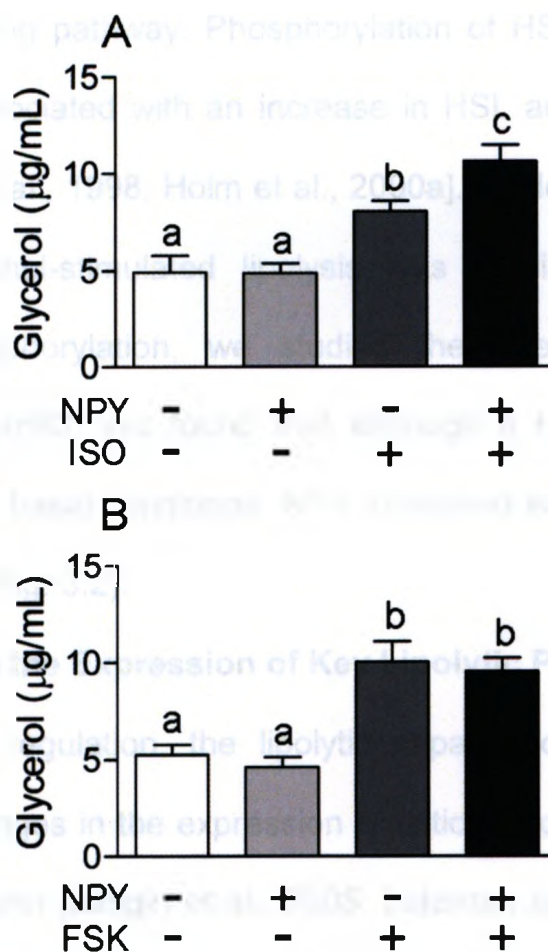
Figure 3.1 Effects of NPY on basal and stimulated lipolysis. Differentiated 3T3-L1 adipocytes were pretreated with NPY (100 nM) for 30 min, and were then treated with (**A**) isoproterenol (ISO; 10nM) or (**B**) forskolin (FSK; 50 nM) for 90 min. Controls were treated at the same time with an appropriate volume of vehicle. At the end of treatment, medium was collected, and lipolysis was determined by measuring glycerol released into the medium using a standard colorimetric assay. Data are presented as means ± SEM of four independent experiments, each performed in triplicate. a *vs.* b, P < 0.05; b *vs.* c, P < 0.05.

3.2 Effects of NPY on HSL Phosphorylation

HSL is the rate-limiting enzyme in lipolysis, and its activity is controlled by the cAMP/PKA signaling pathway. Phosphorylation of HSL by PKA at Ser563, Ser659, Ser660 is associated with an increase in HSL activity and subsequent lipolysis [Anthonsen et al., 1998; Holm et al., 2000a]. To determine whether NPY potentiated isoproterenol-stimulated lipolysis was mediated in part through enhanced HSL phosphorylation, we studied the effect of NPY on HSL phosphorylation at Ser563. We found that although it had no effect on HSL phosphorylation under basal conditions, NPY increased isoproterenol stimulated HSL phosphorylation (Fig. 3.2).

3.3 Effects of NPY on the Expression of Key Lipolytic Proteins/enzymes

Besides acute regulation, the lipolytic capacity of adipocytes can be regulated through changes in the expression of critical lipolytic proteins, such as HSL, ATGL, and perilipin [Langin et al., 2005; Lafontan and Langin, 2009]. The expression of these proteins/enzymes is decreased in obese states and has been shown to be regulated by other hormones such as cortisol and insulin [Kralisch et al., 2005; Jocken et al., 2007]. To ascertain if NPY regulates the expression of these proteins, we treated 3T3-L1 adipocytes with increasing concentrations of NPY for 24 h. As shown in Fig. 3.3, NPY treatment did not change levels of HSL, perilipin, or ATGL protein. Similar results were obtained when adipocytes were treated with NPY for 12 and 48 h (data not shown). Cells appeared normal when checked after treatment periods just prior to protein collection.
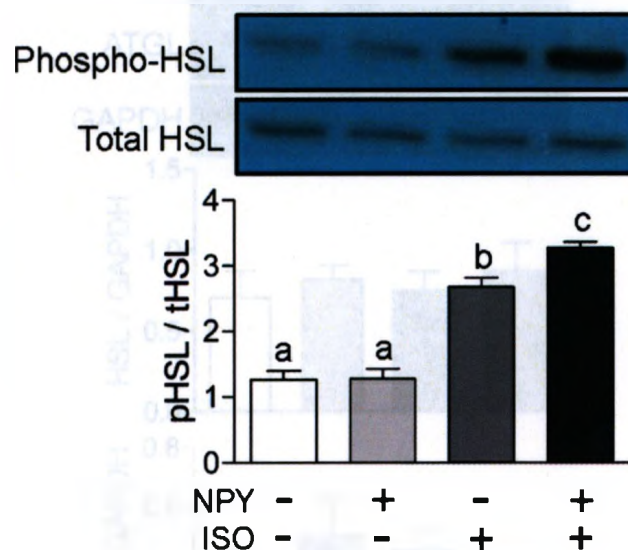
Figure 3.2 Effects of NPY on hormone sensitive lipase (HSL) phosphorylation. Differentiated 3T3-L1 adipocytes were pretreated with NPY (100 nM) for 30 min, and were then treated with isoproterenol (ISO; 10 nM) for 10 min. Controls were treated at the same time with an appropriate volume of vehicle. At the end of treatment, cell lysates were prepared, and HSL phosphorylation at serine 563 was determined by western blotting using antibodies specific for phosphorylated HSL and total HSL proteins. Results of a representative western blotting are shown. Data are presented as means ± SEM of four independent experiments, each performed in triplicate. a *vs.* b, P < 0.05; b *vs.* c, P < 0.05.

Figure 3.3 Effects of NPY on the expression of selected key lipolytic proteins/enzymes. Differentiated 3T3-L1 adipocytes were treated with increasing concentrations of NPY for 24 h. At the end of treatment, cell lysates were prepared, and subjected to western blot analysis. Levels of three key proteins controlling lipolysis were determined using antibodies specific for hormone sensitive lipase (HSL), perilipin and adipose triglyceride lipase (ATGL). GAPDH was used as a loading control. Data are presented as means ± SEM of four independent experiments.

3.4 Effects of NPY on the Expression of Key Lipogenic Enzymes

Energy balance in adipocytes is controlled by lipolysis and lipogenesis. FAS and SCD1 are important lipogenic enzymes that were found to be upregulated in the visceral adipose tissue of obese MPR offspring [Guan et al., 2005]. In other studies, the expression of these enzymes was found to be upregulated in obese animals and correlated with fat accumulation in humans [Boizard et al., 1998; Berndt et al., 2007]. Furthermore, chronic intracerebroventricular NPY administration led to increased adipose tissue lipogenesis in normal rats [Zarjevski et al., 1993]. These findings led us to study the effect of NPY on the expression of FAS and SCD1. We treated 3T3-L1 adipocytes with increasing concentrations of NPY for 24 h. As shown in Fig. 3.4, NPY treatment did not change levels of SCD1 and FAS protein. Similar results were obtained when adipocytes were treated with NPY for 12 and 48 h (data not shown). Cells appeared normal when checked after treatment periods just prior to protein collection.
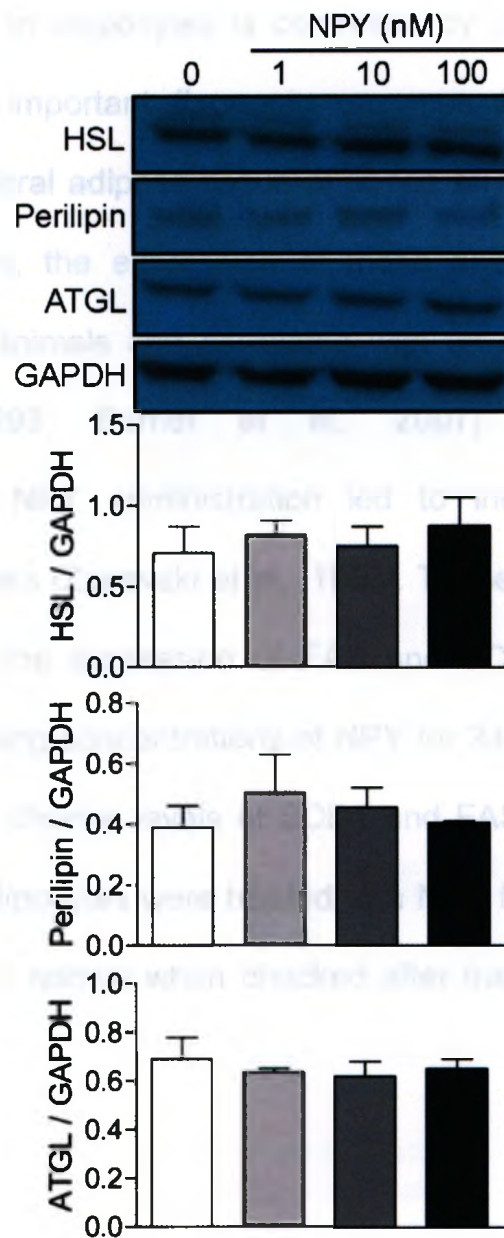
Figure 3.4 Effects of NPY on the expression of selected key lipogenic enzymes. Differentiated 3T3-L1 adipocytes were treated with increasing concentrations of NPY for 24 h. At the end of treatment, cell lysates were prepared, and subjected to western blot analysis. Levels of two critical lipogenic enzymes were determined using antibodies specific for fatty acid synthase (FAS) and stearoyl-CoA desaturase 1 (SCD1). β-tubulin was used as a loading control. Data are presented as means ± SEM of four independent experiments.

3.5 Effects of NPY on Glucose Uptake

Besides energy mobilization and energy storage, glucose uptake is another critical metabolic process in adipocytes [Rosen and Spiegelman, 2006]. Glucose transport is a key regulatory step in glucose utilization in adipocytes. One study found that ICV administration of NPY increased glucose uptake by adipose tissue which could contribute to increased fat accumulation [Zarjevski et al., 1994]. To determine if NPY affects adipocyte glucose uptake, we treated 3T3-L1 adipocytes with NPY in the absence and presence of insulin, the primary stimulator of glucose uptake. As expected, insulin increased glucose uptake by approximately 400%. However, NPY treatment had no effect on basal or insulin stimulated glucose uptake (Fig. 3.5).

Figure 3.5 Effects of NPY on glucose uptake. Differentiated 3T3-L1 adipocytes were treated with NPY (100 nM), insulin (100 nM) or in combination (100 nM each) for 15 minutes, following which 0.1 mM of unlabeled 2-deoxy-D-glucose containing 0.5 µCi [1,2-^{3}H] 2-deoxy-D-glucose was added to the medium. After a 10 min incubation, cells were washed, solubilized and uptake of [1,2-^{3}H] 2-deoxy-D-glucose was measured using a liquid scintillation counter. Data are presented as means ± SEM of three independent experiments, each performed in triplicate. a *vs.* b, P < 0.001.

Chapter 4

Discussion

4.1 General Discussion

Adipose tissue is a novel site of NPY biosynthesis, and NPY acts locally to promote adipogenesis thereby contributing to the pathogenesis of obesity [Kuo et al., 2007; Yang et al., 2008]. Despite these important observations, the role of NPY in regulating adipocyte metabolism is poorly understood. The present findings reveal a novel role for NPY in potentiating β-adrenergic stimulation of lipolysis. Furthermore, our data suggest that this potentiation likely occurs upstream of adenylyl cyclase activation, and is mediated at least in part through enhanced phosphorylation of HSL, a rate limiting enzyme in lipolysis.

Lipolysis is a critical metabolic function of adipocytes, which liberates glycerol and free fatty acids for use by other tissues. In this study, we examined the role of NPY in regulating basal and β-adrenergic stimulated lipolysis. Although it did not affect lipolysis under basal conditions, NPY potentiated isoproterenol-stimulated lipolysis. This is an unexpected finding, and appears to contradict several previous reports in which NPY was found to inhibit both basal and stimulated lipolysis in isolated adipocytes from dogs, rats, and humans as well as 3T3-L1 adipocytes [Valet et al., 1990; Castan et al., 1994; Labelle et al., 1997; Turtzo et al., 2001; Kos et al., 2007]. These discrepancies may be explained by differences in experimental conditions between our present study and those published previously. In previous studies, lipolysis was stimulated by adenosine deaminase, which removes the inhibitory effect of adenosine (a potent inhibitor of lipolysis), and/or isoproterenol. Importantly, the magnitude of lipolytic stimulation in these studies was much greater than that in the present study (~4-

fold vs. ~2-fold) owing to the use of a different stimulus or higher concentrations of isoproterenol (100 nM vs. 10 nM). Thus, the effect of NPY on stimulated lipolysis likely depends on the strength of lipolytic stimulation. In support of this contention, we found that NPY lost its ability to potentiate isoproterenol-stimulated lipolysis at higher concentrations of isoproterenol (>20 nM).

Our present findings that NPY promotes lipolysis by potentiating β-adrenergic receptor signaling are intriguing, and beg the question of the underlying molecular mechanisms. As a first step in deciphering the molecular mechanisms, we examined the effects of NPY on forskolin (a direct activator of adenylyl cyclase) stimulated lipolysis. We found that NPY did not potentiate forskolin stimulated lipolysis, which suggests that NPY acts upstream of adenylyl cyclase to potentiate β-adrenergic stimulated lipolysis. This effect was not due to maximal stimulation of lipolysis by forskolin because lipolysis can be increased by at least several fold in adipocytes [Lafontan and Langin, 2009]. Our present findings are similar to those reported previously showing that NPY potentiated α-adrenergic receptor-mediated vasoconstriction [Edvinsson et al., 1984; Domoso et al., 1993; Fallgren et al., 1993]. Furthermore, NPY had no effect on vasoconstriction when applied alone, which is similar to our observations that NPY did not affect basal lipolysis. Our lab [Yang et al., 2008] and others [Gericke et al., 2009] have reported that the Y_1 receptor subtype is expressed in 3T3-L1 cells and the Y_1 receptor has been implicated in potentiating α-adrenergic mediated vasoconstriction. The crosstalk between NPY and β-adrenergic signaling pathways is significant given the fact that norepinephrine and NPY are

co-stored in sympathetic neurons (Cannon et al., 1986). Thus, we propose that the interaction between these two signaling pathways may provide an additional layer in the precise control of lipolysis (Fig. 4.1).

HSL activity is tightly controlled by the phosphorylation pattern of HSL [Holm et al., 2000a].To determine if NPY potentiation of isoproterenol-stimulated lipolysis is mediated by increased HSL phosphorylation, we examined the effects of NPY on HSL phosphorylation under basal and stimulated conditions. Similar to its effect on lipolysis, NPY did not affect HSL phosphorylation under non-stimulated conditions but enhanced isoproterenol-induced HSL phosphorylation, suggesting that the effect of NPY on β-adrenergic-stimulated lipolysis is mediated at least in part by increased HSL phosphorylation (Fig. 4.1).

Figure 4.1 Schematic of the molecular mechanisms underlying neuropeptide Y (NPY) potentiation of beta-adrenergic stimulated lipolysis. NPY binds to the Y_1 receptor in 3T3-L1 adipocytes and potentiates (+) beta-adrenergic signaling induced by isoproterenol (ISO). This potentiation occurs through an unknown (?) mechanism that is upstream of adenylyl cyclase (AC) activation. Activation of the beta-adrenergic receptor stimulates AC activity leading to an increase in intracellular cAMP and subsequent PKA activity. PKA phosphorylates serine residues of hormone sensitive lipase (HSL) which increases its activity leading to an increase in lipolysis.

Besides acute regulation of lipolysis through the reversible phosphorylation of proteins, lipolysis is also regulated at the level of lipolytic gene expression [Lafontan and Langin, 2009]. When the genes encoding critical lipolytic proteins are knocked out in mice, lipolysis and adipocyte phenotype are significantly affected. Mice deficient in HSL have a 70% reduction in lipolysis in adipose tissue and adipocytes are enlarged two-fold when compared to wild type animals [Osuga et al., 2000; Schweiger et al., 2006]. When ATGL is knocked out, isoproterenol-stimulated lipolysis is reduced by 75% and adipocytes are enlarged by 40% [Haemmerle et al., 2006]. In contrast, basal lipolysis in perilipin knockout mice is increased by 3-fold and adipocytes are 62% smaller [Martinez-Botas et al., 2000]. Although NPY is known to regulate lipolysis, its role in regulating the expression of these important lipolytic proteins had not been previously explored. Therefore, in the present study we examined the effect of NPY on the expression of HSL, ATGL and perilipin. We found that NPY had no effect under a variety of treatment conditions. Taken together, our present findings suggest that NPY regulates lipolysis through mechanisms not involving changes in the expression of lipolytic enzyme/proteins.

The synthesis of lipids and triglycerides involves numerous steps and enzymes [Wolfgang and Lane, 2006]. Our previous DNA microarray data showed that the expression of two critical lipogenic enzymes, FAS and SCD1 , was upregulated in visceral adipose tissue of an early-life programmed rat model of increased visceral adiposity [Guan et al., 2005]. Furthermore, ICV administration of NPY to rats increased lipogenesis in adipose tissue [Zarjevski et al., 1993].

However, it remained unknown if NPY regulates the expression of lipogenic enzymes. Therefore, in the present study we investigated the effect of NPY on the expression of SCD1 and FAS. Under the conditions of the present study, NPY did not affect either SCD1 or FAS expression, suggesting that NPY may regulate lipogenesis through mechanisms that do not involve altered expression of SCD1 and FAS. Alternatively, NPY may affect the expression of other important lipogenic enzymes such as the acyltransferases that catalyze triacylglycerol synthesis.

Adipose tissue is a major insulin sensitive tissue and glucose uptake is another major function of adipocytes [Smith, 2002]. Glucose uptake and utilization is increased in the development of obesity and increased uptake in adipose tissue of our early life model of visceral adiposity could be one mechanism contributing to adipocyte hyperplasia [Shepherd et al., 1993; Guan et al., 2005]. Furthermore, ICV administration of NPY to normal rats increased glucose utilization by adipose tissue but it was not known if NPY has a direct effect on glucose uptake at the adipocyte level [Zarjevski et al., 1994]. We investigated whether NPY affected basal or insulin stimulated glucose uptake but did not find a role for NPY in regulating these processes. This suggests that the effects of NPY on adipose tissue glucose metabolism are mediated centrally and other mechanisms such as increases in preadipocyte proliferation underlie the hyperplastic obesity seen in the early-life rat model of visceral adiposity.

4.2 Summary and Conclusions

In summary, we report here a novel cross talk between NPY and β-adrenergic receptor signaling pathways by demonstrating that NPY potentiates isoproterenol stimulated lipolysis and HSL phosphorylation. This potentiation likely occurs upstream of adenylyl cyclase activation since NPY did not potentiate FSK stimulated lipolysis. We did not find an effect of NPY treatment on lipolytic enzyme/protein expression. Furthermore, we did not find a role for NPY in regulating lipogenic enzyme expression and glucose uptake in adipocytes. Altogether, these present findings add a new dimension to our understanding of the dynamic role NPY plays in regulating energy balance. Overall, this study lays the groundwork for future explorations of the effects of NPY in regulating adipocyte metabolism.

4.3 Future Directions

The novel cross talk between the NPY and β-adrenergic signaling pathways opens up a number of interesting avenues and questions for future investigation. Importantly, the results of this study using the 3T3-L1 cell line will need to be confirmed *in vivo*. The physiological relevance and role of the interaction between these pathways in normal and pathological conditions will need to be assessed. Another logical extension of this study is further investigation into the mechanisms involved in this potentiation effect. Both the signaling events following NPY receptor activation and the molecular changes facilitating the enhancement of β-adrenergic signaling need to be explored.

Furthermore, isoproterenol activates both β1 and β2 adrenergic receptors and it is unclear whether NPY is able to potentiate the signaling of both receptor subtypes.

NPY is co-stored and co-released along with norepinephrine in central neurons and peripheral sympathetic neurons. As such, the NPY potentiation of β-adrenergic signaling could be physiologically relevant in other systems or tissues such as the gut and adrenal glands that are innervated by such neurons. Additionally, in different areas of the brain, NPY is co-stored with other neurotransmitters such as GABA and acetylcholine [Wan and Benjamin, 1995]. To date, NPY signaling has been shown to potentiate the signaling of α-adrenoreceptors and β-adrenoreceptors. The possible interaction of NPY receptor signaling with other neurotransmitter signaling pathways is another interesting future area of questioning.

REFERENCES

Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB. 2001. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature 409:729-733.

Anthonsen MW, Rönnstrand L, Wernstedt C, Degerman E, Holm C. 1998. Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro. Journal of Biological Chemistry 273:215-221.

Barker DJP, Hales CN, Fall CHD, Osmond C, Phipps K, Clark PMS. 1993. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): Relation to reduced fetal growth. Diabetologia 36:62-67.

Bartness TJ, Bamshad M. 1998. Innervation of mammalian white adipose tissue: Implications for the regulation of total body fat. American Journal of Physiology - Regulatory Integrative and Comparative Physiology 275:R1399-R1411.

Berndt J, Kovacs P, Ruschke K, Klöting N, Fasshauer M, Schön MR, Körner A, Stumvoll M, Blüher M. 2007. Fatty acid synthase gene expression in human adipose tissue: Association with obesity and type 2 diabetes. Diabetologia 50:1472-1480.

Bertram CE, Hanson MA. 2001. Animal models and programming of the metabolic syndrome. British Medical Bulletin 60:103-121.

Boizard M, Le Liepvre X, Lemarchand P, Foufelle F, Ferré P, Dugail I. 1998. Obesity-related overexpression of fatty-acid synthase gene in adipose tissue involves sterol regulatory element-binding protein transcription factors. Journal of Biological Chemistry 273:29164-29171.

Brasaemle DL. 2007. The perilipin family of structural lipid droplet proteins: Stabilization of lipid droplets and control of lipolysis. Journal of Lipid Research 48:2547-2559.

Byrne CD, Phillips DI. 2000. Fetal origins of adult disease: Epidemiology and mechanisms. Journal of Clinical Pathology 53:822-828.

Campfield LA, Smith FJ. 1999. The pathogenesis of obesity. Bailliere's Best Practice in Clinical Endocrinology and Metabolism 13:13-30.

Carmen GY, Víctor SM. 2006. Signalling mechanisms regulating lipolysis. Cellular Signalling 18:401-408.

Castan I, Valet P, Quideau N, Voisin T, Ambid L, Laburthe M, Lafontan M, Carpene C. 1994. Antilipolytic effects of $\alpha2$-adrenergic agonists, neuropeptide Y,

adenosine, and PGE1 in mammal adipocytes. American Journal of Physiology - Regulatory Integrative and Comparative Physiology 266:R1141-R1147.

CIHI. 2004. Canadian Community Health Surveyeditor^editors.

Cline GW, Petersen KF, Krssak M, Shen J, Hundal RS, Trajanoski Z, Inzucchi S, Dresner A, Rothman DL, Shulman GI. 1999. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. New England Journal of Medicine 341:240-246.

Coleman RA, Lewin TM, Muoio DM. 2000. Physiological and nutritional regulation of enzymes of triacylglycerol synthesiseditor^editors. Annual review of nutrition. p 77-103.

Coleman RA, Mashek DG. 2011. Mammalian triacylglycerol metabolism: Synthesis, lipolysis, and signaling. Chemical Reviews 111:6359-6386.

De Onis M, Blössner M, Borghi E. 2010. Global prevalence and trends of overweight and obesity among preschool children. American Journal of Clinical Nutrition 92:1257-1264.

Dixon JB. 2010. The effect of obesity on health outcomes. Molecular and Cellular Endocrinology 316:104-108.

Dobrzyn A, Ntambi JM. 2004. The role of stearoyl-CoA desaturase in body weight regulation. Trends in Cardiovascular Medicine 14:77-81.

Dobrzyn A, Ntambi JM. 2005. Stearoyl-CoA desaturase as a new drug target for obesity treatment. Obesity Reviews 6:169-174.

Domoso MV, Boric M, Prado M, Fournier A, St. Pierre S, Edvinsson L, Huidobro-Toro JP. 1993. D-myo-inositol 1,2,6-trisphosphate blocks neuropeptide Y-induced facilitation of noradrenaline-evoked vasoconstriction of the mesenteric bed. Eur. J. Pharmacol. 240:93-97.

Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sul HS. 2007. Regulation of lipolysis in adipocyteseditor^editors. Annual Review of Nutrition. p 79-101.

Edvinsson L, Ekblad E, Hakanson R, Wahlestedt C. 1984. Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels. Br. J. Pharmacol. 83:519-525.

Erickson JC, Hollopeter G, Palmiter RD. 1996. Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. Science 274:1704-1707.

Fallgren B, Arlock P, Edvinsson L. 1993. Neuropeptide Y potentiates noradrenaline-evoked vasoconstriction by an intracellular calcium-dependent mechanism. J. Auton. Nerv. Syst. 44:151-159.

Gericke MT, Kosacka J, Koch D, Nowicki M, Schröder T, Ricken AM, Nieber K, Spanel-Borowski K. 2009. Receptors for NPY and PACAP differ in expression and activity during adipogenesis in the murine 3T3-L1 fibroblast cell line. British Journal of Pharmacology 157:620-632.

Green H, Kehinde O. 1974. Sublines of mouse 3T3 cells that accumulate lipid. Cell 1:113-116.

Gregoire FM, Smas CM, Sul HS. 1998. Understanding adipocyte differentiation. Physiological Reviews 78:783-809.

Guan H, Arany E, Van Beek JP, Chamson-Reig A, Thyssen S, Hill DJ, Yang K. 2005. Adipose tissue gene expression profiling reveals distinct molecular pathways that define visceral adiposity in offspring of maternal protein-restricted rats. American Journal of Physiology - Endocrinology and Metabolism 288:E663-E673.

Haemmerle G, Lass A, Zimmermann R, Gorkiewicz G, Meyer C, Rozman J, Heldmaier G, Maier R, Theussl C, Eder S, Kratky D, Wagner EF, Klingenspor M, Hoefler G, Zechner R. 2006. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. Science 312:734-737.

Hansel DE, Eipper BA, Ronnett GV. 2001. Neuropeptide Y functions as a neuroproliferative factor. Nature 410:940-944.

Higuchi H, Yang HYT, Sabol SL. 1988. Rat neuropeptide Y precursor gene expression. mRNA structure, tissue distribution, and regulation by glucocorticoids, cyclic AMP, and phorbol ester. Journal of Biological Chemistry 263:6288-6295.

Hofbauer KG. 2002. Molecular pathways to obesity. International Journal of Obesity 26:S18-S27.

Holemans K, Aerts L, Van Assche FA. 2003. Fetal growth restriction and consequences for the offspring in animal models. Journal of the Society for Gynecologic Investigation 10:392-399.

Holm C, Osterlund T, Laurell H, Contreras JA. 2000a. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. Annu. Rev. Nutr. 20:365-393.

Holm C, Osterlund T, Laurell H, Contreras JA. 2000b. Molecular mechanisms regulating hormone-sensitive lipase and lipolysiseditor^editors. Annual Review of Nutrition. p 365-393.

Jamdar SC, Cao WF. 1995. Triacylglycerol biosynthetic enzymes in lean and obese Zucker rats. Biochimica et Biophysica Acta - Lipids and Lipid Metabolism 1255:237-243.

James WPT. 2008. WHO recognition of the global obesity epidemic. International Journal of Obesity 32:S120-S126.

Jiang G, Li Z, Liu F, Ellsworth K, Dallas-Yang Q, Wu M, Ronan J, Esau C, Murphy C, Szalkowski D, Bergeron R, Doebber T, Zhang BB. 2005. Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. Journal of Clinical Investigation 115:1030-1038.

Jo J, Gavrilova O, Pack S, Jou W, Mullen S, Sumner AE, Cushman SW, Periwal V. 2009. Hypertrophy and/or hyperplasia: Dynamics of adipose tissue growth. PLoS Computational Biology 5.

Jocken JWE, Langin D, Smit E, Saris WHM, Valle C, Hul GB, Holm C, Arner P, Blaak EE. 2007. Adipose triglyceride lipase and hormone-sensitive lipase protein expression is decreased in the obese insulin-resistant state. J. Clin. Endocrinol. Metab. 92:2292-2299.

Kalra SP, Dube MG, Sahu A, Phelps CP, Kalra PS. 1991. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. Proceedings of the National Academy of Sciences of the United States of America 88:10931-10935.

Kalra SP, Kalra PS. 2003. Neuropeptide Y: A Physiological Orexigen Modulated by the Feedback Action of Ghrelin and Leptin. Endocrine 22:49-55.

Kamiji MM, Inui A. 2007. Neuropeptide y receptor selective ligands in the treatment of obesity. Endocrine Reviews 28:664-684.

Kersten S. 2001. Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO Reports 2:282-286.

Kos K, Harte AL, James S, Snead DR, O'Hare JP, McTernan PG, Kumar S. 2007. Secretion of neuropeptide Y in human adipose tissue and its role in maintenance of adipose tissue mass. American Journal of Physiology - Endocrinology and Metabolism 293:E1335-E1340.

Kralisch S, Klein J, Lossner U, Bluher M, Paschke R, Stumvoll M, Fasshauer M. 2005. Isoproterenol, TNFα, and insulin downregulate adipose triglyceride lipase in 3T3-L1 adipocytes. Mol. Cell. Endocrinol. 240:43-49.

Kuo LE, Kitlinska JB, Tilan JU, Li L, Baker SB, Johnson MD, Lee EW, Burnett MS, Fricke ST, Kvetnansky R, Herzog H, Zukowska Z. 2007. Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. Nat. Med. 13:803-811.

Labelle M, Boulanger Y, Fournier A, St.-Pierre S, Savard R. 1997. Tissue-specific regulation of fat cell lipolysis by NPY in 6-OHDA- treated rats. Peptides 18:801-808.

Lafontan M, Langin D. 2009. Lipolysis and lipid mobilization in human adipose tissue. Prog. Lipid Res. 48:275-297.

Lang A, Froelicher ES. 2006. Management of overweight and obesity in adults: Behavioral intervention for long-term weight loss and maintenance. European Journal of Cardiovascular Nursing 5:102-114.

Langin D. 2006. Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. Pharmacological Research 53:482-491.

Langin D, Dicker A, Tavernier G, Hoffstedt J, Mairal A, Rydén M, Arner E, Sicard A, Jenkins CM, Viguerie N, Van Harmelen V, Gross RW, Holm C, Arner P. 2005. Adipocyte lipases and defect of lipolysis in human obesity. Diabetes 54:3190-3197.

Large V, Peroni O, Letexier D, Ray H, Beylot M. 2004. Metabolism of lipids in human white adipocyte. Diabetes and Metabolism 30:294-309.

Lijnen HR. 2008. Angiogenesis and obesity. Cardiovascular Research 78:286-293.

MacDougald OA, Lane MD. 1995. Transcriptional regulation of gene expression during adipocyte differentiation. Annual Review of Biochemistry 64:345-373.

Martinez-Botas J, Anderson JB, Tessier D, Lapillonne A, Chang BHJ, Quast MJ, Gorenstein D, Chen KH, Chan L. 2000. Absence of perilipin results in leanness and reverses obesity in Lepr(db/db) mice. Nature Genetics 26:474-479.

Menendez JA, Vazquez-Martin A, Ortega FJ, Fernandez-Real JM. 2009. Fatty acid synthase: Association with insulin resistance, type 2 diabetes, and cancer. Clinical Chemistry 55:425-438.

Montague CT, O'Rahilly S. 2000. The perils of portliness: Causes and consequences of visceral adiposity. Diabetes 49:883-888.

Morley R. 2006. Fetal origins of adult disease. Seminars in Fetal and Neonatal Medicine 11:73-78.

Osuga JI, Ishibashi S, Oka T, Yagyu H, Tozawa R, Fujimoto A, Shionoiri F, Yahagi N, Kraemer FB, Tsutsumi O, Yamada N. 2000. Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity. Proc. Natl. Acad. Sci. U. S. A. 97:787-792.

Otto TC, Lane MD. 2005. Adipose development: From stem cell to adipocyte. Critical Reviews in Biochemistry and Molecular Biology 40:229-242.

Ozanne SE. 2001. Metabolic programming in animals. British Medical Bulletin 60:143-152.

Pedersen O, Kahn CR, Kahn BB. 1992. Divergent regulation of the Glut 1 and Glut 4 glucose transporters in isolated adipocytes from Zucker rats. Journal of Clinical Investigation 89:1964-1973.

Pedrazzini T, Pralong F, Grouzmann E. 2003. Neuropeptide Y: The universal soldier. Cellular and Molecular Life Sciences 60:350-377.

Pons J, Kitlinska J, Ji H, Lee EW, Zukowska Z. 2003. Mitogenic actions of neuropeptide Y in vascular smooth muscle cells: Synergetic interactions with the β-adrenergic system. Canadian Journal of Physiology and Pharmacology 81:177-185.

Raposinho PD, Pierroz DD, Broqua P, White RB, Pedrazzini T, Aubert ML. 2001. Chronic administration of neuropeptide Y into the lateral ventricle of C57BL/6J male mice produces an obesity syndrome including hyperphagia, hyperleptinemia, insulin resistance, and hypogonadism. Molecular and Cellular Endocrinology 185:195-204.

Rosen ED, Spiegelman BM. 2006. Adipocytes as regulators of energy balance and glucose homeostasis. Nature 444:847-853.

Schweiger M, Schreiber R, Haemmerle G, Lass A, Fledelius C, Jacobsen P, Tornqvist H, Zechner R, Zimmermann R. 2006. Adipose triglyceride lipase and hormone-sensitive lipase are the major enzymes in adipose tissue triacylglycerol catabolism. Journal of Biological Chemistry 281:40236-40241.

Serradeil-Le Gal C, Lafontan M, Raufaste D, Marchand J, Pouzet B, Casellas P, Pascal M, Maffrand JP, Le Fur G. 2000. Characterization of NPY receptors controlling lipolysis and leptin secretion in human adipocytes. FEBS Letters 475:150-156.

Shepherd PR, Gnudi L, Tozzo E, Yang H, Leach F, Kahn BB. 1993. Adipose cell hyperplasia and enhanced glucose disposal in transgenic mice overexpressing GLUT4 selectively in adipose tissue. Journal of Biological Chemistry 268:22243-22246.

Shepherd PR, Kahn BB. 1999. Glucose transporters and insulin action: Implications for insulin resistance and diabetes mellitus. New England Journal of Medicine 341:248-257.

Silva AP, Cavadas C, Grouzmann E. 2002. Neuropeptide Y and its receptors as potential therapeutic drug targets. Clinica Chimica Acta 326:3-25.

Smith S, Witkowski A, Joshi AK. 2003. Structural and functional organization of the animal fatty acid synthase. Progress in Lipid Research 42:289-317.

Smith U. 2002. Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance - Is insulin resistance initiated in the adipose tissue? International Journal of Obesity 26:897-904.

Takeuchi K, Reue K. 2009. Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis. American Journal of Physiology - Endocrinology and Metabolism 296:E1195-E1209.

Tansey JT, Sztalryd C, Hlavin EM, Kimmel AR, Londos C. 2004. The central role of perilipin A in lipid metabolism and adipocyte lipolysis. IUBMB Life 56:379-385.

Tatemoto K, Carlquist M, Mutt V. 1982. Neuropeptide Y - a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 296:659-660.

Thorsell A, Heilig M. 2002. Diverse functions of neuropeptide Y revealed using genetically modified animals. Neuropeptides 36:182-193.

Tozzo E, Shepherd PR, Gnudi L, Kahn BB. 1995. Transgenic GLUT-4 overexpression in fat enhances glucose metabolism: Preferential effect on fatty acid synthesis. American Journal of Physiology - Endocrinology and Metabolism 268:E956-E964.

Turtzo LC, Marx R, Daniel Lane M. 2001. Cross-talk between sympathetic neurons and adipocytes in coculture. Proc. Natl. Acad. Sci. U. S. A. 98:12385-12390.

Valet P, Berlan M, Beauville M, Crampes F, Montastruc JL, Lafontan M. 1990. Neuropeptide Y and peptide YY inhibit lipolysis in human and dog fat cells through a pertussis toxin-sensitive G protein. J. Clin. Invest. 85:291-295.

Voros G, Maquoi E, Demeulemeester D, Clerx N, Collen D, Lijnen HR. 2005. Modulation of angiogenesis during adipose tissue development in murine models of obesity. Endocrinology 146:4545-4554.

Walley AJ, Asher JE, Froguel P. 2009. The genetic contribution to non-syndromic human obesity. Nature Reviews Genetics 10:431-442.

Wan CP, Benjamin HS. 1995. Neuropeptide Y receptor subtypes. Life Sciences 56:1055-1064.

Wang H, Hu L, Dalen K, Dorward H, Marcinkiewicz A, Russell D, Gong D, Londos C, Yamaguchi T, Holm C, Rizzo MA, Brasaemle D, Sztalryd C. 2009. Activation of hormone-sensitive lipase requires two steps, protein phosphorylation and binding to the PAT-1 domain of lipid droplet coat proteins. Journal of Biological Chemistry 284:32116-32125.

Wang Y, Voy BJ, Urs S, Kim S, Soltani-Bejnood M, Quigley N, Heo YR, Standridge M, Andersen B, Dhar M, Joshi R, Wortman P, Taylor JW, Chun J, Leuze M, Claycombe K, Saxton AM, Moustaid-Moussa N. 2004. The Human Fatty Acid Synthase Gene and De Novo Lipogenesis Are Coordinately Regulated in Human Adipose Tissue. Journal of Nutrition 134:1032-1038.

Wolfgang MJ, Lane MD. 2006. Control of energy homeostasis: role of enzymes and intermediates of fatty acid metabolism in the central nervous system. Annu. Rev. Nutr. 26:23-44.

Yang K, Guan H, Arany E, Hill DJ, Cao X. 2008. Neuropeptide Y is produced in visceral adipose tissue and promotes proliferation of adipocyte precursor cells via the Y1 receptor. FASEB J. 22:2452-2464.

Yulyaningsih E, Zhang L, Herzog H, Sainsbury A. 2011. NPY receptors as potential targets for anti-obesity drug development. British Journal of Pharmacology 163:1170-1202.

Zarjevski N, Cusin I, Vettor R, Rohner-Jeanrenaud F, Jeanrenaud B. 1993. Chronic intracerebroventricular neuropeptide-Y administration to normal rats mimics hormonal and metabolic changes of obesity. Endocrinology 133:1753-1758.

Zarjevski N, Cusin I, Vettor R, Rohner-Jeanrenaud F, Jeanrenaud B. 1994. Intracerebroventricular administration of neuropeptide Y to normal rats has divergent effects on glucose utilization by adipose tissue and skeletal muscle. Diabetes 43:764-769.

Zechner R, Kienesberger PC, Haemmerle G, Zimmermann R, Lass A. 2009. Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. Journal of Lipid Research 50:3-21.

Zhang T, Guan H, Arany E, Hill DJ, Yang K. 2007. Maternal protein restriction permanently programs adipocyte growth and development in adult male rat offspring. Journal of Cellular Biochemistry 101:381-388.

Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, Zechner R. 2004. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 306:1383-1386.