Role of nuclear receptors in the regulation of human adipose tissue metabolism

PRASAD G. KAMBLE
Abstract

Nuclear receptors modulate expression of genes involved in adipose tissue (AT) metabolism. Their improved understanding may provide new treatment options for metabolic disorders such as obesity, insulin resistance (IR) and type 2 diabetes (T2D).

This thesis explored the role of nuclear receptors, mainly, glucocorticoid and estrogen receptors (GR and ER, respectively) and peroxisome proliferator-activated receptor gamma (PPARγ), and their interplay in the regulation of metabolic function and dysfunction in human AT.

In Paper I, the regulation of adipokine lipocalin 2 (LCN2) expression by synthetic glucocorticoid, dexamethasone and effect of LCN2 on glucose and lipid metabolism in AT were studied. In pre-menopausal but not post-menopausal women or men, dexamethasone upregulated LCN2 gene expression, which also correlated with markers of obesity and IR. LCN2 inhibited adipocyte glucose uptake.

In Paper II, the effect of estrogen (E2) and its interaction with GR in LCN2 regulation in AT from post-menopausal women were examined. E2 increased LCN2 expression, what seems to be mediated by ERβ. E2 and dexamethasone co-treatment increased LCN2 gene expression in presence of ERα but not ERβ antagonist. Dexamethasone decreased ERα, while increased ERβ gene expression.

In Paper III and IV, the feasibility of genotype-based recall (GBR), a participant recruitment approach, was tested by undertaking clinical and AT phenotyping of different PPARγ Pro12Ala carriers. The baseline characteristics were comparable between genotypes. Compared to fasting, a decreased hormone-sensitive lipase gene expression in Pro/Pro group also accompanied with a higher antilipolytic effect of insulin after oral glucose. Adipocyte glucose uptake and adipogenesis remained unchanged between genotypes.

Overall, LCN2 can induce IR in human AT and may mediate metabolic defects by excess glucocorticoids in pre-menopausal women. GR selectively interacts with ERα and ERβ, the latter two acts oppositely to control LCN2 expression in AT. PPARγ Pro12Ala had no major effect on clinical and adipose phenotype, likely due to a small sample size in relation to the modest effect the Ala variant or tissues other than adipose could be critical in conferring protection by Pro12Ala against T2D risk. Further, the GBR approach deemed feasible, however, would be more suitable in the characterization of rare genetic variants.

Keywords: human adipose tissue, nuclear receptors, glucocorticoids, estrogen, PPARγ, lipocalin 2, glucose uptake, lipolysis, adipogenesis, genotype-based recall

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ISSN 1651-6206
ISBN 978-91-513-0401-4
urn:nbn:se:uu:diva-357119 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-357119)
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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I  **Prasad G Kamble**, Maria J Pereira, Cherno O Sidibeh, Sam Amini, Magnus Sundbom, Joey Lau Börjesson, Jan W Eriksson

II  **Prasad G Kamble**, Maria J Pereira, Gretha J Boersma, Kristina Almby, Jan W Eriksson
   “Effect of estrogen and its interaction with glucocorticoids in the regulation of lipocalin 2 in human adipose tissue: a role for estrogen receptor β pathway in insulin resistance?” Manuscript

III  **Prasad G Kamble**, Stefan Gustafsson, Maria J Pereira, Per Lundkvist, Naomi Cook, Paul Franks, Lars Lind, Tove Fall, Jan W Eriksson*, Erik Ingelsson*

IV  **Prasad G Kamble**, Maria J Pereira, Stefan Gustafsson, Per Lundkvist, Casimiro Castillejo- López, Tove Fall, Erik Ingelsson*, Jan W Eriksson*

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List of papers not included in the thesis:

I Per Lundkvist, Maria J Pereira, Prasad G Kamble, Petros Katsogiannos, Anna Maria Langkilde, Russell Esterline, Eva Johnsson, Jan W Eriksson
“Glucagon Levels during Short-term SGLT2 Inhibition are Largely Regulated by Glucose Changes in Type 2 Diabetes Patients.” Accepted in Journal of Clinical Endocrinology & Metabolism. 2018.

II Maria J Pereira, Per Lundkvist, Prasad G Kamble, Joey Lau Börjesson, Julian G Martins, C. David Sjöström, Volker Schnecke, Anna Walentinsson, Eva Johnsson, Jan W Eriksson

III Cherno O Sidibeh, Maria J Pereira, Joey Lau Börjesson, Prasad G Kamble, Stanko Skrtic, Petros Katsogiannos, Magnus Sundbom, Maria K Svensson, Jan W Eriksson
Abbreviations

AS160  PKB/Akt substrate of 160 kDa
ATGL  Adipose triglyceride lipase
ATP  Adenosine triphosphate
AUC  Area under curve
Beta ADR  Beta-adrenergic receptor
BMP4  Bone morphogenetic protein 4
BMP7  Bone morphogenetic protein 7
cAMP  Cyclic adenosine monophosphate
CAPN10  Calpain 10
CEBP  CCAAT enhancer-binding proteins
CGI 58  Comparative gene identification 58
CNR1  Cannabinoid receptor type 1
DG  Diglycerides
DPP4  Dipeptidyl peptidase 4
ERα  Estrogen receptor alpha
ERβ  Estrogen receptor beta
FABP4  Fatty acid binding protein 4
FFA  Free fatty acids
FGF21  Fibroblast growth factor 21
FKBP5  FK506 binding protein 5
FPG  Fasting plasma glucose
FPI  Fasting plasma insulin
GBR  Genotype-based recall
GLUT1  Glucose transporter 1
GLUT4  Glucose transporter 4
GSV  GLUT4 storage vesicle
GWAS  Genome-wide association studies
HDL  High-density lipoprotein
HOMA-IR  Homeostatic model assessment of insulin resistance
HPA axis  Hypothalamus-pituitary-adrenal axis
HRE  Hormone response element
HSL  Hormone-sensitive lipase
HSP  Heat shock protein
IL1  Interleukin 1
IL6  Interleukin 6
IRS1  Insulin receptor substrate 1
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRS2</td>
<td>Insulin receptor substrate 2</td>
</tr>
<tr>
<td>KCNJ11</td>
<td>Potassium voltage-gated channel subfamily J member 11</td>
</tr>
<tr>
<td>KRH</td>
<td>Krebs-Ringer media</td>
</tr>
<tr>
<td>LCN2</td>
<td>Lipocalin 2</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
</tr>
<tr>
<td>MAF</td>
<td>Minor allele frequency</td>
</tr>
<tr>
<td>MCP1</td>
<td>Monocyte chemoattractant protein 1</td>
</tr>
<tr>
<td>MG</td>
<td>Monoglycerides</td>
</tr>
<tr>
<td>MGL</td>
<td>Monoacyl glycerol lipase</td>
</tr>
<tr>
<td>mTORC2</td>
<td>Mammalian target of rapamycin complexed with Rictor</td>
</tr>
<tr>
<td>OGGT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>PD3B</td>
<td>Phosphodiesterase 3B</td>
</tr>
<tr>
<td>PDK1</td>
<td>Phosphoinositide-dependent kinase 1</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>PI2</td>
<td>Phosphatidylinositol 4,5-bisphosphate</td>
</tr>
<tr>
<td>PI3</td>
<td>Phosphatidylinositol 3,4,5-triphosphate</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein kinase A</td>
</tr>
<tr>
<td>PKB</td>
<td>Protein kinase B</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Peroxisome proliferator-activated receptor gamma</td>
</tr>
<tr>
<td>QUICKI</td>
<td>Quantitative insulin-sensitivity check index</td>
</tr>
<tr>
<td>SH2</td>
<td>Src homology 2</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SVF</td>
<td>Stromal vascular fraction</td>
</tr>
<tr>
<td>T2D</td>
<td>Type 2 diabetes</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>Transcription factor 7 like 2</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>WFS</td>
<td>Wolframin ER transmembrane glycoprotein</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>
Introduction

Obesity and type 2 diabetes

Obesity has emerged as a leading global health concern. The predisposing factors include high-calorie or high-fat diet, physical inactivity, and a shift towards a well-developed sedentary lifestyle (1, 2). As a result, obesity prevalence has nearly doubled worldwide since 1980. According to the World Health Organization (WHO), in 2014, more than 39% of the adults, who were 18 years or older, were overweight with 13% being obese. Moreover, about 41 million children below 5 years were overweight or obese (3). Obesity is a significant risk factor for a number of diseases including type 2 diabetes (T2D), cardiovascular disease, hypertension, or certain types of cancer (4). Of which, T2D is strongly associated with obesity.

The global burden of diabetes is rising steadily, and the number of diagnosed cases worldwide increased from 108 million in 1980 to 422 million in 2014 according to the WHO. The most common types of diabetes are type 1 and type 2. Type 1 diabetes is an autoimmune disease associated with the destruction of pancreatic beta cells with subsequent loss of their ability to produce insulin. The highly prevalent T2D which accounts for about 90% of all diabetes cases is related to insulin resistance at the cellular level together with a relative insulin deficiency. Gestational diabetes develops in some women during pregnancy and usually goes away after childbirth, but may increase the risk of T2D in the future. Other forms of diabetes that are less common include monogenic diabetes, pancreatic or endocrine disease-related, and drug or chemical-induced diabetes (5, 6).

Pathophysiology of T2D

Two main pathophysiological conditions underlie T2D are insulin resistance and beta-cell failure. A common cause of insulin resistance is obesity. Adipose tissue controls metabolic homeostasis by regulating the production and release of free fatty acids (FFA), glycerol, hormones like leptin and adiponectin, and proinflammatory cytokines (7). Dysfunction of adipose tissue, as commonly seen in obesity leads to (1) production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin 1 and 6 (IL1 and IL6) and retinol binding protein 4 (RBP4) and (2) ectopic lipid
deposition into non-adipose tissue such as muscle, heart and liver causing insulin resistance in these organs. Consequently, pancreatic beta cells produce insulin in increasing amounts to overcome insulin resistance in these tissues ultimately lead to their failure causing hyperglycemia and eventually T2D (8, 9).

Genetic component of T2D

Underlying role of both genetic and environmental factors in the pathogenesis of T2D is well accepted. Known environmental factors contributing to the disease progression include physical inactivity, diet, cigarette smoking, generous alcohol consumption, and stress. Intriguingly, these factors do not affect all individuals in the same way (10). Despite similar environmental exposure, some individuals display increased susceptibility to the disease than others, which suggests an underlying inherited predisposition towards the disease. The heritability estimates of T2D varies from 20 to 80% in a variety of population, family, and twin-based studies (11, 12). The estimated risk of developing T2D is 40% and 70%, respectively, in individuals with one or both parents with T2D (13). Furthermore, the concordance rate of T2D is 70% in monozygotic and 20 to 30% in dizygotic twins. Moreover, the prevalence of T2D is different among different populations as with a low prevalence in European Caucasians to about 50% prevalence in Pima Indians in Arizona (14).

Approaches to finding genetic determinants of T2D

Recent years have seen advancement in the development of genetic research tools. Until the discovery of genome-wide association studies (GWAS), genetic linkage and candidate gene approaches were primarily used to identify gene loci associated with the disease. Examples of gene loci about T2D that are identified using genetic linkage approach are CAPN10 (15) and TCF7L2 (16). Whereas IRS1, IRS2, KCNJ11, WFS and PPARγ were identified using a candidate gene approach (17-23). A breakthrough in the identification of genetic loci associated with T2D came after a successful implementation of GWAS. This method screens for single nucleotide polymorphisms (SNPs) across thousands to millions of markers in the genome that occur at a higher (or lower) frequency in individuals with a particular disease compared to individuals without the disease (24). Researchers were thus able to analyze millions of SNPs at the same time. Since GWAS examine SNPs across the whole genome in an unbiased and hypothesis-free manner, this method holds promise to study complex, common diseases where genetic variation is a
contributing factor. To date, GWAS have identified over 150 gene variants associated with obesity and over 100 variants associated with T2D (13, 25).

Genotype-based recall
As described above, over a hundred genetic variants have been identified with different genetic research methodologies, but the fact that remains mostly unresolved is the mechanisms through which a genetic variant alters the phenotype. Advanced molecular techniques have helped to some extent in characterizing critical subphenotypes underlying the disease. However, challenges incur when one is interested in studying the common genetic variants. Because of a modest effect size of common gene variants, the large sample size is required to detect any phenotypic differences. This may not always be feasible considering time and cost-effectiveness of such large studies.

An alternate approach called genotype-based recall (GBR) is gaining popularity these days as it offers a unique way to study genotype-phenotype relationships. In this method, the research participants are selected from a larger cohort based on their genotype to take part in a subsequent study which targets more detailed phenotypic characterization (Figure 1). As the genotype of participants is already known, only the required number of participants can be invited to thorough phenotypic assessment, which otherwise would be infeasible to undertake in an overall cohort. The decision to invite a participant is based on whether a participant is carrying a genotype of interest or not, a comprehensive analysis of the genotype is then stratified. A further advantage of GBR approach is that given similar sample sizes, compared to a random sampling approach, GBR is considerably more potent for the assessment of genetic effects and gene × treatment interactions (26, 27).
Adipose tissue

This thesis focuses on white adipose tissue unless otherwise mentioned.

Originally, adipose tissue was thought merely to be a reservoir to store energy in the form of triglycerides. However, it is now widely regarded as a metabolically active endocrine organ. It produces and secretes numerous signaling proteins, which play a pivotal role in the regulation of metabolic homeostasis (7). Indeed, dysregulation of adipose tissue function is centrally linked to metabolic disorders such as obesity, insulin resistance, T2D, and lipodystrophy (7). Thus, interest in understanding adipose tissue biology has grown over the last decades. Adipose tissue is mainly composed of adipocytes. However, it also contains stromal vascular fraction, which is a composite of different cell types including blood cells, endothelial cells, macrophages, pericytes, fibroblasts and adipose precursor cells (Figure 2).
In adult humans, approximately 10% of total body fat regenerates each year (28). The process which forms new adipocytes is known as adipogenesis. It is a transcriptionally driven process. Two main features of adipogenesis are precursor cell proliferation and its differentiation. The morphological and functional changes in preadipocytes that occur during differentiation are tightly regulated by alterations in the expression and activity of transcription factors such as CCAAT/enhancer-binding proteins (CEBPs) and peroxisome proliferator-activated receptor gamma (PPARγ) (29). These changes ultimately lead to the formation of an adipocyte with a characteristic shape and lipid accumulation (30). The ability of preadipocytes to differentiate into mature adipocytes throughout life enables hyperplastic expansion (increase in cell number) of adipose tissue when storage demand is higher (31).

Similarly, mature adipocytes can undergo hypertrophic expansion (increase in size) and begin to accumulate lipids in an undefined manner to meet increasing storage demand, as in the case of overnutrition, and this is thought to be an initial event that triggers obesity (32). As a result, adipocyte number and morphology changes in response to energy balance via the biochemical processes involved in lipid uptake, esterification, lipolysis, glucose uptake and differentiation of preadipocytes. In humans, the white adipose tissue is located throughout the body in subcutaneous and visceral compartments. Functionally, these two different depots play a distinct role. Accumulation of visceral adipose depot tissue as seen during the progression of obesity eventually is associated with insulin resistance and T2D (33). On the other hand, subcutaneous adipose tissue depots are considered to be offering better glucose tolerance.

Moreover, sex differences exist in adipose tissue biology (34). Women have more body fat than men. However, due to a distinct pattern of fat deposition in women (more in hips and thigh), in contrast to men who accrue fat in the abdomen, they are more protected from metabolic complications. The sexual differences are not only limited to the amount and distribution but also modulate the metabolic capacity and function of adipose tissue (35). For example, adipocyte lipolysis has been shown to be higher in women than men (36-38). Also, women have higher circulating levels of leptin than men, even after correcting for the difference in BMI and body fat content (39, 40). The sexual differences in adipocyte function could be either inherent or regulated by hormones. Regardless, such differences could causally affect the whole body insulin sensitivity and metabolism between men and women.
Figure 2. Adipose tissue composition. Adipose tissue digestion with collagenase can isolate adipocytes. The digested cell suspension contains adipocytes that float and the SVF which contains different types of mature, progenitor and stem cells. SVF; stromal vascular fraction.

Glucose uptake

Cellular glucose uptake provides a critical energy source for mammalian cells and is vital for the effective regulation of energy homeostasis. Insulin is a principal mediator of cellular glucose uptake. After intake of diet rich in carbohydrates, the rise in blood glucose levels leads to secretion of insulin from pancreatic beta cells. Insulin then mediates the uptake of excess circulating glucose in peripheral tissues such as skeletal muscle, liver, and adipose tissue (41-43). Skeletal muscle accounts for most of the glucose uptake which takes up approximately 75 to 80% of free glucose (44). Two primary glucose transporters, glucose transporter 1 (GLUT1) and glucose transporter 4 (GLUT4) serves an important role to facilitate glucose entry into the cell. GLUT1 is expressed ubiquitously, whereas GLUT4 is expressed mainly in insulin-sensitive tissues where it plays a predominant role in insulin-stimulated glucose transport (45).

The insulin receptor is a transmembrane protein. It consists of two extracellular alpha subunits that bind to insulin molecules and two transmembrane beta subunits with cytosolic protein kinase activity (46).
Insulin binding leads to autophosphorylation of tyrosine residues which results in insulin receptor kinase activity towards other substrates such as insulin receptor substrate 1 and 2 (IRS1/2). Phosphorylated IRS1/2 activates phosphoinositide 3-kinase (PI3K) at the plasma membrane leading to initiation of downstream signaling. The PI3K is a heterodimer consisting of regulatory and catalytic subunits, each of which occurs in several isoforms. Binding of Src homology 2 (SH2) domains in the regulatory subunits of PI3K to tyrosine-phosphorylated IRS proteins leads to its activation. The activation of PI3K catalytic subunit rapidly phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP₂) to phosphatidylinositol 3,4,5-triphosphate (PIP₃) localized on the plasma membrane (47). The transient increase in PIP₃ to the plasma membrane in response to insulin receptor activation recruits proteins containing pleckstrin homology (PH) domain to the cell surface. These include serine/threonine protein kinase, protein kinase B (PKB or Akt), and phosphoinositide-dependent kinase 1 (PDK1). Akt, once recruited to plasma membrane it can be activated by phosphorylation on threonine 308 by PDK1 and Serine 473 by mammalian target of rapamycin complexed with Rictor (mTORC2) (48, 49). Numerous studies have demonstrated the importance of Akt signaling in glucose uptake. For example, constitutively active Akt mutant could reiterate the effect of insulin-stimulated GLUT4 translocation and glucose uptake (50). Insulin-stimulated GLUT4 translocation and glucose uptake were shown to be inhibited by dominant-negative Akt mutant, siRNA knockdown, antibodies or Akt inhibitors (51-54). Moreover, PKBβ knockout mice have been shown to develop insulin resistance and T2D, implicating an essential role for this protein in insulin-mediated glucose uptake. Downstream of Akt is an Akt substrate of 160 kDa (AS160), also called TBC1D4 (55, 56).

Activation of Akt phosphorylates AS160 leading to translocation of GLUT4 from the cytosol, which then docks and fuses with the plasma membrane, augmenting an entry of glucose into the cell (Figure 3). Studies have shown a reduction in GLUT4 levels in insulin resistance and T2D (57). Heterozygous GLUT4 knockout mice showed a significant decrease in insulin-dependent glucose uptake in adipocytes (58). Adipocyte-specific GLUT4 knockout mice showed a marked decline in glucose uptake in adipocytes as expected, but it also produced insulin resistance in other organs such as muscle and liver suggesting an essential role of adipocyte GLUT4 in maintaining glucose homeostasis (59). Conversely, transgenic mice overexpressing GLUT4 in adipose tissue are insulin sensitive and glucose tolerant (60).
Figure 3. A simplified overview of pathways involved in insulin-mediated glucose uptake. Insulin binding to its receptor leads to an activation of the downstream signaling cascade. This further causes the translocation of GLUT4 from the cytoplasm to the plasma membrane, which then takes up glucose from extracellular spaces into the cell. AS160; PKB/Akt substrate of 160 kDa, GSV; GLUT4 storage vesicle, GLUT4; glucose transporter 4, IRS1 and IRS2; insulin receptor substrate 1 and 2, mTORC2; mammalian target of rapamycin complexed with Rictor, PDK1; phosphoinositide-dependent kinase 1, PKB; protein kinase B, PI3 K; phosphoinositide 3-kinase, PIP 2; phosphatidylinositol 4,5-bisphosphate, PIP 3; phosphatidylinositol 3,4,5-triphosphate.

Lipid regulation

Systemic FFA levels are determined by the balance between their storage and release from adipose tissue. Impaired postprandial trapping of FFA, as well as their release by adipocyte results in increased FFA levels in circulation (61). This increased circulating lipids then flow to peripheral tissues, and ectopically accumulates in the pancreas, heart, skeletal muscle or liver causing insulin resistance in these tissues. Likewise, in lipodystrophy, a condition associated with loss of body fat, a defective lipid partitioning is linked to insulin resistance, hepatic steatosis, hypertriglyceridemia, impaired insulin secretion and eventually T2D (62). Thus, effective functioning of adipose tissue is essential for efficient lipid partitioning to avoid its spill over into non-adipose tissues.

Postprandially increased FFA are taken up by adipocytes from triglyceride-rich lipoproteins and store them in the form of triglycerides, a process known as lipogenesis (63). Here, adipocyte, via lipoprotein lipase (LPL), degrades the triglyceride-rich chylomicrons and very-low-density lipoprotein to release FFA. The transporter proteins such as fatty acid
binding protein or CD36, which are located at the cell surface then facilitate
the entry of FFA inside the cell. Also, within adipocytes, FFA liberated by
lipolysis can also be re-esterified into newly synthesized glycerol. Triglyceride synthesis requires activation of FFA into Acyl-CoA by the
enzyme acyl-CoA synthetase. Acyl-CoA is then esterified with glycerol-3-
phosphate and undergoes a series of enzymatic reactions to ultimately form
triglycerides that are eventually stored in the lipid droplet (64).

In contrast, under fasting state or during increasing energy demands (e.g.,
after exercise), triglycerides stored in adipocytes are hydrolyzed to FFA and
glycerol, a process known as lipolysis. Fasting acutely stimulates lipolysis,
increasing the serum concentration of FFA and glycerol, which act as oxida-
tive substrates to be utilized by other metabolic tissues. Catecholamines
(e.g., epinephrine) are initiators of fasting-induced lipolysis. They bind to
beta-adrenergic receptors on the plasma membrane of adipocytes. These
receptors are coupled with $G_s$ proteins that transmit a stimulatory signal to
adenyl cyclase to elevate cyclic adenosine monophosphate (cAMP) levels.
cAMP then binds to protein kinase A (PKA), dissociates regulatory subunits
and activates catalytic subunits leading to an increased enzymatic activity of
PKA (65). The activated PKA then phosphorylates serine residues on
hormone-sensitive lipase (HSL) leading to its translocation from the cytosol
to the lipid droplet. PKA activation also causes phosphorylation of perilipin,
a protein that coats lipid droplets, leading to its dissociation from the lipid
droplet, further enhancing lipolysis. In addition to HSL, which was thought
to be the main lipase responsible for triglyceride hydrolysis, Zimmermann et
al. discovered (66) another lipase called adipose triglyceride lipase (ATGL).
ATGL has high substrate specificity for triglycerides in contrast to HSL.

Contrary to murine adipocytes, in human adipocytes, HSL enzyme often
seems to be the major lipase for catecholamine-induced lipolysis, whereas
ATGL appears to play a role in basal lipolysis (67, 68). Studies suggested
that ATGL activation is independent of PKA mediated phosphorylation (66,
69). An activator protein comparative gene identification 58 (CGI 58) has
been shown to regulate ATGL action (70). Both ATGL and HSL carry out
most of the triglyceride hydrolysis. Finally, monoacylglycerol lipase (MGL)
is necessary for the complete hydrolysis of monoglycerides into one FFA
and glycerol molecule which then enters the bloodstream. Insulin, on the
other hand, inhibits lipolysis. It activates phosphodiesterase 3B, which
degrades cAMP, thus inhibits the PKA mediated lipolysis (71). Altogether,
the storage of excess energy in the form of triglycerides and its mobilization
to meet energy demands represents a highly adaptive metabolic response by
adipose tissue. Figure 4 summarizes in a simplified manner the regulation of
lipid storage by adipocytes.
Figure 4. A simplified overview of lipid turnover in the adipocyte. Fatty acids are taken up by adipocyte and are stored in the form of triglyceride in a lipid droplet known as lipogenesis (black arrow). Fatty acids released after lipolysis can also be re-esterified to triglyceride (blue arrow). Activation of beta-adrenergic receptors stimulates PKA mediated triglyceride hydrolysis (shown in green arrows), whereas, insulin, inhibits lipolysis by degrading cAMP (shown red arrows). ATGL, adipose triglyceride lipase, ATP; adenosine triphosphate, Beta ADR; beta-adrenergic receptor, cAMP; cyclic adenosine monophosphate, DG; diglycerides, FFA; free fatty acid, HSL; hormone-sensitive lipase, IRS1 and IRS2; insulin receptor substrate 1 and 2, LPL; lipoprotein lipase, MG; monoglyceride, MGL; monoacyl glycerol lipase, mTORC2; mammalian target of rapamycin complexed with Rictor, PDK1; phosphoinositide-dependent kinase 1, PI3K ; phosphoinositide 3-kinase, PKA; protein kinase A, PKB; protein kinase B, PIP2; phosphatidylinositol 4,5-bisphosphate, PIP3; phosphatidylinositol 3,4,5-trisphosphate, TG; triglycerides.

Adipose tissue-derived hormones - adipokines

Adipose tissue produces and secretes numerous protein factors collectively called adipokines. By their signaling properties, adipokines can act in either an autocrine or a paracrine manner to modulate adipose tissue as well as whole-body metabolism. Within adipose tissue, adipokines have been shown to influence adipogenesis, immune cell infiltration into adipose tissue and adipocyte function. At the systemic level, these signaling proteins have been shown to modulate different biochemical processes in various target organs.
such as the brain, skeletal muscle, liver, pancreas and heart. The physiological processes regulated by adipokines include insulin secretion (e.g., apelin), insulin sensitivity (e.g., leptin, adiponectin and RBP4), glucose metabolism (e.g., leptin, adiponectin, fibroblast growth factor 21 (FGF21) and dipeptidyl peptidase 4 (e.g., DPP4)), lipid metabolism (e.g., apelin), appetite and satiety (e.g., leptin and vaspin), inflammation (e.g., TNFα, IL6 and IL1β), blood pressure (e.g., angiotensinogen), immune cell infiltration (e.g., monocyte chemoattractant protein 1(MCP1)) (72). The production and secretion of adipokines are regulated mainly by the availability of nutrients, hormones and stress conditions (73-75). Altered adipose tissue mass or its dysregulation as seen in obesity or lipodystrophy can change the adipokine secretory profile (76, 77). Since both these conditions are related to the metabolic disorder, it would be essential to characterize the role of adipokines in the pathogenesis of human metabolic disorders. Characterization of the role of adipokines could potentially unravel novel aspects of adipose tissue biology and would be useful in understanding the pathophysiology of multifactorial diseases including T2D (72). Table 1 summarizes a list of a few adipokines with their role in metabolism.

Table 1: A list of adipokines with their role in different biochemical processes. (adapted from (72)).

<table>
<thead>
<tr>
<th>Adipokine</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td>Improves insulin sensitivity, antidiabetic and anti-inflammatory</td>
</tr>
<tr>
<td>Adipsin</td>
<td>Activates the alternative complement pathway</td>
</tr>
<tr>
<td>Apelin</td>
<td>Inhibits insulin secretion</td>
</tr>
<tr>
<td>BMP4</td>
<td>Precursor cell commitment and differentiation</td>
</tr>
<tr>
<td>BMP7</td>
<td>Stimulates brown adipogenesis, reduces food intake and increases energy expenditure</td>
</tr>
<tr>
<td>Chemerin</td>
<td>Chemoattractant protein regulates adipogenesis</td>
</tr>
<tr>
<td>DPP4</td>
<td>Degradation of glucagon-like peptide 1 and gastric inhibitory polypeptide</td>
</tr>
<tr>
<td>FGF21</td>
<td>Promotes adipocyte glucose uptake, thermogenesis, increase energy expenditure and fat utilization</td>
</tr>
<tr>
<td>IL6 and IL1β</td>
<td>Proinflammatory</td>
</tr>
<tr>
<td>Leptin</td>
<td>Controls appetite, food intake and energy expenditure</td>
</tr>
<tr>
<td>MCP1</td>
<td>Involved in adipose tissue inflammation</td>
</tr>
<tr>
<td>Omentin</td>
<td>Anti-inflammatory and insulin sensitizing</td>
</tr>
<tr>
<td>TNFα</td>
<td>Proinflammatory</td>
</tr>
</tbody>
</table>
Nuclear receptors

Nuclear receptors represent a family of ligand-regulated transcription factors. The family comprises receptors for steroids, retinoid, thyroid, vitamin D and other orphan receptors of unknown ligands. Unlike other intracellular messengers, which act in response to receptor activation at the cell surface, ligands for nuclear receptors can permeate through the plasma membrane and directly interact with them inside the cell (78, 79).

Structurally, nuclear receptors share common features (Figure 5). They consist of a variable N-terminal domain that includes different transactivation regions (A/B domain also called AF1 domain). There is also a central highly conserved region (C domain) which contains two zinc fingers motifs. These motifs allow the nuclear receptor to bind to the consensus sequence (also known as hormone response element, HRE) on the target gene as a homodimer or heterodimer with retinoid X receptor (RXR). Followed by the C domain is a short D domain responsible for nuclear localization. The C-terminal ligand binding domain (LBD or the E domain) is involved in ligand binding, receptor dimerization and interacts with co-activators or co-repressors. Some nuclear receptors also possess a short tail towards C-terminal (F domain) with an unknown function (80).

![Figure 5. The structure of the nuclear receptor. AF-1 and AF-2; activation function1 and 2. DBD; DNA binding domain, LBD; ligand binding domain.](image)

Based on their mode of action, nuclear receptors fall into four different types. Type I receptors such as glucocorticoid receptors, estrogen receptors, and androgen receptors reside in the cytoplasm bound to chaperone proteins (e.g., heat shock protein 90). Ligand binding relieves the binding of chaperone protein to the receptor, allowing it to form a homodimer or heterodimer complex which then translocates into the nucleus. Once inside the nucleus, the dimerized complex (active complex) then binds to the HRE in the promoter region of the target gene. The active complex allows the recruitment of transcription co-activators and carries out the transcription of its target gene. Type II receptors, in contrast to type I are present in the nucleus, bound to their specific response element even in the absence of ligand.
These receptors generally form a heterodimer with RXR, and in the absence of ligands are present in the repressive state due to interaction with co-repressor. Ligand binding dissociates the co-repressor and replaces it with the co-activator that helps open chromatin and helps transcribe the target gene. Example of type II nuclear receptors includes thyroid hormone receptor and the retinoic acid receptor. Type III functions similar to type I receptors except they differ in their HRE organization. Type IV receptors bind as monomers to half site HREs (78).

By their transactivation and transrepression ability, nuclear receptors regulate the expression of many different genes involved in adipogenesis, glucose and lipid metabolism and inflammation thereby maintain metabolic homeostasis (81). Given the range of actions of nuclear receptors, their dysregulation can lead to metabolic perturbations (81, 82). As these receptors can bind to small molecules, they are also attractive targets for therapeutic intervention, and therefore, an increasing understanding of these receptors is required. Figure 6 summarizes a brief overview of a classical nuclear receptor signaling.

Figure 6. A classical nuclear receptor signaling. Ligand binding leads to conformational changes and activation of the nuclear receptor, which then binds to the HRE on the target gene to carry out its transcription. HRE; hormone response element, HSP; heat shock protein, NR; nuclear receptor, RXR, retinoid X receptor.
PPARγ

PPARγ is one of the well-known nuclear transcription factors which is also a target of antidiabetic thiazolidinedione compounds such as rosiglitazone and pioglitazone. It is expressed in several different tissues and regulates processes such as bone remodeling, cell differentiation, and inflammation (83, 84). However, the significant actions of PPARγ are confined mainly to adipose tissue. It mediates its insulin-sensitizing effect via increasing adipogenesis, glucose uptake, insulin signaling, lipid storage, stimulating and suppressing the expression of anti and pro-inflammatory adipokines, respectively. Studies have also shown a role of PPARγ in adipose tissue browning (85). Alternate transcription initiation sites and alternative splicing results in two different PPARγ mRNA isoforms such as PPARγ1 and PPARγ2. The difference between these two isoforms is an additional stretch of 28 amino acids towards N-terminal of PPARγ2 (86). PPARγ1 is expressed in most tissues, whereas PPARγ2 is expressed predominantly in fat cells (87).

Pro12Ala and T2D

T2D is a multifactorial disease, and twin studies have clearly shown that T2D underlies a genetic component (88). However, only a few genetic variants associated with T2D have been discovered due to substantial heterogeneity as well as polygeneity among people. Scanning of mutations in the coding region of the PPARγ gene (both isoforms) in type 2 diabetic Caucasians identified a missense mutation (CCGPro→GCGAla) at the 12th codon in exon B of PPARγ2 (89). Functionally, the Pro12Ala variant has reduced ligand binding and transactivation ability. However, it offers protection against the development of T2D via unspecified mechanisms.

Deeb and colleagues in 1998 first reported an association between Pro12Ala and T2D in Japanese-Americans in which the frequency of Ala variant was 9.3% and 2.2% in normal glucose tolerant subjects and T2D patients, respectively (90). However, a challenge with such types of genetic studies is that they lack reproducibility (91). Moreover, it can further result in false positives due to population stratification. To circumvent this issue, Altshuler and colleagues used a robust method of transmission disequilibrium testing in 333 Scandinavian parent-offspring trios with T2D or abnormal glucose tolerance. In their study, of 16 previously published genetic variants associated with T2D or related disorders, only Pro12Ala polymorphism remained significantly associated (92). Two later studies from Finland and Japan re-confirmed an association between Pro12Ala and T2D in over 4000 subjects (93, 94). A recent meta-analysis in 47,456 controls and 32,849 T2D cases from North America, Europe, and East Asia showed that high-risk Pro allele is related with a 16% increase in the risk of T2D (95). In a more recent large-scale association study combining from GWAS and the custom array, Metabochip, in 34,840 T2D cases and 114,981 controls
predominantly of European ancestry confirmed an increase in T2D risk by 13% with deleterious Pro allele (96).

Since the discovery of Pro12Ala, numerous studies have sought to find its association with T2D risk. However, the results are heterogeneous among different ethnic groups, reporting either protective, predisposing or no effect of the Ala variant (97). The most likely explanation for differences between studies is that many are underpowered. Nevertheless, a meta-analysis of these studies including 42,910 subjects showed an average 19% reduction in T2D risk. The body mass index (BMI) seems to be a significant factor determining the effect of Pro12Ala on T2D risk since the risk prevention with Pro12Ala is higher when BMI is lower. Risk reduction in the carriers of Pro12Ala was higher in the Asian population (35%) followed by North Americans (18%) and Europeans (15%) compared to their Pro12 allele controls (98). However, when adjusted for the BMI of controls, the difference between Asians and Europeans was no longer significant. The heterogeneity also observed in the effect of Pro12Ala among Europeans. In Northern Europeans, Ala carriers had T2D risk reduction of 26%, whereas in Central and Southern Europeans it was 10% and 0% respectively (98). While the BMI can explain heterogeneity in the effect of Asians and Europeans, this was not the case in Europeans indicating that other factors such as environmental factors and genetic variations might be important underlying the heterogeneity among European populations. In fact, prior studies have shown that dietary lipids can modulate the protective effect of Pro12Ala. In a study including subjects from Ethiopia, Benin, Ecuador, Italy and world population, protection against T2D among a population where energy from lipids is higher than 30% of total energy intake was observed (99). Moreover, lipid composition of the diet is also a significant factor as long-term consumption of trans fatty acids, and saturated fatty acids can elevate the risk of T2D and impaired glucose tolerance in Pro12Ala carriers over Pro12Pro carriers (100).

Moreover, prior studies have also demonstrated that interaction between Pro12Ala and other genetic variants can influence the risk of developing T2D. Higher plasma adiponectin levels in subjects with Pro12Ala and Gly972Gly variant of IRS1 gene than Pro12Pro with Gly972, Gly genotype was observed (101). In Mexican-Americans, subjects with Pro12Ala become obese only when they also carry the Trp64Arg of Beta-3 adrenergic receptor polymorphism (102). An interaction between Pro12Ala and Trp64Arg of the β3-adrenergic receptor in dizygotic twins was associated with higher BMI, waist to hip ratio and body fat % and blood glucose (103). Such interaction was also shown to attribute an increased risk of obesity in children and adolescents (104). Moreover, an interaction between Pro12Ala and a single nucleotide polymorphism in PGC-1α (Gly482Ser) has been reported to contribute to the shift from impaired glucose tolerance to T2D (105). Altogether, these studies highlight the importance of considering other gene mutations
and their interactions with Pro12Ala when determining its effect on T2D risk.

**Pro12Ala and BMI**

Soon after the discovery of Pro12Ala, an independent study suggested that this polymorphism results in decreased transactivation ability of PPARγ2 and a lower BMI (90). These findings were in agreement with the later shown inhibitory effect of Pro12Ala on the differentiation capacity of 3T3-L1 cells with regards to its decreased transcriptional ability (106). However, later studies in different ethnic subgroups showed that the effect of Pro12Ala on BMI is somewhat perplexed. A relationship between Pro12Ala and decreased BMI was confirmed in diabetic, non-diabetic and healthy individuals (107-110).

Moreover, the inverse association was observed between Pro12Ala and BMI in African American and white American population suggesting that the same genetic mutation has a different role in different ethnic populations (111, 112). However, some studies in different ethnic subgroups indicated a higher BMI in carriers of the PPARγ Pro12Ala (113). Meta-analysis of 57 studies on non-diabetic subjects further confirmed the positive association between Pro12Ala and BMI and also indicated that the effect of Pro12Ala on BMI was highest among Caucasians (115). These results indicate that a moderate reduction in transcriptional activity of PPARγ2 because of Pro12Ala mutation can have a significant impact on lipid accumulation in adipose tissue. However, the contrasting association between increased BMI and decreased risk of T2D can be explained by that the Pro12Ala might favor the lipid accumulation in relatively safer adipose tissue depot (subcutaneous adipose tissue) or by increasing adipogenesis which would form small and more insulin sensitive adipocytes.

It is not clear how Pro12Ala results in different effect in different ethnic groups. A possible reason could be that most studies were smaller with respect to the minimal variance explained by the Ala variant. Given the pro-adipogenic role of PPARγ (116), one could expect that the modest reduction in the transcriptional activity of PPARγ2 due to Pro12Ala can result in lower BMI. This further clearly shows that the regulation of PPARγ2 in human adipose tissue due to polymorphic mutation is complex. Several studies suggest that genetic and environmental factors would be critical in regulating the effect of Pro12Ala on adiposity. Prior studies have shown the significant effect of the ratio of dietary polyunsaturated fatty acid to saturated fatty acid (P:S ratio) on BMI in Ala12 allele carriers. Thus, intake of a diet containing higher P:S ratio results in lower BMI, whereas lower P:S ratio was inversely related with BMI in carriers of the Ala12 allele (117). Likewise, intake of 26
monounsaturated fatty acid also showed such an effect in Ala12 allele carriers (118, 119). In another study, Pro12 homozygotes were associated with higher BMI with increased consumption of total fat and saturated fat, while Ala12 homozygotes remained protected (120). A recent review by Lapice and Vaccaro (121) nicely summarized an interaction between gene-diet. This review concluded that carriers of the Ala allele might be more susceptible to weight gain when exposed to the obesogenic environment (i.e., high energy, high carbohydrates and to some extent high fat diet). In addition to diet-gene interactions, the presence of other polymorphisms can influence the effect of Pro12Ala on BMI. For example, either Pro12Ala or G174C in the promoter region of IL6 gene have been shown to reduce body fat mass or prevent weight gain after body weight loss and the presence of both variants has an additive effect (108, 122). On the other hand, subjects carrying both Pro12Ala and Trp64Arg mutation of the β3-adrenergic receptor are more prone to develop obesity when compared to those bearing only a single mutation in a case-control study (104), whereas Ala12 allele carriers become more obese only when they also carry the Trp64Arg variant in a Mexican American population (102). These data suggest complex interactions between genes that both affect lipid metabolism.

To date, most of the studies are based on the association between PPARγ Pro12Ala and anthropometric and metabolic variables. However, the cellular mechanisms underlying the protective effect of PPARγ Pro12Ala remain unclear. Considering the expression of PPARγ2 which harbor Pro12Ala mutation is exclusive to adipose tissue (123), any protective effects mediated by this polymorphism would be expected to be secondary to alteration in adipose tissue metabolism.

**Glucocorticoids**

Glucocorticoids constitute a major subclass of steroid hormones that regulate metabolic, cardiovascular, immune and behavioral processes (124). The anatomical drivers of stress response located in the central nervous system and peripheral tissues control the systematic release of glucocorticoids. Main effectors of stress response are present in the paraventricular nucleus (PVN) of the hypothalamus, the anterior lobe of the pituitary gland and the adrenal gland. Together these structures are referred to as the hypothalamic-pituitary-adrenal (HPA) axis. Hypophysiotropic neurons located in the medial parvocellular subdivision of the PVN produces and secretes corticotropin-releasing factor (CRF), the central regulator of the HPA axis. In response to stress, CRF is released into hypophysial portal vessels that access the anterior pituitary gland. Binding of CRF to its receptors on pituitary corticotropes stimulates the release of adrenocorticotropic hormone (ACTH) into the systemic circulation. The primary target for ACTH is the adrenal cortex where it induces glucocorticoid synthesis and secretion. Glucocorticoids are down-
stream effectors of the HPA axis and regulate physiological changes via its ubiquitously present intracellular receptors. The biological effects of glucocorticoids are usually adaptive; however, uncontrolled activation of the HPA axis may contribute to the development of pathologies (125).

In humans, cortisol is an endogenous glucocorticoid. Its elevated levels as seen in Cushing's syndrome or chronic exposure to glucocorticoids results in a variety of metabolic complications including distribution of peripheral fat to intra-abdominal areas, hyperlipidemia, insulin resistance and T2D (126, 127). Glucocorticoids are widely used for their anti-inflammatory and immunosuppressive properties. The incident rate of glucocorticoid-induced hyperglycemia has been reported to be 12% (128). In patients on chronic glucocorticoid treatment after organ transplantation, the prevalence of abnormal glucose metabolism has been reported to be 17 to 32% (129). Moreover, Gulliford and colleagues (130) reported that in primary care population 2% of incident cases of diabetes were on oral glucocorticoid treatment. A recent meta-analysis suggested that the rates at which patients on glucocorticoid treatment develop hyperglycemia and diabetes were 32.3% and 18.6%, respectively (131).

In adipose tissue, ex vivo treatment with a synthetic glucocorticoid, dexamethasone has been shown to reduce glucose uptake and increase lipolysis in isolated adipocytes, although the latter effect was observed only in adipose tissue obtained from women donors (132-135). The above effects of glucocorticoid on the metabolic activities of adipose tissue may be in part by regulating the downstream target genes such as FKBP51 and CNR1 (133, 136). Thus, identifying and characterizing the role of the adipose tissue-specific glucocorticoid-regulated genes may provide novel mechanisms underlying metabolic disease and could be used for therapeutic interventions.

Estrogen

Estrogens are synthesized from cholesterol primarily in ovaries which represents a significant source of circulating estrogens in pre-menopausal women. In addition, the placenta also contributes to a large extent to the circulating estrogens levels during pregnancy (137).

Amid the fertile years of women life, estrogens production in ovaries is regulated by the feedback mechanism that involves the hypothalamic – pituitary – ovarian – axis. Estrogens occur in three primary physiological forms such as estrone (E1), estradiol (E2, or 17-β-estradiol), and estriol (E3). E2 is a primary product of the whole biosynthesis and is also the most active estrogen in pre-menopausal women. E1 is vital after menopause. The least potent form E3 is derived from E1 and plays a crucial role during pregnancy when the placenta abundantly produces it. Estrogen degradation occurs via its metabolism. E2 converts to less potent forms E1 and E3 and sulfation of
E2 by estrogen sulfotransferase to a metabolite which can no longer interact with estrogen receptors (ERs). Moreover, a deficiency of lipocalin 2 (LCN2), a recently characterized adipokine can limit E2 synthesis by aromatase in adipose tissue in female mice (138). Thus, the circulating levels of estrogens are determined by the balance between their synthesis and degradation.

With menopause and cessation of ovarian function, a drop in systemic estrogens levels is compensated by non-gonad organs mainly adipose tissue and liver. Estrogens are formed by aromatization of androgen catalyzed by enzyme aromatase (CYP19A1) (139). Aromatase is expressed in the gonadal tissue of both sexes (139). Estradiol is synthesized from testosterone, and estrone is produced from androstenedione. In humans, it has been demonstrated that in subcutaneous adipose tissue, aromatase activity, as well as its mRNA expression, increases with age (140). Likewise, in ovariectomized rats, the activity of aromatase increases with time in subcutaneous adipose tissue and liver (141). Furthermore, glucocorticoids, IL6, and TNFα can also increase aromatase activity (142-145). Therefore, the production of estrogen in extra-ovarian tissues in postmenopausal women is a result of the availability of androgen substrate and activity of metabolizing enzyme. Hence, the circulating levels of estradiol in post-menopausal women merely reflect its peripheral production rate.

**Estrogen signaling**

Estrogen mediates its actions mainly via ERα and ERβ. These nuclear receptors act as ligand-regulated transcription factors. By their transactivation or transrepression ability, these receptors regulate the expression of numerous genes and hence control a variety of biochemical processes in target tissues (137). Additionally, ERs may act via non-genomic or ligand-independent pathways (137). ERα and ERβ often have opposite effects, and when co-expressed, ERβ may inhibit ERα signaling (146). Thus, the balance between these two receptors may be necessary for estrogen signaling. Human adipose tissue expresses both ERα and ERβ (147-149). ERβ expression was reported to be higher in subcutaneous adipose tissue than the visceral adipose depot. The expression of ERα was similar in subcutaneous and visceral adipose tissue depots (148, 149). Moreover, only ERα is expressed in preadipocytes, whereas both ERα and ERβ are expressed in mature adipocytes. To date, most studies have attempted to understand the contribution of ERα in adipose tissue and implicated its protective role against metabolism disturbances. In contrast, very few studies have investigated the role of ERβ in adipose tissue metabolism. A few animals and human studies have suggested its pro-diabetogenic actions (150-153).

In ER knockout (ERKO) mice, studies imply that ERα is obesogenic, while ERβ produces anti-obesity effects (154). Thus, αERKO mice, which have an intact ERβ signaling, had increased body weight, adipose tissue
mass, hyperplasia and hypertrophy (154, 155). Moreover, αERKO mice also had increased plasma estrogen levels, which could essentially promote ERβ signaling (156). Additionally, ovariectomized αERKO mice had reduced body weight; however, this was counteracted with estrogen treatment (154, 157). In contrast, βERKO mice had an average body weight (154). Double ER knockout mice and aromatase knockout mice lack estrogen signaling altogether due to ER or estrogen deficiencies, respectively. These mice become obese with increased intra-abdominal fat depots (154, 158).

**Lipocalin 2**

Lipocalin 2 (LCN2) also known as neutrophil gelatinase-associated lipocalin, siderocalin, 24p3, and uterocalin is a member of the lipocalin superfamily. The members of the lipocalin family share a common tertiary structure made up of segments called lipocalin folds (159). These folds consist of 8 antiparallel β-sheets that surround a hydrophobic pocket and allow lipocalins to function as transport or carrier proteins. LCN2 was first isolated from human neutrophils and was shown to have its role in innate immune response to infection by binding to iron-laden bacterial siderophores and therefore limiting bacterial grown (160). In 2007, Wang and colleagues reported a positive association of LCN2 with obesity, insulin resistance, and inflammation in mice and humans (161). In the following studies, LCN2 has been characterized as an adipose-derived cytokine, and its deficiency has been shown to attenuate insulin resistance and related comorbidities in mice (161, 162). In humans, LCN2 gene expression was shown to be higher in adipose tissue obtained from obese individuals (163, 164). Pro-inflammatory cytokines such as TNFα, IL6, and lipopolysaccharides or agents that promote insulin resistance such as dexamethasone, can increase LCN2 expression in adipose tissue, whereas thiazolidinediones can suppress it (165-167). Moreover, in T2D, circulating levels of LCN2 have been reported to be elevated (161, 168, 169).
Aim

The overall aim of this thesis was to explore the role of nuclear receptors, mainly, glucocorticoid and estrogen receptors, and PPARγ and their interplay in the regulation of metabolic function or dysfunction in human adipose tissue.

The specific aims are as below.

**Paper I:** To study the regulation of LCN2 expression in adipose tissue by glucocorticoids. To explore the effect of LCN2 on glucose and lipid metabolism in adipose tissue. To study the effect of LCN2 on metabolic and pro-inflammatory genes in adipose tissue.

**Paper II:** To assess the direct effect of estrogen on LCN2 expression in subcutaneous adipose tissue obtained from post-menopausal women. Further, to explore any possible relationship between estrogen and glucocorticoids in the regulation of *LCN2* gene expression in adipose tissue.

**Paper III:** To test the feasibility and practical implications of using a GBR approach by performing comprehensive clinical phenotyping of individuals with PPARγ Pro12Ala variant.

**Paper IV:** To investigate the adipose tissue related mechanisms underlying the effect of PPARγ Pro12Ala variant.
Methods

Ethical approval for studies

*Paper I and II*

The Regional Ethics Review Boards in Gothenburg (Dnr 336-07) and Uppsala (Dnr 2013/330 and Dnr 2013-183/494) approved the study protocol.

*Paper III and IV*

The Regional Ethics Review Board in Uppsala (Dnr 2013/246) approved the study protocol. All study subjects provided their written informed consent.

Subjects and adipose tissue samples

Subjects with diabetes and other endocrine disorders, cancer or other major illnesses, as well as ongoing treatment with beta-adrenergic blockers, systemic glucocorticoids or immune-modulating therapies, were excluded from all studies.

*Paper I*

Paired subcutaneous and omental adipose tissue samples were obtained from non-diabetic subjects that underwent kidney donation or bariatric surgery (7 men/14 women, mean age 19-76 years, BMI 23.9-55.8 kg/m²). Also, subcutaneous adipose tissue was obtained from non-diabetic volunteers (8 men /26 women, mean age 20-77 years, BMI 20.2-37.3 kg/m²) by needle biopsy from the lower part of the abdomen after administration of local anesthetics Xylocaine; AstraZeneca, Sweden.

*Paper II*

Subcutaneous adipose tissue was obtained from two different cohorts by needle biopsy. In the first cohort, adipose tissue was obtained from pre- (n=23, mean age 32 ± 9 years, BMI 31.5 ± 3.1 kg/m²) and post-menopausal (n=51, mean age 66 ± 5 years, BMI 26.1 ± 3.2 kg/m²) women and men (n=32, mean age 51 ± 17 years, BMI 29.2 ± 9.5 kg/m²). The tissue was snap-frozen in liquid nitrogen and stored at -80 °C to measure LCN2 gene expres-
sion. In the second cohort, the adipose tissue was obtained only from post-menopausal women (n=48, mean age 67 ± 7 years, BMI 27.3 ± 5.6 kg/m²) and exclusively used for ex vivo incubations.

*Paper III and IV*

2,500 participants were genotyped from the EpiHealth cohort in Uppsala. From this, 40 age and sex-matched Caucasians were recruited based on their carrier status for PPARγ Pro12Ala polymorphism. Out of 40 that agreed to participate, 12 were Pro homozygotes (Pro/Pro, 4 men /8 women, mean age 64 ± 9 years, BMI 26.8 ± 3.3 kg/m²), 15 were Pro/Ala heterozygotes (Pro/Ala, 6 men /9 women, mean age 63 ± 9 years, BMI 24.3 ± 3.2 kg/m²), and 13 were Ala homozygotes (Ala/Ala, 4 men/9 women, mean age 64 ± 8 years, BMI 26.6 ± 3.6 kg/m²), respectively. Subcutaneous adipose tissue was obtained by needle biopsy upon fasting and after a 3 h oral glucose tolerance tests (OGTT). Part of the tissue was snap-frozen in liquid nitrogen and stored at -80 °C, and the other part was used to perform ex vivo metabolic assays including glucose uptake and lipolysis, and adipogenesis and cell size measurement.

**Anthropometric and biochemical measurement**

*Papers I-IV*

All subjects completed a detailed investigation of their medical history and a physical examination, including height (cm), weight (kg), waist (cm) and hip (cm) circumference, which was carried out according to the WHO guidelines. Body composition was determined by bioelectrical impedance (Kroppsanalysator BC-418MA, Tania). BMI was calculated as weight (kg) divided by height (m²). Also, the fasting baseline blood sample was used for the analysis of glucose, insulin and lipid profile (triglyceride, total cholesterol, high-density cholesterol [HDL], and low-density lipoprotein [LDL]). Blood plasma and serum samples were analyzed at the Department of Clinical Chemistry at Sahlgrenska University Hospital or Uppsala University Hospital. Fasting glucose and insulin values were used to calculate a homeostatic model assessment of insulin resistance (HOMA-IR), which was used as an estimate of insulin resistance (170). In addition, glucose, insulin, and non-esterified fatty acids (NEFA) levels obtained during OGTT were used to calculate insulin sensitivity indices (ISI), which were determined by the Matsuda Index, and a revised quantitative insulin sensitivity check (QUICKI) (171, 172).

*Paper III*
A 3 h OGTT (75 g oral glucose load) was performed, and blood samples for analysis of glucose, insulin, NEFA and glycerol were drawn at baseline, 15, 30, 60, 90, 120, and 180 min. NEFA was measured using NEFA fluorometric assay kit (Cayman Chemicals, Ann Arbor, MI, USA), and glycerol was quantified using free glycerol reagent (Sigma Chemicals Co., St Louis, MO, USA). Plasma glucose at 2 h after glucose load was used as an additional indicator of insulin resistance (173).

Adipose tissue incubation

Paper I

Subcutaneous and omental adipose tissue samples obtained from surgical biopsies were cut into small pieces (approximately 5 to 10 mg). Tissue was incubated with Dulbecco's Modified Eagle Medium (DMEM) containing 6 mM glucose (Invitrogen Corporation, Paisley, USA), 10% fetal calf serum (FCS, Invitrogen) and 1% penicillin-streptomycin (PEST, Invitrogen) in the presence or absence of dexamethasone (Sigma) at concentrations ranging from 0.003 to 3 μM for 24 h at 37 °C and 5% CO₂. At the end of the incubation period, tissue was collected and snap-frozen for further analysis of LCN2 gene expression. Likewise, subcutaneous adipose tissue was incubated only with 0.3 μM dexamethasone to study its effects on LCN2 protein levels. Also, subcutaneous adipose tissue obtained by needle biopsy was incubated in DMEM medium with or without recombinant human lipocalin 2 (rhLCN2) 100 ng/ml for 24 h as described above. After incubation, a part of the adipose tissue sample was snap-frozen in liquid nitrogen for the gene, and protein expression and portion were digested with collagenase (Sigma) as described below. Isolated adipocytes were then filtered through a 250 μm pore size nylon mesh and used to perform glucose uptake and lipolysis and cell size measurement. In addition, isolated adipocytes were also used to study the effect of short-term incubation with rhLCN2 (30 min) on glucose uptake capacity. Moreover, subcutaneous adipose tissue from needle biopsies was further incubated for 24 h with dexamethasone to assess its effect on LCN2 gene expression.

Paper II

Subcutaneous adipose tissue obtained from needle biopsies was incubated as described above in phenol red-free DMEM in the presence or absence of E2 (0.01 to 100 nM, Sigma), dexamethasone (0.3 μM) or the ERβ agonist 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN, 100 nM, Tocris) and co-incubated with E2 (1 nM) with either the ERα antagonist 1,3-bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy) phenol]-1H-pyrazole dihydrochloride (MPP, 100 nM, Sigma) or the ERβ antagonist 4-[2-phenyl-5,7-
bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol (PHTPP, 100 nM, Sigma), or with E2 (1 nM) and dexamethasone (0.3 µM) with either ERα antagonist, MPP 100 nM or ERβ antagonist, PHTPP 100 nM. At the end of the incubation period, tissue was collected, and snap-frozen in liquid nitrogen for further analysis of gene or protein expression.

Isolation of adipocytes

Adipose tissue was digested in a shaking water bath at 37 °C for 1 h with collagenase A (1.2 mg/ml) obtained from bacteria *Clostridium histolyticum* (Roche). Digestion was performed using Hank’s media added with 4% bovine serum albumin (BSA, Sigma), 150 nM adenosine (Sigma) and pH 7.4. After digestion, the cell suspension was filtered through a 250 µm pore size nylon mesh and rested for 5 min until adipocytes float. Adipocytes were isolated by aspirating the infranatant, which then collected into a separate tube. The infranatant was then used for the isolation of stromal vascular fraction (SVF) as described below. Isolated adipocytes were used to perform metabolic assays such as glucose uptake and lipolysis.

*Ex vivo* glucose uptake in primary adipocytes

*Paper I and IV*

Isolated adipocytes were washed three times with 5 min intervals in glucose-free Krebs–Ringer media (KRH) supplemented with 4% BSA, 150 nM adenosine and pH 7.4. Adipocytes were then diluted ten times in KRH media and incubated with or without insulin (25 and 1000 μU/ml, Actrapid, Novo Nordisk, Bagsvaerd, Denmark) for 15 min at 37 °C in a gently shaking water bath, followed by an additional 45 min of incubation with D-[U-14C] glucose (0.26 mCi/L, 0.86 µM, Perkin Elmer, Boston, MA, 161 USA). The reaction was stopped by transferring the cell suspension into pre-chilled vials followed by separation from the medium by centrifugation through silicon oil (SERVA Electrophoresis GmbH, Heidelberg, Germany). Radioactivity associated with cells was then determined using a scintillation counter. Cellular glucose uptake was mainly determined by the rate of transmembrane glucose transport using the following formula: cellular clearance of medium glucose = (cell-associated radioactivity × volume) / (radioactivity of medium × cell number × time).
**Ex vivo lipolysis in primary adipocytes**

*Paper I and IV*

Isolated adipocytes were washed three times with 5 min intervals in Hank’s media supplemented with 4% BSA, 150 nM adenosine and pH 7.4. Cells were then suspended (3–5% lipocrit) in vials containing Hank's medium and incubated in a gently shaking water bath at 37 °C for 2 h to measure basal lipolysis. Also, to measure stimulated lipolysis and antilipolytic effect of insulin, the medium was supplemented with isoproterenol (0.5 μM, Sigma) or isoproterenol (0.5 μM) and insulin (0.1 to 100 μU/ml), respectively. The reaction was stopped after 2 h by transferring the vials to ice. Glycerol released into the medium was assessed by a quantitative enzymatic reaction using Free Glycerol reagent (Sigma), and absorbance was read at 540 nm using a microplate reader (Infinite®200, Tecan, and Männedorf, Switzerland). Glycerol released into the medium was taken as an index of lipolysis and normalized for 10⁵ cells.

**SVF isolation and adipogenesis**

*Paper IV*

**Isolation and culture of human SVF**

After adipose tissue digested with collagenase, the SVF containing preadipocytes was separated from mature adipocytes into a Falcon tube. The SVF was centrifuged at 1200 RPM for 3 min, and the pellet was cultured in preadipocytes medium containing DMEM-F12 supplemented with 10% FCS, 1% PEST, and 17 ng/ml basic fibroblast growth factor (bFGF, Sigma) at 37 °C. Media was replaced after every 2 days.

**Ex vivo differentiation of preadipocytes**

Differentiation was induced when preadipocytes reached 100% confluence by adding a differentiation cocktail containing DMEM-F12, 1% PEST, 100 nM insulin, 17 μM pantothenate (Sigma), 33 μM biotin (Sigma), 1 μM dexamethasone, 1 μM rosiglitazone (Sigma), 250 μM 3-isobutyl-1-methylxanthine (IBMX, Sigma), 10 μg/ml transferrin (Sigma), 2 nM triiodothyronine (T3, Sigma) for 5 days. After 5 days, the differentiation cocktail was replaced by maintenance media (composition is the same as that of the differentiation cocktail except for IBMX) to continue differentiation until 14 days. The medium was replenished every 2 days. To assess the differentiation rate the expression levels of differentiation markers were measured in cells collected at the confluence and 2, 4, 8 and 14 days post induction. On the 14th day of differentiation, media was removed, and cells
were washed with PBS (Medicago, Uppsala, Sweden) and fixed with 4% formaldehyde (Histolab, Gothenburg, Sweden) for 30 min at room temperature. The cells were again washed two times with PBS and lipid was stained with 0.5% red oil dissolved in isopropanol (Sigma) for 30 min. After washing, nuclei were counterstained using 20 µM 2-(4-aminophenyl)-1H -indole-6-carboxamidine (DAPI, Thermo Fisher). The images of differentiated cells were acquired from three different randomly selected areas under a microscope (Evos, Invitrogen). The total area covered by red oil (lipid stains) and a number of nuclei stained with DAPI were measured. The percent differentiation was calculated using the following formula: percent differentiation = area covered by lipid/number of nuclei ×100.

Genetic analysis

*Paper III*

**Genotyping**

Genotyping in the EpiHealth cohort was carried out with the Illumina HumanCoreExome-12 v1.0 BeadChip including 522,731 autosomal markers. The genotype data were called using Illumina GenomeStudio 2011.1 GenCall followed by zCall version 3.3 (174). Sample exclusion filters applied prior to the genotype calling with zCall were: (1) discordant sex information when comparing reported sex and sex determined by the X-chromosome; (2) outlying, non-European ancestry based on the first two components in a multidimensional scaling analysis (>3 standard deviations [SD] from the mean); (3) outlying heterozygosity rate (>5 SD from the mean based on markers with a minor allele frequency [MAF] <1% or markers with MAF ≥1%); and (4) low sample call rate (<98%). Markers with a call rate <97%, a Fisher’s exact test p-value for Hardy–Weinberg equilibrium <10^{-4}, a cluster separation score <0.4, or a GenTrain score <0.6 were also excluded. After genotype calling with zCall, markers with a call rate <99% or a Fisher’s exact test p-value for Hardy–Weinberg equilibrium <10^{-4} were also excluded. In total, 2432 samples passed the quality control, and 2378 samples remained after further exclusion of related individuals. All quality filters were applied using PLINK v.1.0.7 (175).

*Paper I-II and IV*

**Quantitative real-time PCR**

Total RNA from adipose tissue was harvested using the RNeasy lipid tissue mini kit (Qiagen, Hilden, Germany) and reverse transcribed using High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Foster City, CA, USA). The protocol was carried out as per manufacturer guidelines. The
concentration and purity of total RNA were measured with the Nanodrop (Thermo Scientific) to ensure the reliability of subsequent RNA processing and mRNA assessments. TaqMan gene expression assays (Thermo Fisher) were used to study the expression levels of different transcripts. The gene expression was detected using the QuantStudio 3 sequence detection system (Applied Biosystems). The data were calculated using a relative standard curve method or 2-delta Ct as specified wherever necessary. The results are plotted as relative quantification using 18S ribosomal RNA (18S rRNA) (Paper I, II and IV) or glucuronidase beta (GUSB) (Paper II and IV) as an endogenous control. Both are standard housekeeping genes and have been validated in studies of human adipose tissue (176, 177). For adipogenesis assays, GUSB was exclusively used given high variability in the expression of 18S rRNA in response to treatment with differentiation cocktail. In addition, GUSB has also been shown to be stably expressed during preadipocyte differentiation [45]. All samples were run in duplicates.

Immunoblotting analysis of adipose tissue

Paper I and II

Adipose tissue was processed according to the previously described method (165). Briefly, after incubation with or without different compounds as described above, tissue was washed three times with PBS and homogenized in ice-cold lysis buffer (25 mM Tris-HCl (Sigma), pH 7.4; 0.5 mM EGTA (Sigma); 25 mM NaCl (Sigma); 1% Nonidet P-40; 1 mM Na3Vo4; 10 mM NaF; 100 mM okadaic acid (Alexis Biochemicals, Lausen, Switzerland), 1X Complete protease inhibitor cocktail (Roche, Indianapolis, IN, USA) and 1 mM orthovanadate (Sigma). Next, the samples were rocked for 2 h at 4 ºC and centrifuged at 12000g for 15 min at 4 ºC. The lysate was collected, and protein concentration was measured using BCA protein assay kit (Pierce, Thermo Scientific, Rockford, IL, USA). SDS-PAGE separated (4−15% gradient) proteins (15 or 20 μg) were transferred to nitrocellulose membranes and blocked for 1 h at room temperature with 0.05% tween-phosphate buffer saline (Medicago, Uppsala, Sweden) with 5% non-fat dry milk (Biorad). Membranes were incubated overnight with primary antibodies: anti-GLUT1, anti-GLUT4, anti-phospho-AKT Ser473, anti-phospho-AKT Thr308, anti-AKT, anti-estrogen receptor α (Cell Signaling Technology, Beverly, MA, USA) or anti-lipocalin 2 (R and D Systems, Minneapilos, MN, USA). Antiglyceraldehyde-3-phosphate dehydrogenase (GAPDH, Millipore, and Temecula, CA, USA) was used as a loading control protein. Membranes were then washed with phosphate buffer saline with 0.05% tween and incubated with appropriate horseradish peroxide conjugated anti-rabbit (Cell Signaling Technology) or anti-goat (R and D Systems) secondary antibody. Immu-
noreactive protein bands were then visualized using enhanced chemiluminescence with the high-resolution field (ChemiDoc™ MP System, Bio-Rad) and quantified with Image Lab software, Biorad.

Cell viability assay

*Paper I*

Water-soluble tetrazolium-colorimetric assay (WST-1, Roche) was used to study the effect of rhLCN2 treatment on adipocyte viability. After 24 h of incubation with rhLCN2, adipose tissue was digested with collagenase, and isolated adipocytes were placed in a 96 well plate at 3-5% lipocrit in Hank's medium supplemented with 5.6 mM glucose, 4% BSA, 150 nM adenosine with pH 7.4. The WST-1 reagent was added to each well, and the cells were incubated for 30 min, 1 h and 3 h and absorbance was read at 450 nm.

Statistical analyses

IBM SPSS statistical software version 22 (*Paper I-II and IV*) or STATA 13.1 (StataCorp LP, College Station, TX, USA) or R 3.0.0 was used (*Paper III*) and all data are presented as mean ± SEM unless otherwise indicated. All data were first checked for the distribution of normality using Shapiro-Wilk test and visual inspection of a histogram (*Paper I-IV*). In some cases, to promote neutrality, the data were log transformed (*Paper III*). Associations between a number of minor alleles and phenotypes were tested under an additive model using linear regressions adjusted for age, sex, and ancestry components (*Paper III*). Comparison between two paired samples was made using a paired t-test or Wilcoxon test for normally and non-normally distributed data, respectively (*Paper I, II and IV*). Comparisons of glucose uptake, lipolysis, gene expression and adipocyte size between Pro/Pro, Pro/Ala, and Ala/Ala groups were done using one-way ANOVA or Kruskal-Wallis test depending upon normally and non-normally distributed data, respectively. Analysis of repeated measures was done using Friedman's test for nonparametric data. Bivariate tests for paired or non-paired analysis was then used as appropriate, for post-hoc testing. Association between dexamethasone-induced *LCN2* gene expression (*Paper I*) and Dexamethasone-induced *ERα* gene reduction (*Paper II*) was done using Spearman’s correlation. A p-value <0.05 was considered to be statistically significant.
Results

Paper I

Lipocalin 2 produces insulin resistance and can be upregulated by glucocorticoids in human adipose tissue

Dexamethasone treatment increased \( LCN2 \) gene expression by ~3- and ~4-fold in subcutaneous and omental adipose tissue, respectively, obtained from pre-menopausal women, but not from post-menopausal women or men. Dexamethasone also upregulated LCN2 protein expression by ~3.5-fold in subcutaneous adipose tissue from pre-menopausal women. Furthermore, the dexamethasone-induced \( LCN2 \) gene expression was positively correlated with HbA1c and fasting plasma glucose in subcutaneous and omental adipose tissue, respectively. Under non-treated incubation, \( LCN2 \) gene expression in both adipose depots was positively associated with BMI, fasting plasma insulin and glucose, HOMA-IR, subcutaneous and omental adipocyte size and negatively with HDL-cholesterol.

Within adipose tissue, SVF constituted the majority of LCN2 protein levels than isolated primary adipocytes. Subcutaneous adipose tissue from both genders treated for 24 h with rhLCN2 significantly inhibited basal and insulin-stimulated glucose uptake in isolated adipocytes by about 30%. At the molecular level, rhLCN2 treatment decreased the expression of GLUT1 and GLUT4 glucose transporters by ~40% in subcutaneous adipose tissue. Additionally, rhLCN2 treatment increased the gene expression of pro-inflammatory cytokine \( IL6 \) by 120%, while it decreased the expression of \( PPAR\gamma \) by 22% and \( ADIPOQ \) by 32%. rhLCN2 treatment did not affect lipolysis in isolated adipocytes.

Paper II

Effect of estrogen and its interaction with glucocorticoids in the regulation of lipocalin 2 in human adipose tissue: a role for estrogen receptor \( \beta \) pathway in insulin resistance?

In subcutaneous adipose tissue from post-menopausal women, a 24 h E2 treatment dose-dependently increased \( LCN2 \) gene expression by 3.5-fold.
Moreover, E2 also increased LCN2 protein levels by 2.7-fold when compared to untreated control.

ERα antagonist alone increased the E2 mediated LCN2 gene expression levels by 4.8-fold. However, ERβ antagonist abrogated this effect by ~75% when compared to ERα antagonist, and 40% when compared to E2 alone.

Dexamethasone, alone or together with E2, showed only a non-significant trend to increase LCN2 gene expression when compared to control. However, dexamethasone together with E2 significantly increased LCN2 gene expression by about 20-fold in the presence of ERα antagonist, but not in the presence of ERβ antagonist. Furthermore, adipose tissue treated with ERβ-specific agonist, DPN, showed a 3.5-fold increased LCN2 gene expression than that of control.

E2 treatment did not affect ERα gene or protein expression in adipose tissue from post-menopausal women when compared to that of control. However, E2 increased ERβ gene expression in adipose tissue by 1.8-fold. Dexamethasone exposure decreased ERα gene and protein expression by 1.25- and 1.5-fold, respectively. In contrast, dexamethasone increased the expression of ERβ gene by 2.3-fold.

Dexamethasone-induced reduction of ERα gene expression was positively correlated with waist circumference. Whereas, no significant association was found for dexamethasone-induced ERβ gene expression with any of the anthropometric or biochemical variables assessed.

Men had 1.6-fold higher LCN2 gene expression in subcutaneous adipose tissue compared to women. When women were stratified based on their menopausal state, men had a significantly higher expression of the LCN2 gene (2-fold) than pre-menopausal women, but not post-menopausal women. LCN2 gene expression tended to be higher in post-menopausal women than pre-menopausal women.

**Paper III**

Genotype-based recall to study metabolic effects of genetic variation: a pilot study of PPARG Pro12Ala carriers

In total, we invited 39, 34, and 30 individuals with 0, 1, and 2 copies of the minor Ala12 allele, respectively. With a participation rate of 31%, 44%, and 40%, we recruited 12 Pro12Pro, 15 Pro12Ala, and 13 Ala12Ala individuals in this GBR study. As a result, no significant differences were found in anthropometric or metabolic variables assessed between the three genotype groups (Table 2).
Table 2. A summary of anthropometric and biochemical variables between the three genotype groups.

<table>
<thead>
<tr>
<th>The measurement between three genotype groups</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric variables</strong></td>
<td></td>
</tr>
<tr>
<td>BMI, waist-hip ratio, body fat %</td>
<td>No difference</td>
</tr>
<tr>
<td><strong>Biochemical variables</strong></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose, fasting insulin, C-peptide, HbA1c, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides</td>
<td>No difference</td>
</tr>
<tr>
<td><strong>Substrate utilization during oral glucose tolerance test</strong></td>
<td></td>
</tr>
<tr>
<td>AUC glucose, AUC insulin, AUC FFA, AUC glycerol</td>
<td>No difference</td>
</tr>
<tr>
<td><strong>Insulin sensitivity</strong></td>
<td></td>
</tr>
<tr>
<td>Matsuda index, HOMA-IR, revised QUICKI</td>
<td>No difference</td>
</tr>
</tbody>
</table>
Paper IV

Role of peroxisome proliferator-activated receptor gamma Pro12Ala polymorphism in human adipose tissue: assessment of adipogenesis and adipocyte glucose and lipid turnover

The effect of PPARγ Pro12Ala was studied on the expression of PPARγ target genes in subcutaneous adipose tissue and glucose uptake and lipolysis in isolated adipocytes during fasting and after OGTT. During fasting, only HSL gene expression was higher, by about 50%, in the Pro/Pro and Pro/Ala groups, compared to Ala/Ala. In comparison to fasting, the HSL gene expression levels were decreased by about 35% in the Pro/Pro and Pro/Ala groups following OGTT.

During fasting, no significant effect was seen on the adipocyte basal, isoproterenol-stimulated lipolysis or anti-lipolytic effect of insulin between the three genotype groups. After OGTT, the antilipolytic effect of insulin was significantly higher in Pro/Pro group than Pro/Ala, but not when compared to Ala/Ala. Compared to fasting, Ala carriers showed a significant reduction in isoproterenol-stimulated lipolysis after OGTT.

There was no major effect of Pro12Ala on adipocyte basal or insulin-stimulated glucose uptake capacity between carriers and non-carriers, both during fasting and after OGTT. Adipocyte size was also similar across genotypes. Finally, the degree and rate of adipocyte differentiation remained unchanged between the three different genotype groups.
Insulin resistance is a fundamental aspect of the development of T2D. A large body of research into mechanisms underlying insulin resistance has focused on insulin signaling transduction and other mitochondrial and cytosolic pathways. Despite compelling evidence showing a crucial role of transcriptional contributors in insulin resistance, they have, however, received relatively little attention (81). Because nuclear receptors control the expression of numerous genes in many tissues, synthetic ligands usually produce favorable therapeutic effects but also unwanted side effects that limit their clinical use (178). Thus, improved understanding of the mechanisms underlying the actions of nuclear receptors in cell types and ways to selectively modulate their activities should be the principal goals in the field of nuclear receptors research (179). In accordance, we investigated the role of nuclear receptors for hormones and nutrients in human adipose tissue metabolism. Specifically, we studied pathways regulated by glucocorticoid and estrogen receptors and PPARγ in human adipose tissue. We mainly used subcutaneous adipose tissue; however, we also utilized visceral (omenta) adipose tissue for a few experiments (Paper I).

The effect of glucocorticoid and estrogen receptors activation and their interaction in the regulation of LCN2 expression in adipose tissue was examined in Paper I and II. In this perspective, we also investigated the role of LCN2 in adipose tissue glucose and lipid metabolism and explored underlying mechanisms. In Paper III and IV, the feasibility of employing a GBR approach, which we used to recruit participants with different PPARγ Pro12Ala gene variant, was tested. At this end, a detailed clinical examination and metabolic phenotyping of subcutaneous adipose tissue obtained from subjects with different PPARγ Pro12Ala variant were also carried out.

Effect of dexamethasone on LCN2 expression in adipose tissue
In Paper I, we demonstrate that dexamethasone treatment dose-dependently increased LCN2 gene expression in subcutaneous and omental adipose tissue from pre-menopausal women, but not post-menopausal women or men. It also increased LCN2 protein levels in subcutaneous adipose tissue from pre-menopausal women. Our findings agree with prior studies in different cell lines, including 3T3-L1 adipocytes (180, 181). However, to date, limited studies have examined the relationship between glucocorticoids and LCN2 expression in human adipose tissue. To the best of our knowledge, only one
study, which was also in compliance with ours, showed an increased LCN2 expression by dexamethasone in omental adipose tissue obtained exclusively from women (182). We took these findings ahead and explored the role of dexamethasone on LCN2 expression in both adipose tissue depots from both genders.

Interestingly, the stimulating effect of dexamethasone on \textit{LCN2} gene expression was found to be regulated by gender and menopausal state in women, suggesting a plausible role of sex hormones. In fact, a study has identified an estrogen response binding element in the promoter region of \textit{LCN2} gene and \textit{LCN2} gene as a putative target of estrogen (183). We followed up these findings in \textit{Paper II} and studied the direct effect of estrogen and its possible interaction with glucocorticoids in the regulation of LCN2 expression in adipose tissue.

High systemic glucocorticoids level, as observed in Cushing’s syndrome or from chronic glucocorticoids treatment result in metabolic distress causing redistribution of fat from peripheral to intra-abdomen, hyperlipidemia, insulin resistance and eventually T2D (127, 184). We found a positive association of dexamethasone-induced \textit{LCN2} gene expression with clinical variables including BMI, waist circumference, fasting glucose, fasting insulin, HbA1c and adipocyte size. These findings may suggest LCN2 as a potential mediator between glucocorticoids induced insulin resistance in adipose tissue.

\textbf{LCN2 in adipose tissue metabolism}

Studies have reported high circulating levels of LCN2 protein in obese individuals and T2D patients (161, 168, 169, 185). Studies have suggested the LCN2 is abundantly produced by adipose tissue (161, 186, 187). However, the role of LCN2 in glucose and lipid metabolism in human adipose tissue was unknown. In \textit{Paper I}, the direct effects of LCN2 on adipocyte glucose and lipid metabolism were investigated, and underlying mechanisms were explored.

Adipose tissue treated with rhLCN2 reduced basal and insulin-stimulated glucose uptake capacity of isolated adipocytes. Our results comply with prior studies in cell lines and animal models (162, 180). At the molecular level, rhLCN2 treatment significantly reduced the expression of glucose transporters, GLUT1 and GLUT4 (188), which can explain the observed reduction in adipocyte glucose uptake.

LCN2 has been shown as an inflammatory marker associated with obesity, insulin resistance, and T2D (161). We showed that rhLCN2 treatment increased \textit{IL6} gene expression in subcutaneous adipose tissue, suggesting its possible role in low-grade inflammation of adipose tissue. rhLCN2 also suppressed \textit{PPARγ} and \textit{ADIPOQ} gene expression. Inhibitory effect of LCN2 on PPARγ, a master regulator of adipogenesis, implicates its possible role in adipocyte differentiation and should be addressed in future studies.
Effect of E2 on LCN2 expression in adipose tissue

In Paper II, we demonstrate that E2 increased LCN2 gene and protein expression in subcutaneous adipose tissue from post-menopausal women. Post-menopausal women were recruited exclusively to circumvent any confounding effects which could have been expected due to the high amount of estrogen and testosterone in pre-menopausal women and men, respectively. Our results are in accordance with a previous animal study (189). Of note, a study has shown an ER binding element in the promoter region of the LCN2 gene and LCN2 as a putative target of estrogen (183).

Estrogen actions are mediated via ERs. Thus, altered ERs expression could influence metabolic outcomes in pre- and post-menopausal women (153, 190). Using ER-specific antagonists, we showed that E2 increased LCN2 gene expression only in the presence of ERα antagonist, but not ERβ antagonist. This suggests that both ERα and ERβ may act in the opposite direction to regulate LCN2 expression. This was again confirmed by the observed stimulating effect of ERβ specific agonist, DPN, on LCN2 gene expression. Moreover, E2 in our culture conditions showed no effect on ERα gene or protein expression, while it increased ERβ gene expression.

Since the discovery of ERs, several studies have established a protective role of ERα against dysregulation of adipose tissue metabolism (191), while the function of ERβ remains elusive. Nevertheless, a few studies in animal models and humans have suggested its pro-diabetogenic actions (150-152, 192). A study on humans showed a positive association of ERβ gene expression in subcutaneous adipose tissue in post-menopausal women with the activity of an enzyme which converts inactive cortisone to cortisol, as well as the expression of its corresponding gene, HSD11B1 (153). In this case, our findings implicate LCN2 as a link between ERβ pathways and insulin resistance.

The interaction between dexamethasone and estrogen in the regulation of LCN2 expression

In Paper I, our findings suggest an involvement of sex hormones in regulating the gender and menopausal-specific effect of dexamethasone on the expression of the LCN2 gene in adipose tissue. Thus, we investigated whether estrogen interacts with the action of glucocorticoids to regulate LCN2 gene expression. Our findings indicate that any possible interactions between glucocorticoids and estrogen appears to be in an ER selective manner and may potentiate the ERβ mediated transcriptional pathways. This may further align with the observed suppressing and inducing effect of dexamethasone on ERα and ERβ expression, respectively; which is also in concordance with previous studies (153, 193).

The ER levels and their different metabolic effects in adipose tissue may influence the predisposition of pre- and post-menopausal women to
metabolic dysregulation (190, 194). Higher ERα gene expression in pre-menopausal women, but higher ERβ gene expression in post-menopausal women have been reported (149, 153, 190, 194). In this context, dexamethasone-mediated inhibition of ERα gene and protein, contrary to induction of ERβ gene expression, may partly explain our previous findings of gender-specific effect dexamethasone on LCN2 gene expression in adipose tissue (Paper I) (165). Of note, glucocorticoids have also been shown to increase local estrogen production in subcutaneous adipose tissue from women by elevating aromatase activity (145). Thus, downregulation of ERα and upregulation of ERβ expression, along with locally produced estrogen, may act in an autocrine or paracrine fashion, triggering ERβ-mediated transcriptional pathways to regulate LCN2 expression.

In the present study, we also observed a correlation of dexamethasone-induced reduction of ERα gene expression with metabolic variables related to insulin resistance such as BMI, waist circumference, fasting insulin, and C-peptide. The stimulation of ERβ and inhibition of ERα gene expression after dexamethasone treatment could contribute to changes in LCN2 levels. However, whether these changes play any role in the development of insulin resistance in human adipose tissue due to chronic glucocorticoid exposure needs further analysis. Based on our results, a proposed model of estrogen and its interaction with glucocorticoids in the regulation of LCN2 expression in adipose tissue is shown in Figure 7.

![Figure 7. Illustration of proposed mechanisms of the effect of dexamethasone and E2, and possible interaction between them in the regulation of LCN2 expression in adipose tissue.](image)

E2 increased LCN2 expression in subcutaneous adipose tissue from post-menopausal women, and this seems to be exerted via ERβ. ERα and ERβ appear to have the opposite effect on LCN2 expression, and they seem to interact with glucocorticoids. Altogether, our data suggest that dexamethasone selectively interacts with ERs to promote ERβ pathways that may play an essential role in the regulation of LCN2 expression in adipose tissue and thereby insulin resistance. AT; adipose tissue. E2; 17-β-estradiol, ERα and ERβ; estrogen receptor alpha and beta. IL6; interleukin 6. LCN2; lipocalin 2, PPARγ; peroxisome proliferator-activated receptor gamma. 
Effect of PPARγ Pro12Ala on clinical variables and adipose tissue metabolism

PPARγ Pro12Ala has been shown to protect against the development of T2D via unspecified mechanisms (92). Under normal physiological conditions, the expression of PPARγ2 which also harbors Pro12Ala mutation is confined to adipose tissue. Thus, any protective effects mediated by the Ala variant could be assumed to be due to changes in adipose tissue metabolism. In paper III and IV, we performed a detailed clinical examination and metabolic phenotyping of adipose tissue obtained before and after 3 h OGTT from 0, 1 or 2 copies of the minor Ala allele. We found no major effect of the Ala variant on baseline anthropometric and clinical variables. Moreover, the response to OGTT also did not alter between genotypes. A possible explanation could be a small sample size in relation to the modest effect of the Ala variant.

Synthetic PPARγ agonists used in the management of T2D have been shown to increase adipocyte glucose uptake (195, 196). Thus, we asked whether adipocyte glucose uptake could be a factor underlying insulin sensitization in Ala12 carriers. However, we found no change in glucose uptake capacity of adipocytes from different genotypes. Our results are in contrast to a prior study in PPARγ2 Pro12Ala knockin mice (197). However, different experimental models could explain the discrepancy. In agreement with ours, an in vivo study in humans did not find any change in adipose tissue but found increased glucose uptake in skeletal muscle under hyperinsulinemic-euglycemic clamp in Ala carriers (198, 199). This finding suggests that other tissues might be contributing to insulin sensitization in Ala carriers, whereas adipose tissue, on the other hand, regulates the release of FFA and adipokines to improve insulin sensitivity.

During fasting, no significant effect was observed on ex vivo adipocyte lipolysis regulation. However, after OGTT, the antilipolytic effect of insulin was significantly higher in Pro/Pro group than Pro/Ala, but not when compared to Ala/Ala group. Our findings are in discordance with the previously shown insulin sensitizing role of PPARγ Pro12Ala. We have no clear explanation for the observed effect of OGTT on lipolysis and also did not find any studies assessing ex vivo adipocyte lipolysis after OGTT. Nevertheless, after OGTT, significantly low lipolysis stimulation in Pro/Ala and Ala/Ala groups could explain in part the resultant decrease in the antilipolytic effect of insulin in these two groups when compared to Pro/Pro. Also, after OGTT, a decreased gene expression of the lipolytic enzyme HSL from fasting levels could explain the observed higher antilipolytic effect of insulin in Pro/Pro group. Although a similar decrease in HSL gene expression was observed in the Pro/Ala group, the presence of both alleles might have counter-regulated the antilipolytic effect in this group. In Ala/Ala group, HSL gene expression during fasting was lower than the two other
groups, which also did not change after OGTT like in the other two groups. This could partly explain a lower antilipolytic effect in Ala/Ala after OGTT. Phosphorylation mainly regulates the HSL activity and we acknowledge that the change in HSL gene expression only does not entirely explain our findings on lipolysis (200).

PPARγ is a master regulator of adipogenesis; a process that leads to the formation of smaller and more insulin-sensitive adipocytes (201). Thus, we studied if adipogenesis was affected by the Ala variant. However, we did not observe any difference in either the rate or degree of adipogenesis between Pro/Pro and Ala/Ala homozygotes. A study in 3T3-L1 cells and primary preadipocytes obtained from Pro12Ala knockin mice showed reduced adipogenesis with Ala variant (106, 197). This difference can be explained by the use of a different experimental model. Like our findings, a study in human SGBS cell line showed a non-significant effect on differentiation in Pro12Ala compared to wild-type (202). Taken together, our results suggest that Pro12Ala may not have major effect on human adipogenesis.

Only a few subjects were included due to the limited size of recruitment cohort, which decreased the probability of finding a statistically significant difference, particularly when studying a common variant with modest effect size. However, our study was estimated to have 80% power to detect a 20% difference between carriers and non-carriers in adipocyte metabolic measures such as glucose uptake and lipolysis. Such a difference concerning lipolysis could have been expected between Pro and Ala homozygote groups (or even Pro/Ala) according to some previous in vivo studies (27, 203) and between insulin-resistant individuals and control subjects (44). Reductions of that magnitude are also found concerning glucose uptake in common insulin-resistant conditions, such as obesity or T2D (204).

This may indicate that changes in adipose tissue may not solely exert protective effects of Ala variants. Other mechanisms like signaling via adipokines to other tissues such as skeletal muscle and liver, where metabolic effects may occur could be crucial. Additionally, due to limited access, we could not study the effect of the Ala variant in visceral (omentum) adipose tissue depot, which needs to be addressed in future. Also, gene-gene, gene-environmental interactions, which can influence the impact of gene variant on phenotype was not controlled in our study and should be considered in future. Moreover, future investigations using estimates of polygenic risk score (205) or other genetic research methodologies should be employed to identify any confounding risk genes that could be different between different groups and could influence the Pro12Ala effects.

GBR- learnings from our study

Genome-wide association studies have substantially contributed to the discovery of genetic variants associated with disease risk (206). However, their phenotypic characterization entails more detailed follow-up studies, and this
is challenging to undertake in large study populations where gene variants are identified. In this case, the GBR approach can prove to be an asset in the functional analysis of gene variants. Many researchers have recommended this approach, yet only a few studies have been performed to date (27, 207). In paper III, we addressed the feasibility and practical implications of employing a GBR approach by conducting a pilot study to examine the effect of PPARγ Pro12Ala variant on clinical phenotype.

As genotypes of participants in the parent cohort were already known, it was possible to invite the desired number of individuals with the desired genotype distribution. This is an advantage of the GBR approach over conventional recruitment models in which the genotypes are assigned randomly. This is primarily critical when oversampling of homozygotes of an uncommon variant is a requirement. Another advantage of the GBR strategy is that it also allows matching of baseline characteristics of invitees, for example, in our case; participants were matched for age and sex. This is crucial, notably when such factors other than genotype can modulate the phenotype (26).

Besides its potential, the GBR approach also has some ethical challenges (208), mainly if there is a disclosure of genotype to the participants. In our study, none of the participants asked to reveal their genotype information. However, in such case, following the principle of autonomy, the information about the participants genotype would have been provided to them (by breaking the code). We acknowledge that given minimal variance explained, knowledge about the Pro12Ala may not be the most informative for a research participant. However, this could be harder to justify, taking into consideration the sensitivity of the information and consequences that participants, as well as his/her relatives, could face in case of more predictive variant such as high-risk BRCA variants.

The participation rate as highlighted in our study is another critical aspect of a GBR study. This is mainly important when studying the rare variant and when the size of the parent cohort is limited. Despite such common gene variant with minor allele frequency (MAF) of 14% and with considerably larger genotype cohort (n=2,500), we ended up with 561 Pro12Ala and 31 Ala12Ala carriers. Hence, based on our previous experience of participation rate (about 50%), it was possible to include only 15 Ala homozygotes. This was also a reason we included Ala heterozygotes although the statistical power would be higher by recruiting only homozygotes. Even for a relatively common variant as Pro12Ala, we did not have enough homozygotes to study only homozygotes. In case of rare variants (MAF<0.05) where the effect on phenotype can be expected to be larger, it was impractical to perform a GBR study based on our parent cohort, as we had only five or few individuals that were homozygous for the minor alleles.

Thus, for future GBR endeavors, appreciably larger number of genotyped, at least 10-fold larger or preferably sequenced (to capture rare and private variants) individuals would be ideal. The larger pool can allow the selection
of rarer variant with substantial effects, which would make the GBR approach more efficient and increase the statistical power.

In the present study, due to limited samples (n=2,500), we were unable to consider rarer variants. Moreover, based on a power calculation, it was not surprising that we did not find any significant impact of the common PPARγ Pro12Ala variant with a moderate effect on the clinical phenotype. Figure 8 summarizes the findings of the effect of Pro12Ala on clinical and adipose tissue phenotype.

**Figure 8: Illustration of the effect of PPARγ Pro12Ala on clinical and adipose tissue phenotypes in the present study.**
We showed that LCN2 could induce insulin resistance in human adipose tissue. Dexamethasone increased \textit{LCN2} gene expression in a gender-specific manner with an effect observed in both adipose tissue depots obtained only from pre-menopausal women, but not from post-menopausal women and men. \textit{LCN2} gene expression in response to dexamethasone was associated with markers of insulin resistance. Taken together, it suggests that in pre-menopausal women LCN2 could be a mediator of adverse metabolic effects due to excess glucocorticoids. However, further investigation is required to understand a direct link between these two factors. An adipose-specific LCN2 knockout model, therefore, would be useful. The \textit{ex vivo} nature of our study does not take into account the complex interplay between different tissues that occurs \textit{in vivo}. Thus, in future studies, the LCN2 expression should be assessed in adipose tissue obtained from donors with or without long-term glucocorticoids treatment.

Several studies have shown an association of LCN2 with insulin resistance and T2D, making it an attractive candidate for further validation. Also, being a secretory protein, it is easy to measure in blood, as well as non-invasively in urine and feces. However, there also exists some discrepancy in the literature about the role of LCN2, mainly due to different experimental settings. Thus, an improved understanding of the pathophysiological role of LCN2 in metabolic disease could facilitate to develop new therapies to counteract its deleterious effects.

In post-menopausal women, who are more susceptible to develop T2D than pre-menopausal women, estrogen increased LCN2 expression in subcutaneous adipose tissue. This may affect the systemic LCN2 levels and may contribute to the development of insulin resistance and T2D. ER\textalpha and ER\textbeta have opposite effects on LCN2 expression in human adipose tissue. Estrogen appears to act via ER\textbeta to elevate the \textit{LCN2} gene expression in adipose tissue, whereas ER\textalpha seems to suppress it. These findings would be critical from the clinical point of view in choosing treatment options using selective estrogen receptor modulators (SERMs) in women. Whether LCN2 is directly linked to metabolic complications due to activation of ER\textbeta needs to be studied further. Given the depot differences in ER levels, in the future, it would be interesting to investigate the effect of ERs on LCN2 in omental adipose tissue.

Dexamethasone seems to selectively interact with the ER to regulate LCN2 expression in adipose tissue in post-menopausal women. Dexamethasone
reduced ERα, whereas increased ERβ expression, which may affect the systemic levels of LCN2. In addition, prior studies have shown that dexamethasone can increase local estrogen production by increasing aromatase activity in adipose tissue from women. The locally produced estrogen can act either in an autocrine or paracrine fashion to further potentiate ERβ transcriptional pathway to regulate LCN2 expression. However, this remains to be validated and should be addressed in future. Our data suggest a gender-specific regulation of LCN2 expression in adipose tissue. However, the differences between the sexes and menopausal state in women do not only consist of estrogen as other hormones such as testosterone and progesterone could also be important and should be considered in future.

The effect of Ala variant on T2D prevention is modest. However, due to the large frequency of the high-risk Pro allele in the general population, the population attributable risk is enormous. Thus, understanding mechanisms through which this polymorphism confers protection may lead to the discovery of novel mechanisms that can be used in the diagnosis, prevention and potentially to create new treatment strategies. We found no major effect of PPARγ Pro12Ala on clinical phenotypes in vivo or in subcutaneous adipose tissue ex vivo. The most likely explanation for this observation is a small sample size with regards to the modest effect of the common Pro12Ala variant. However, an alternative explanation is that mechanisms, other than we studied or in other tissues such as muscle, liver, as well as omental adipose tissue, could be more critical in mediating the protective effect of Pro12Ala against the risk of development of T2D.

Furthermore, our results suggest that the GBR approach, which we used to recruit participants based on their carrier status for Pro12Ala, was feasible to perform. This approach will be more potent for functional characterization of rare genetic variants which are expected to have larger effects on metabolic phenotypes that would be detectable even with limited sample sizes. However, for this to work, the parent cohort will have to be much larger, probably at least 10-fold of the sample size that we had access to for our project.

In conclusion, our data suggest that glucocorticoids or selective activation of estrogen receptors in human adipose tissue regulate and thereby can influence systemic LCN2 levels. LCN2 can act in autocrine or paracrine fashion and can contribute to the development of insulin resistance and eventually T2D. This may have significant clinical implications in patients on long-term glucocorticoids or estrogen treatment. Protective effects of PPARγ Pro12Ala against the development of T2D could be mediated via pathways in adipose tissue other than we studied or via other tissues like skeletal muscle, liver, as well as visceral adipose tissue where metabolic effects may occur. This needs to be addressed in the future. Finally, our GBR study may provide new directions in designing strategies in future for undertaking such an approach.
Fetma och typ 2-diabetes är i allt större utsträckning globala hälsoproblem. Ur behandlingssynpunkt är det därför viktigt att hitta nya biologiska markörrer eller mekanismer som kan användas vid diagnos, för förebyggande åtgärder eller för att skapa nya behandlingsalternativ. Uttrycket av många gener i många vävnader regleras av kärnreceptorer. Läkemedel som verkar genom dessa receptorer och som används vid hanteringen av metabolisk sjukdom ger vanligen gynnsamma terapeutiska effekter. Deras kliniska användning har dock begränsats p.g.a. oönskade biverkningar. En djupare förståelse av dessa receptorer kan leda till upptäckten av nya mekanismer vid metaboliska störningar såsom fetma, insulinresistens och typ 2-diabetes.

I denna avhandling undersökte vi effekten av kärnreceptorer, huvudsakligen glukokortikoid- och östrogenreceptorer samt peroxisomproliferatoraktiviserad receptor gamma (PPARγ), och deras samspel i regleringen av metabolic funktion och dysfunktion i mänsklig fettvävnad.


Artikel II behandlar en uppföljande studie efter artikel I. Här studerade vi den direkta effekten av östrogen samt dess möjliga relation med glukokorti-
koidreceptorer vid reglering av LCN2-uttryck i fettvävnad. För detta ändamål behandlades underhudsfett från postmenopausala kvinnor med eller utan östrogen eller sambehandlades med eller utan östrogen och dexametason för att mäta deras effekter på LCN2-uttryck. LC2-gen- och proteinnivåerna ökade av 17-P-östradiol (E2) i underhudsfett från postmenopausala kvinnor, och detta samband verkar vara medierat av östrogenreceptorn ERβ. Sambehandling med E2 och dexametason ökade LCN2-genuttryck i närvaro av ERα -antagonist men inte i närvaro av ERβ -antagonist. Dexametason minskade gen- och proteinuttryck för ERα samtidigt som det ökade genuttrycket för ERβ. Dexametason-inducerad reduktion av ERα -genuttryck var positivt associerat med markörer för fetma och insulinresistens.


Sammanfattningsvis kan glukokortikoider eller selektiv aktivering av östrogenreceptorer i mänsklig fettvävnad reglera och därigenom påverka LCN2-nivåerna i blodet. LCN2 kan vidare verka på fettvävnad eller andra vävnader i kroppen och kan bidra till utvecklingen av insulinresistens och så småningom T2D. Detta kan ha signifikanta kliniska konsekvenser för patienter på långvarig glukokortikoid- eller östrogenbehandling.

Skyddande effekter av PPARγ Pro12Ala mot utvecklingen av T2D kan förmedlas via andra vägar i fettvävnad än de vi studerade eller via andra vävnader där metaboliska effekter kan uppstå, såsom skelett Muskulatur, lever samt visceral fettvävnad. Detta måste adresseras i framtida forskning. Vår GBR-studie kan ge vägledning för hur framtida strategier ska utformas för detta ändamål.
लढ़ण प्रकार 2 चा मधुमेह, या आरोग्यविषयक समस्या जगाला भेयसावत आहे. त्यामुळे उपचारांच्या रूपांतराने, नवनवीन जैविक मार्कर किंवा यंत्रणा शोधणे अत्यावश्यक आहे, ज्या रोगाचे निदान, त्याचा प्रतिबंध किंवा नवीन उपचार पद्धती तयार करण्यासाठी वापरणे येऊ शकतील. अनेक उत्तमधील असंख्य जनुकीय अभिव्यक्तीतील नियमन न्युक्लिअर रिसेप्टर्स करतात. अशा रिसेप्टर्सच्या कार्य करणारी आणि चयापचय रोगांच्या व्यवस्थापनातील वापरली जाणारी औषधी व्याच्या अनुकूल उपचारांमध्ये अभिव्यक्तीचे ज्यामुळे उपचारांमध्ये नवीन कार्यप्रक्रियांचे आणि उपचारांमध्ये नवीन कार्यप्रक्रियांचे शोध म्हणून अशा नसल्यासेचा अनेक उत्तमधील असंख्य जनुकीय अभिव्यक्तीतील नियमन न्युक्लिअर रिसेप्टर्स करतात. अशा रिसेप्टर्सच्या कार्य करणारी आणि चयापचय रोगांच्या व्यवस्थापनातील वापरली जाणारी औषधी व्याच्या अनुकूल उपचारांमध्ये अभिव्यक्तीचे ज्यामुळे उपचारांमध्ये नवीन कार्यप्रक्रियांचे आणि उपचारांमध्ये नवीन कार्यप्रक्रियांचे शोध म्हणून अशा नसल्यासेचा अनेक उत्तमधील असंख्य जनुकीय अभिव्यक्तीतील नियमन न्युक्लिअर रिसेप्टर्स करतात. अशा नसल्यासेचा अनेक उत्तमधील असंख्य जनुकीय अभिव्यक्तीतील नियमन न्युक्लिअर रिसेप्टर्स करतात.

या प्रबंधामध्ये आम्ही मुख्यतः ग्लुकोकॉर्टिकॉइड, इस्ट्रोजेन आणि पेरॉक्सिज़न प्रॉलिफेटर ऑक्टेवेटेड रिसेप्टर गॅमा (PPARγ) हे न्युक्लिअर रिसेप्टर्स, आणि मानवी चरबीयुक्त उतीमधील कार्य आणि विधान यांतील परस्पर संबंधाचा शोध घेतला आहे.

शोधपत्र १ मध्ये, आम्ही कृत्रिम ग्लुकोकॉर्टिकॉइड आणि डेक्झामियथेज़ ह्या चांगली चरबीयुक्त उतीमधील असंख्य राइपोकॅलीन २ (LCN2) वर होणारा परिणाम तपासून पाहिला. चरबीयुक्त उती LCN2 प्रथम बनवतात, ज्याचा इंसुलिनला कमी प्रतिसाद आणि प्रकार २ चा मधुमेह व्यवस्थापनातील असल्याचे पूर्वांच सिद्ध केले गेले आहे. म्हणून आम्ही सुष्टा LCN2 चा, चरबीयुक्त पेशींच्या ग्लुकोज आणि लिपिडच्या चयावर चयावर होणारा येत प्रभावाचा आणि त्यामुळे यंत्रणा अभ्यास केला. डेक्झामियथेज़ ह्यांनी, रजोनिवृत्ती आतील महिलांमध्ये चरबीयुक्त उतीमधील LCN2 जनुकाची अभिव्यक्ती वाढवली, परंतु रजोनिवृत्तीनंतरच्या महिलेसाठी पुष्पांधराचा हा फक्त दिसला नाही. डेक्झामियथेज़ ह्यांनी LCN2 च्या अभिव्यक्तीतील झालेली वाढ हे लढ़णाचे मार्कस सारखे इंसुलिनला कमी प्रतिसाद, याच्या निगडीत आहे.
LCN2 is a glucocorticoid-associated adipogenic factor, which enhances the expression of LCN2 under conditions of glucose uptake. Insulin diminishes the glucocorticoid signaling, thereby reducing the expression of LCN2, where LCN2 is involved in the glucose uptake of insulin-resistant cells. However, LCN2 is expressed in the insulin-sensitive cells.

Section 2.1 demonstrates that LCN2 facilitates the glucose uptake under conditions of decreased insulin signaling, indicating that the glucose uptake is enhanced by LCN2.

To circumvent this, an alternative approach was explored. Glucocorticoids are known to enhance the expression of LCN2, and the study further investigates the role of glucocorticoids in regulating LCN2 expression. The results show that glucocorticoids enhance the expression of LCN2, thereby facilitating glucose uptake under insulin-resistant conditions.

Section 3.4 introduces the role of PPARγ in regulating LCN2 expression. PPARγ is a nuclear receptor that regulates lipid metabolism and energy balance. The study shows that PPARγ modulates the expression of LCN2, thereby influencing glucose uptake. Additionally, the study explores the role of glucocorticoids in regulating PPARγ expression, indicating that glucocorticoids enhance the expression of PPARγ, thereby facilitating glucose uptake under insulin-resistant conditions.

Overall, the study demonstrates the role of glucocorticoids and insulin in regulating glucose uptake and adipogenesis, highlighting the importance of understanding the molecular mechanisms underlying these processes.
मौखिक ग्लूकोज थेलट्यानंतर उपाशीपोटी केलेल्या चाचणीच्या तुलनेत, Pro/Pro मध्ये, संप्रेरक - संवेदनशील लायपेड्ज जनुकाची अभिव्यक्ती कमी झाली तर मेद - अपघटन विरोधी परिणाममात वाढ झाली. विविध जनुकप्रकार गटांमध्ये चाचणीची ग्लूकोज ग्रहण क्षमता, पेशिव्य आकार आणि मेद निर्मितीमध्ये लक्षणीय फरक दिसला नाही.

सारांश, मानवी चाचणीच्या उतीमध्ये, ग्लुकोकार्टीकॉइड किंवा इस्ट्रोजेन रिसेप्टरसम्बन्धी निवडक संक्रियकरण LCN2 याचे नियमन करत आणि त्यामुळे रक्ततातील LCN2 च्या पातळीवर परिणाम होऊ शकतो. LCN2 पुढे जाऊन चाचणीच्या उती किंवा इतर उतीवर परिणाम करणे ज्यामुळे इंसुलिनच्या प्रतिसादात अवगत कमी होऊन शैवती प्रकार 2 या मधुमेह होऊ शकतो. खप काळ, ग्लुकोकार्टीकॉइड किंवा इस्ट्रोजेनचा वापर करत असणाऱ्या रुग्णांच्या उपचार पद्धतीवर ह्याचा लक्षणीय परिणाम होऊ शकतो. PPARγ Pro12Ala चा, हा संरक्षणात्मक परीक्षण जो प्रकार 2 च्या मधुमेहाची लागण रोखण्यासाठी मदत करतो, तो चाचणीच्या उतीमध्ये आम्ही अभ्यासलेल्या मार्गावर तितक्क इतर मार्गाने ह्या ठरू आणार. येथे तसेच स्नायू उती, यकृत, आँत्रिकांतील चाचणीच्या उती सारख्या इतर उती जिथे चयापचय क्रिया होऊ शकते, त्याच्या ठरू तो च इतर म्हणून साधारण ह्याचा भविष्यात विचार करणे गरजेचे आहे. अंत्तर्गत, आमचा GBR अभ्यासाने, भविष्यातील अशा प्रकारच्या घडामोडूना नवीन मार्ग दाखवू शकेल.
Acknowledgments

With a compilation of this thesis, my journey as a PhD student is coming to an end, and this thesis would be incomplete without acknowledging those who immensely contributed, and without them, this work would not have been possible.

Jan Eriksson, my main supervisor. Thank you for giving me the opportunity to perform my PhD in your group. Thank for your invaluable support, caring and patience and also for being a healthy critic, which has helped me improved as a researcher. Thanks for always encouraging for going to conferences, which has built my confidence over the last few years.

Maria Pereira, my co-supervisor. Thanks for introducing me to adipose tissue experiments. You were always approachable and have been instrumental in shaping up my thesis with your great inputs. Your energy is contagious!

Erik Ingelsson, my co-supervisor. Your knowledge about the field is fascinating and inspiring. Thank you for all the support over the last few years. Although you were at a distance but very approachable.

Tove Fall, my co-supervisor. Thank you for all the support and valuable feedbacks during manuscript preparations.

Cherno Sidibeh, I missed your presence in the lab. We had lots of fun in and outside the lab. Thank you for all the support over the last four years and all the chats, laughs, conferences and our Budapest trip. My best wishes for your future endeavors.

To all the nurses for trying hard to make sure we have biopsies for smooth running of our work. Big thanks to Anna Ehrenborg, Sofia Löfving, Caroline Woxberg, Monika Gelotte, Carola Almström, Lovisa Nordliner, Tanja Putger and Caroline Moberg.
All the physicians, Per Lundkvist, Petros Katsogiannos Kristina Almby, Magnus Sundbom, Niclas Abrahamsson, and Sam Amini for your precious support in obtaining biopsies. Big thanks!

Assel and Cátia, you both are highly energetic and hardworking. Thank you for all the discussions, laughs, and helps whenever needed. I wish you best luck with your PhD and everything you want to achieve in life.

Anders Karlsson, your curiosity about science is inspiring.

Jan Hall and Susanne, thank you for your support at work and for your hospitality outside the lab.

Xexus Abalo (Chus), I adore your passion for microscopy and imaging. We had a great time, both in and outside the lab.

Gretha Boersma, thank you for being so enthusiastic about everything and for always being there to discuss whatever questions I had.

Petros, you were the cool companion in warm in Orlando. I enjoyed our time in Rome and the states.

Per Lundkvist, your interpersonal skills are admirable. I enjoyed your company in Madrid.

Casimiro Castillejo-lópez, your experience, and knowledge in the field are inspiring. Thanks for all scientific and non-scientific discussions during lunch and help with image analysis.

Dariush Mokhtari, you are fun to be around. I had a great time with you in Banff. You are my first ski teacher.

Desirée Eden, thanks for all the discussions and laugher. Best wishes for your PhD.

Greg Panayiotou, your zeal for science is appreciable. Although short, we had a great time, especially the cruise trip and Coldplay concert were the best. Thank you for your friendship.

Ana Fonseca, a very hard working person I came across. Thanks for all the discussion we have had.

Joey Lau Börjesson, thank you for all your support.
Ulf Hammar, thanks for helping with Swedish summary.

Stefan Gustafsson, for a nice collaboration during GBR study.

Ruth Birner-Gruenberger (Graz Uni, Austria), my master thesis supervisor. Thank you for giving me an opportunity to work in the field of proteomics and also allowing me to present a poster at a conference in Innsbruck. Katarina Fritz (co-supervisor), thank you for teaching me the basics of proteomics experiments. It was exciting to watch those silver stained protein spots appearing on the gel. My best wishes to you.

Chintan (Bhai), Thanks for taking care of me in Graz.

I would also like to thank all our colleagues on the floor, Malin Grönberg, Abir Ali, Azita Monazzam, Masoud Razmara and Su-Chen Li, Hanqian Zhang (Larry), Elham Barazeghi, Simone Weström, Birgitta Bondeson, Samuel Backman, Dominic-Luc Webb and Anas Al-Saffar. Thank you for support over the last years, for sharing lab equipment and for all the small talks.

All present and past members of Uppsala Cricket team (Hari, Sethu, Yashraj, Shardul, Srinu A, Srinu T, Manoj B, Majoi N, Srisailam, Kiran, Varun, Naresh, Mahesh, Shambhu and Prakash K). Last all five seasons have been really great.

My friends back home for all your support over the last years (Juber, Ganesh P, Ganesh B, Nitin, Sagar, Adil M, Kunal, Dnyanoba, Jillu, Bilal, Pankaj and Jaywant).

Phani, Chandu, Abhiyan, Ram, Rajani, Ketan, Pratik, Chandra, Sumit, I don’t need to say much about you guys, you are more than friends to me. Thanks for being there whenever I needed. Piyush, Munna, Nisi, Saurabh and Anish for being around.

Arun Bhalerao for caring during my one year at ReeLabs.

My sincere gratitude to all staff at SPS college, Satara.

All my relatives, Kanoje family, Pandharpatte family, Bhandare family and Kadav family for support to pursue my higher education in Sweden.

Ganesh Akade and Vanita Akade for being supportive.

Pranav, for taking care of mom and dad while I am here.
Haware family, words are not enough to express my gratitude. Your role in shaping up my life is second to none. Thank you for your unconditional support and for always being there in ups and downs.

Last but not least, Megha, my beloved wife, thank you for all the love, care and support over the past year.

Special thanks to all the volunteers, without whom this work would never have been possible. Also, I gratefully acknowledge the help from the funding organizations.
This work was supported by the Swedish Diabetes Foundation, EXODIAB (Excellence of Diabetes Research in Sweden), ALF grants from the Swedish government, the Swedish Society for Medical Research, Ernfors Foundation, Swedish Heart and Lung Foundation project, Göran Gustafsson Foundation [Göran Gustafsson Prize 2015], Swedish Heart-Lung Foundation [grant no. 20140422], Knut och Alice Wallenberg Foundation [grant no. 2013.0126].
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