UNIVERSITAT DE BARCELONA

The interplay of the adipokine neuregulin-4 on the inflammation, autophagy, oxidative stress and insulin responsiveness in adipocytes and skeletal muscle

Francisco Díaz Sáez

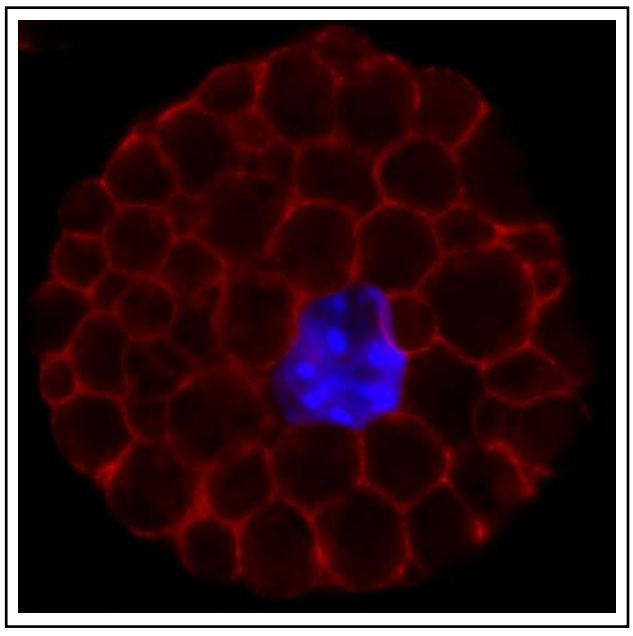
UNIVERSITAT DE BARCELONA

FACULTAT DE BIOLOGIA

DEPARTAMENT DE BIOQUÍMICA I BIOMEDICINA MOLECULAR

The interplay of the adipokine neuregulin-4 on the inflammation, autophagy, oxidative stress and insulin responsiveness in adipocytes and skeletal muscle

La interacción de la adipoquina neuregulina-4 en la inflamación, autofagia, estrés oxidativo y respuesta a la insulina en adipocitos y músculo esquelético



Francisco Díaz Sáez

Barcelona, 2020

UNIVERSITAT DE BARCELONA

FACULTAT DE BIOLOGIA

DEPARTAMENT DE BIOQUÍMICA I BIOMEDICINA MOLECULAR

The interplay of the adipokine neuregulin-4 on the inflammation, autophagy, oxidative stress and insulin responsiveness in adipocytes and skeletal muscle

La interacción de la adipoquina neuregulina-4 en la inflamación, autofagia, estrés oxidativo y respuesta a la insulina en adipocitos y músculo esquelético

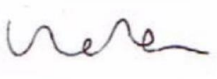
A THESIS SUBMITTED BY:

Francisco Díaz Sáez

FOR THE AWARD OF:

Doctor's degree by the University of Barcelona. Programme in Biomedicine

Supervisor: Anna Maria Gumà Garcia Co-Supervisor: Marta Camps Camprubí



Tutor: Anna Maria Gumà Garcia Author: Francisco Díaz Sáez

Barcelona, 2020

I. Abstract

Neuregulin-4 has emerged as a novel adipokine in recent years. Several authors have previously explored the endocrine role of this adipocyte-secreted growth factor in the liver metabolism. In those studies, it has been shown that neuregulin-4 is a potent factor that protects against some of the deleterious effects induced by obesity and metabolic syndrome in the liver. However, the local role of this factor in adipocyte physiology remains unknown. Thus, in this study, we shed some light into the role of the adipose-tissue secreted neuregulin-4 in the physiology of adipocytes. To this end, we knocked down the expression of *Nrg4* and its receptor, *Erbb4*, in 3T3-L1 adipocytes. Here, we show that the neuregulin-4/ErbB4 signalling axis disruption causes insulin resistance in adipocytes by promoting GLUT4 storage vesicle protein degradation through autophagy. In addition, neuregulin-4 and *Erbb4*-silenced adipocytes display cell-autonomous inflammation via NF-κB activation, which causes the down-regulation of the insulin receptor gene expression. Besides that, some studies have analysed the role of neuregulin-4 in the macrophage inflammation in inflammatory colitis. Inflammation drives insulin resistance and metabolic syndrome in pathologies such as type 2 diabetes. Therefore, we further explored the effects in the macrophage inflammation and polarization of the adipocyte-secreted neuregulin-4. To this end, we analysed the expression of proinflammatory and anti-inflammatory genes in bone marrow-derived and RAW 264.7 macrophages upon treatment with NRG4 derived from adipocytes. In this study, we show that macrophages treated with NRG4 from control adipocytes prevent and recover FROM the M1 polarization induced by lipopolysaccharide treatment. Besides the local effects of neuregulin-4 in the adipocyte and macrophage physiology, the distal effects of these adipokines in the skeletal muscle physiology are yet to be explored. Previous studies have described that neuregulin-1 promotes oxidative metabolism and mitochondrial biogenesis in skeletal muscle. Therefore, we analysed the role of the adipokine neuregulin-4 in skeletal muscle physiology and whether this has consequences on the crosstalk of muscle with other metabolic tissues. Hence, we analysed the phenotype of muscle-specific *Erbb4* gene-deleted mice at 2 and 6 months. Here, we show that the absence of neuregulin-4 action in skeletal muscle triggers inflammation in this tissue. Furthermore, the hepatic expression of neuregulin-4 is indirectly ablated in these mice. Upon high-fat diet feeding, 6-months-old muscle-specific *Erbb4* gene-deleted mice display less triacylglyceride accumulation in skeletal muscle and diminished the white adipose tissue mass, which is consistent with the emergence of systemic inflammation upon *Erbb4* gene deletion in skeletal muscle. In all, in this study, we highlight the importance of the novel adipokine neuregulin-4 as an anti-inflammatory factor with local and systemic effects that contribute to the preservation of insulin sensitivity in the organism.

II. Contents

CONTENTS

1. Abbreviations

#
(p): Phospho
2DG: 2-D-deoxyglucose
4E-BP1: E4-binding protein 1

A
Ab: Antibody
Ab3: Anti-ErbB4 neutralizing antibody
ABHD5: α/β Hydrolase domain-containing protein 5
ACC1: Acetyl-CoA carboxylases 1
ACLY: ATP-citrate lyase
ADAM: Metalloprotease domain
Adipoq: Adiponectin gene
ADP: Adenosine diphosphate
aFABP: Adipocyte fatty acid-binding protein
AIM2: Absent in melanoma 2
AKT: Ak strain transforming
ALBP: Adipocyte lipid-binding protein
AMP: Adenosine monophosphate
AMPK: 5'AMP-activated protein kinase
aP2: Activator protein 2
apoE: Apolipoprotein E
APS: Ammonium persulfate
Arg1: Arginase-1
ASC: Apoptosis-associated speck-like protein containing CARD
ATCC: American Type Culture Collection
ATG: Autophagy-related
ATGL: TAG lipase
ATP: Adenosine triphosphate
AUC: Area Under the Curve

B
Baf: Bafilomycin A1
BAFFR: B-cell activating factor receptor
BAT: Brown Adipose Tissue
BCAT: Branched-chain amino acid aminotransferase
BMDM: Bone Marrow-Derived Macrophages
BNIP3: BCL2/Adenovirus E1B 19 kDa protein-interacting protein 3
BNIP3L: BNIP3-like
BSA: Bovine Serum Albumin

C

c/EBP: CCAAT/enhancer-binding protein
CaMKII: Calmodulin-dependent protein kinase II
cAMP: Cyclic adenosine monophosphate
CAV1: Caveolin-1
CCiTUB: Scientific and Technological Centre of the UB
CCL: Chemokine (C-C motif) ligand
CD: Cluster of Differentiation
cGAS: Cyclic GMP-AMP synthase
CoA: Coenzyme A
CoQ: Coenzyme Q
Cre-GFP: Cre recombinase-green fluorescent protein
CS: Calf Serum
CT: Cycle Threshold
CTP: Citrate transport protein
Ctrl: Control

D
D: Day of differentiation
DAG: Diacylglyceride
DAMP: Damaged-Associated Molecular Patterns
DEPTOR: DEP domain-containing mTOR interacting protein
DMEM: Dulbecco's Modified Eagle Medium
DMOG: Dimethyloxalylglycine
DMSO: Dimethyl sulfoxide
DRP1: Dynamin-related protein 1
DSS: Dextran Sodium Sulfate

E
EBSS: Earle's Balanced Salts
EDL: Extensor Digitorum Longus
EDTA: Ethylenedinitrilotetraacetic acid
EEA1: Endosome antigen 1
EGTA: Glycol ether diamine tetraacetic acid
EIF4G: Eukaryotic translation initiation factor 4G
ErbB: Avian erythroblastosis virus B

F
FAK: Focal adhesion kinase
FASN: Fatty acid synthase
FATP1: Fatty acid transport protein-1
FBS: Foetal Bovine Serum
FFA: Free Fatty Acid
FGF21: Fibroblast growth factor 21
FIP200: Focal adhesion kinase family interacting protein of 200 kDa
FIS1: Fission protein 1 homolog
FOXO1: Forkhead box class O1

G
GAB1: GRB-associated-binding protein 1
GAP: GTPase-activating protein
GC: Gas Chromatography
GDH: Glycerol-3-P-dehydrogenase
GK: Glycerol kinase
GLUT: Glucose transporter
GMP: Guanosine monophosphate
GRB2: Growth factor receptor bound 2
GSV: GLUT4 Storage Vesicle
GTT: Glucose Tolerance Test

H
HAS: Human skeletal actin
HDAC3: Histone deacetylase 3
HEK: Human Embryo Kidney
HER: Human epidermal growth factor receptor
HFD: High-Fat Diet
HIF1α: Hypoxia-inducible factor 1α
HRG: Recombinant human heregulin-1β
HRP: Horseradish peroxidase
HSL: Hormone-sensitive lipase

I
IBMX: 3-Isobutyl-1-methylxanthine
IFG1R: Insulin-like growth factor receptor
iFlox *Erbb2*: Primary immortalised adipocytes from *Erbb2* Flox mice
iFlox *Erbb4*: Primary immortalised adipocytes from *Erbb4* Flox mice
IgG: Immunoglobulin G
IKK: IκB kinase
IL: Interleukin

INF: Interferon
INS: Insulin
InsR: Insulin Receptor
IRAP: Insulin-regulated aminopeptidase
IRB: Institute for Research in Biomedicine of Barcelona
IRF: Interferon regulatory factor
IRS: Insulin receptor substrate
ITT: Insulin Tolerance Test
iWT: Primary immortalised adipocytes from WT mice
IκB: Inhibitor of nuclear factor-κ-B

J
JAK: Janus kinase
JNK: c-Jun N-terminal kinase

K
KD: Knockdown
KO: Knockout
KRHB: Krebs-Ringer-HEPES Buffer

L
LAMP2: Lysosomal membrane protein 2
LC3B: Light chain 3B
LDH: Lactate dehydrogenase
LPL: Lipoprotein lipase
LPS: Liposaccharide
LRP1: Low-density lipoprotein receptor-related protein 1
LSB: Laemmli Sample Buffer
LTβR: Lymphotoxin β receptor
LXR: Liver X receptor

M
MAG: Monoacylglyceride
MAPK: Mitogen-activated protein kinase
MCSF: Macrophage colony-stimulating factor
MFN: Mitofusin
MGL: Monoacylglyceride lipase
mLST8: Mammalian lethal with Sec13 protein 8
MOI: Minimum Multiplicity of Infection
MS: Mass Spectrometry
mSIN1: Mammalian stress-activated protein kinase-interacting protein 1
MtDNA: Mitochondrial DNA
mTOR: Mammalian target of rapamycin
mTORc: Mechanistic target of rapamycin complex
MTP18: Mitochondrial protein 18 kDa
mtROS: Mitochondrial Reactive Oxygen Species

N
NAC: N-acetylcysteine
NAD: Nicotinamide Adenine Dinucleotide
NASH: Nonalcoholic steatohepatitis
NCBI: National Centre for Biotechnology Information
ND: Normal diet
NEMO: NF-κB essential modulator
NF- κB: Nuclear factor κ-light-chain-enhancer of activated B
NIH**:** National Institute of mental Health
NIK: NF-κB inducing kinase
NLR: NOD-like receptor
NLRP3: NOD-like receptor protein 3
NRG: Neuregulin
ns: Not significant

O

OPA1: Optic atrophy 1
OXPHOS: Oxidative phosphorylation system

P

PAMP: Pathogen-Associated Molecular Patterns
PBS: Phosphate Buffer Saline
PDK1: 3-Phosphoinositide-dependent protein kinase 1
PEI: Polyethyleneimine
PGC1α: Proliferator-activated receptor γ coactivator 1-α
PHD: Prolyl hydroxylases
PHD3: Egl-9 family hypoxia-inducible factor 3
PI3K: Phosphoinositide 3-kinase
PINK1: Phosphatase and tensin homolog-induced kinase 1
PIP_3: Phosphatidylinositol-3,4,5-triphosphate
PKA: Protein kinase A
PKB: Protein kinase B
PKC: Protein kinase C
PLC: Phospholipase C
Plin1: Perilipin-1 gene
PPARγ: Proliferator-activated receptor γ
PPRE: PPAR responsive elements
PRAS40: Proline-rich AKT substrate of 40 kDa
PRR: Pattern-Recognition Receptor
PtdIns3K: Phosphatidylinositol 3-kinase
PVDF: Polyvinylidene fluoride

Q

qPCR: Quantitative Polymerase Chain Reaction

R

Rab: Ras-associated binding
Rab11: Ras-related protein 11
RANK: Receptor activation of nuclear factor κB
RDAU: Relative Densitometric Arbitrary Units
RHEB: Ras homolog enriched in brain
Rho: Ras homologous
rNRG4: Human-recombinant NRG4
ROS: Reactive Oxygen Species
RT: Room Temperature
RT-PCR: Reverse-Transcription Polymerase Chain Reaction
RXR: Retinoid X receptor

S

S6K: S6 kinase
Sali: Salicylate
Scr: Scramble
SDS: Sodium Dodecyl Sulphate
SNAP23: Synaptosomal-associated protein 23
SNARE: N-Ethylmaleimide sensitive factor attachment
SOD: Superoxide dismutase
SOS: Son of sevenless
SREBP1: Sterol regulatory element-binding protein 1
STAT: Signal transducer and activator of transcription
Stx6: Syntaxin-6 gene
SYK: Spleen tyrosine kinase

T
T2D: Type II Diabetes
TAG: Triacylglycerides
TBC1D4: TBC1 domain family member 4
TBS: Tris Buffer Saline
TCA: Tricarboxylic acid
TCR: T-cell receptor
TERT: Telomerase reverse transcriptase
TFEB: Transcription factor EB
TGFα: Transforming growth factor α
TGN: Trans-Golgi-Network
TIM44: Translocase subunit 44
TIP47: Tail-interacting protein 47
TLR: Toll-like receptor
TMRM: Tetramethylrhodamine methyl ester
TNFR: TNFα receptor
TNFα: Tumour necrosis factor α
TSC: Tuberous sclerosis
TUG: UBX domain for GLUT4-ubiquitin-like

U
UB: University of Barcelona
UCP1: Uncoupling protein 1
ULK1: Unc-51 like autophagy activating kinase 1

V
VA: Visceral Adipocyte fraction from WAT
VEGF: Vascular endothelial growth factor
VLDL: Very low-density lipoproteins
VS: Visceral Stromal fraction from WAT

W
WAT: White Adipose Tissue
WT: Wild-Type

2. Introduction

2.1. Adipocyte physiology

2.1.1. White adipose tissue, much more than a metabolic tissue

White adipose tissue (WAT) plays a central role in the regulation of whole-body energy and glucose homeostasis. It is mainly constituted by adipocytes, which, upon an excessive caloric intake, they synthesize triacylglycerides (TAGs) to form lipid droplets as an energy reservoir. Afterwards, when food is scarce or the body energy expenditure increases, TAGs are broken down into free fatty acids (FFAs) and glycerol, which can be transported through the blood to be consumed by other tissues such as muscle and the liver. Therefore, adipocytes regulate the distribution of lipids through the body and modulate the whole-body energy balance (*reviewed in Luo et al., 2016*). These metabolic processes are stimulated by external stimuli, such as low energy levels, or even by other molecules such as cytokines, adipokines and other hormones. These factors can be produced by different cells and signal in a paracrine or endocrine manner. Alternatively, they can be produced by adipocytes and signal in an autocrine manner. Some of the molecules that can regulate the metabolism of adipocytes are insulin, cortisol, catecholamines, growth hormones, FFAs and numerous cytokines such as tumour necrosis factor α (TNFα). Some of these factors are secreted by adipocytes and thus, WAT functions as an endocrine organ by regulating the release of different adipose-derived secreted factors or adipokines. Some of the adipokines that have been described are metabolites (FFA, lactate), enzymes (lipoprotein lipase (LPL), adipsin), growth factors (vascular endothelial growth factor (VEGF)), cytokines (TNFα, interleukin-6 (IL-6) among others) and metabolic hormones (leptin, resistin, adiponectin among others) (*reviewed in Mora et al., 2002; reviewed in Fischer-Posovszky et al., 2007; Takahashi et al., 2019*). These factors can circulate through the blood and reach other metabolic organs such as muscle, liver, pancreas and brain, modulating their metabolism. Besides that, adipocytes can regulate the inflammatory state of other stromal cells such as tissue-infiltrated macrophages to regulate WAT inflammation. Furthermore, the autonomous inflammation of adipocytes under stress conditions drives the recruitment of immune cells and the polarization of macrophages (*reviewed in Huh et al., 2014*). Therefore, alterations in the expression of these factors upon obesity or lipodystrophy lead to metabolic alterations in WAT and other tissues, which contributes to the development of Type II Diabetes (T2D). Recently, in 2014, it has been discovered that the growth factor neuregulin-4 (NRG4) is highly expressed in mice adipose tissue, especially enriched in brown fat (*Wang et al., 2014*). The authors conclude that NRG4 reduces *de novo* lipogenesis in the liver from mice. However, the role of this adipokine in the regulation of the biology of adipocytes remains largely obscured.

2.1.2. Differentiation of adipocytes

To study the adipocyte function and metabolism, several different *in vivo* and *in vitro* models have been developed. Specifically, different pre-adipocytic cell lines have been generated such as the immortalised 3T3-L1 cell line. In addition to the generation of secondary cell lines, primary adipocytes from different adipose tissue pads have been generated to study adipocyte biology (*reviewed in Gregoire et al., 1998*). Recently, conditional primary immortalised adipocytes have been generated since they constitute a more physiological model than the secondary cell lines (*Church et al., 2015*). In this thesis, 3T3-L1 and primary immortalised adipocytes have been used as cellular adipocyte models. Indeed, 3T3-L1 cells are the most used secondary cell line to study adipocytes. Specifically, 3T3-L1 cells were isolated from the *Swiss* 3T3 cells from mice embryos between day 17-19 of development (*Todaro et al., 1963*). These cells are similar to fibroblast during the growth stage, as it is the case with primary preadipocytes. Upon cellular confluency, adipogenic differentiation is induced by the treatment with different factors. After 7 days, adipocytes display a rounded morphology and accumulate lipid droplets, acquiring the biochemical and functional characteristics of differentiated adipocytes. The specific protocol to induce adipogenesis is explained in section 7.3.3.1. This differentiation requires 2 interconnected events, the growth of the pre-adipocyte and its differentiation. When pre-adipocytes reach cellular confluency, cell growth is arrested. Afterwards, the stimulation with the correct hormonal cocktail resumes the cell cycle and triggers the clonal expansion, which begins the differentiation process. Insulin can induce the clonal expansion by binding to the insulin-like growth factor receptor (IGF1R), the signalling of which activates mitogen-activated protein kinase (MAPK) and protein kinase B (PKB)/Ak strain transforming (AKT) pathways (*Beguinot et al., 1988*). AKT has also been shown to induce peroxisome proliferator-activated receptor γ (*Pparg*) expression by inhibiting the forkhead box class O1 (FOXO1) transcription factor (*Nakae et al., 2003*). After the clonal expansion, cell growth is arrested again until the end of the differentiation process. In this final step, cells induce the expression of different specific genes that determine the final phenotype of the adipocyte. In the end, adipogenesis is caused by the activation of different transcription factors PPARγ, CCAAT/enhancer-binding proteins (c/EBPs) and sterol regulatory element-binding protein 1 (SREBP1) at different stages. First, insulin and glucocorticoids such as dexamethasone activate c/EBPβ, c/EBPδ and SREBP1, which induce the transcription of *Pparγ*. Then, the terminal differentiation occurs by the expression of adipogenic genes upon the action of the transcription factors PPARγ, c/EBPα and SREBP1 (*reviewed in Fajas, 2003; Farmer et al., 2005; Liu et al., 2001; Farmer et al., 2006*). Some adipogenic markers up-regulated by the adipogenic differentiation are caveolin-1 (*Cav1*), glucose transporter 4 (*Glut4*), perilipin-1 (*Plin1*), adiponectin (*Adipoq*), insulin receptor (*InsR*) and *Lpl*.

The PPAR transcription factors regulate the expression of genes that modulate cellular metabolism, differentiation and apoptosis. Upon the binding of ligands such as vitamin A, steroids or fatty acids, these receptors heterodimerize with retinoid X receptor (RXR) (*Juge-Aubry et al., 1995*). The heterodimers then activate the expression of genes with PPAR responsive elements (PPRE). The anti-diabetic agents of the family of the thiazolidinediones such as rosiglitazone can bind and activate PPARγ and thus, they are used in the differentiation cocktail of 3T3-L1 cells. As mentioned previously, *Pparγ* expression is induced by the activity of c/EBPβ, c/EBPδ and SREBP1. Both insulin and corticosteroids can activate these transcription factors. In addition, the reagent 3-isobutyl-1-methylxanthine (IBMX) can also induce the expression of *Pparγ* and its endogenous ligands by increasing the levels of cyclic adenosine monophosphate (cAMP) (*Hamm et al., 2001*; *reviewed in Farmer et al., 2005*; *Kim et al., 1998*) and thus, this reagent is also used in the differentiation cocktail of 3T3-L1 cells.

The c/EBP transcription factor plays a central role in the progression of adipogenesis (*reviewed in Gregoire et al., 1998); reviewed in Farmer et al., 2005*). Upon their dimerization, these transcription factors induce the expression of adipogenic genes. Different isoforms of c/EBP are expressed during the differentiation process (*Cao et al., 1991; Yeh et al., 1995; Hamm et al., 2001*). Thus, c/EBPβ and c/EBPδ induce the expression of *Pparγ* in the early stages of the differentiation process (*Wu et al., 1996*) and later, c/EBPα is induced, which increases the expression of adipogenic markers in adipocytes such as *Glut4* and the *InsR* (*reviewed in Darlington et al., 1998*). On the other hand, c/EBPζ has been shown to have a role in growth arrest (*Barone et al., 1994*) and thus, it has a role in the induction of adipogenesis. The action of IBMX and dexamethasone increases the expression of c/EBPβ and c/EBPδ during the adipogenic differentiation.

SRBBP1 belongs to the family of SRBB transcription factors. It induces the expression of genes that regulate lipid and cholesterol biosynthesis (*reviewed in Bertolio et al., 2019*). In addition, SRBP1 regulates adipogenesis by controlling the expression of PPARγ ligands (*Kim et al., 1998*). This protein translocates from the endoplasmic reticulum to the Golgi apparatus upon cholesterol deprivation. Afterwards, it gets cleaved and translocates to the nucleus. On the other hand, the action of proinflammatory cytokines such as TNFα reduce the gene expression of *Pparγ* and c/EBPδ (*Zhang et al., 1996; Ruan et al., 2002*) in adipocytes and thus, they disrupt the differentiation process and down-regulate the expression of adipogenic genes. In addition, TNFα signalling leads to inhibitor of nuclear factor-κ-B (IκB) degradation, which allows the nuclear co-repressor histone deacetylase 3 (HDAC3) to enter to the cell nucleus inhibiting the transcription of PPARγ and c/EBPδ target genes (*Kudo et al., 2004*; *reviewed in Ye, 2008*).

2.1.3. Insulin signalling in adipocytes

Glucose homeostasis is a finely tuned process controlled by their production and uptake by different tissues. In physiological conditions, the blood glucose levels are maintained in a narrow range by the action of different hormones (*reviewed in Cherrington et al., 1999*). When the blood glucose levels increase, the β-pancreatic cells respond by increasing the production of insulin. Mechanistically, β-pancreatic cells uptake glucose increasing the intracellular adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio. This increase results in the closing of K^+ ATP channels, which induces the secretion of insulin-containing granules (*reviewed in Newgard et al., 1995*). Afterwards, insulin stimulates the glucose uptake in WAT and muscle since they express the insulin-dependent glucose transporter GLUT4. Besides, insulin inhibits gluconeogenesis and glycogenolysis in the liver, which contributes to the maintenance of the physiological blood glucose levels. In addition to the stimulation of the glucose uptake, insulin regulates a plethora of aspects in adipocytes. For instance, insulin promotes adipocyte differentiation as we previously described (*Beguinot et al., 1988*). Furthermore, it stimulates the uptake of FFA and glucose to synthesize TAGs (*Stahl et al., 2002*), while it inhibits lipolysis by the inhibition of hormone-sensitive lipase (HSL) (*Ferrannini et al., 1983*). Therefore, despite skeletal muscle is the main consumer of glucose upon insulin stimulation, insulin signalling is key in the regulation of metabolic homeostasis in WAT. As a matter of fact, the gene deletion of *Glut4* in WAT from mice causes insulin resistance in muscle and the liver (*Abel et al., 2001*). That study highlights the importance of WAT as an endocrine organ that controls the insulin sensitivity of other insulin-sensitive tissues.

2.1.4. GLUT

Glucose is a hydrophilic molecule and thus, it cannot passively diffuse through the cell membrane. Therefore, to enter into the cell, it requires membrane transporters that facilitate their diffusion. Mammalian cells uptake glucose by facilitative diffusion using transporters of the GLUT family. In humans, there are 14 different GLUT isoforms, which are differentially expressed between tissues (*Scheepers et al., 2004*). Structurally, GLUT transporters present 12 transmembrane domains with both extremes C and N-terminus in the cytosol. Specifically, the GLUT1-4 mediate glucose uptake and display different regulatory properties. GLUT1 is ubiquitously expressed and regulates the basal uptake of glucose (*reviewed in Mueckler et al., 1995*). However, it is mainly expressed in erythrocytes and endothelial, foetal, and cancer cells (*Pardridge et al., 1990; Ganapathy et al., 2009*). GLUT2 is only expressed in intestinal and kidney epithelial cells, pancreatic β-cells and hepatocytes (*Uldry et al., 2002*). In addition, it has the lowest glucose affinity among other GLUT transporters.

GLUT3 has a high affinity for glucose and thus, it is expressed in tissues with high glucose consumption such as neurons (*Kayano et al., 1988*). Finally, GLUT4 is the most expressed GLUT in WAT and muscle (*Fukumoto et al., 1989*). GLUT4 has a high affinity for glucose, however, it is not constitutively present in the cellular membrane. Instead, GLUT4 is stored in GLUT4 Storage Vesicles (GSVs) and, upon the action of insulin, it gets translocated to the cell surface, increasing the glucose transport (*reviewed in Bryant et al., 2002*). In adipocytes, the amount of GLUT4 in the membrane increases by 50% upon insulin stimulation (*reviewed in Watson et al., 2004*). Therefore, GLUT4 is especially relevant in the regulation of the blood glucose homeostasis upon feeding. Regarding the other GLUT members, GLUT5, 7, 8 and 11 are fructose transporters, whereas GLUT6, 8, 10, 12 and the myoinositol transporter HMIT1 are structurally atypical members of the GLUT family.

2.1.5. GLUT4 trafficking in adipocytes

GLUT4 trafficking constitutes an equilibrium between GLUT4 endocytosis and exocytosis. In basal conditions, this equilibrium is displaced towards the endocytosis, while, upon insulin stimulation, it is displaced towards the exocytosis of GSVs. Thus, there is low-rate insulin-independent recycling of GLUT4 in basal conditions (*Carvalho et al., 2004*). Not only do the GSVs contain GLUT4, but they also contain other cargo proteins such as insulin-regulated aminopeptidase (IRAP), sortilin and low-density lipoprotein receptor-related protein 1 (LRP1). LRP1 is a transmembrane protein that internalizes lipoproteins bearing apolipoprotein E (apoE) (*reviewed in Strickland et al., 2002*). It also interacts with other receptors such as the InsR, regulating the trafficking of this receptor in different tissues (*reviewed in Dato et al., 2018*). IRAP regulates the degradation of vasopressin (*Wallis et al., 2007*). Sortilin is a scaffold protein that recruits clathrin to promote the budding of GSVs (*Nielsen et al., 2001*). Furthermore, other proteins can interact with GSV cargo proteins such as TBC1 domain family member 4 (TBC1D4, also known as AS160) with IRAP and LRP1 (*reviewed in Klip et al., 2019*). Besides that, GSVs contain N-ethylmaleimide sensitive factor attachment (SNARE) proteins such as VAMP2 and syntaxin-6 (*Perera et al., 2003*). Upon insulin stimulation, GLUT4 is translocated to the plasma membrane. GSVs are translocated upon insulin signalling through the activation of Ras-associated binding (Rab) proteins such as Ras-related protein 11 (Rab11) by the AKT pathway (*Ren et al., 1998; Wilcke et al., 2000*). Insulin also activates the Ras homologous (Rho) GTPase TC10, which is necessary for the exocytosis of GLUT4 (*Watson et al., 2001*). Finally, the fusion of GSVs with the cellular membrane is mediated by the SNAREs VAMP2 (*Ramm et al., 2000*), syntaxin-6 (*Perera et al., 2003*), synaptosomal-associated protein 23 (SNAP23) (*Kawanishi et al., 2000*) and syntaxin-4 (*Olson et al., 1997*).

Regarding GLUT4 internalization, 2 different mechanisms have been proposed: clathrin- (*Robinson et al., 1992*) and caveolae-dependant (*Ros-Baro et al., 2001*), which are not mutually exclusive. Clathrin dependent GLUT4 internalization is the same than for GLUT1 and transferrin receptor recycling and requires the recruitment of the clathrin adaptor protein activator protein 2 (aP2), the GTPase dynamin and Rab5. Contrarily, lipid raft dependant GLUT4 internalization is independent of aP2 and may occur as a consequence of caveolae fission (*Blot et al., 2006*). Afterwards, GLUT4 co-localizes with endosomal markers such as early endosome antigen 1 (EEA1) and transferrin receptor (*Ramm et al., 2000*). Upon endocytosis, GLUT4 and other GSV cargo proteins are recycled from the cellular membrane to the recycling compartment (*reviewed in Foley et al., 2011*). From there, GLUT4 and other GSV cargo proteins are sorted and translocate to the Trans-Golgi-Network (TGN) or to the GSV pool to become functional again. However, it is known that endosomes can progress to late endosomes (*reviewed in Eskelinen et al., 2009*), and from there, one may think that some GSVs could be degraded through autophagy. Specifically, early endosomes can evolve into late endosomes and fuse with autophagosomes to form amphisomes and undergo lysosomal degradation. Besides that, it has been previously described that the recycling endosomes contribute to the formation of autophagosome (*reviewed in Puri et al., 2018).* However, the role of autophagy in GSV degradation has not been explored yet.

2.1.6. Insulin signalling induces GLUT4 translocation

Insulin binds to the InsR and, with less affinity, to the IGF1R (*Steele-Perkins et al., 1988*). Importantly, the InsR exists in 2 isoforms: InsR-A and InsR-B. These are produced by alternative splicing; however, it remains unclear the different physiological roles of each isoform (*reviewed in Malakar et al., 2016*). It has been reported that InsR-A form is predominantly expressed in preadipocytes, while InsR-B form increases in differentiated adipocytes (*Belfiore et al., 2017*). Furthermore, the ratio InsR-A:InsR-B in WAT is altered in T2D patients (*Sesti et al., 1991*). The InsR is a tyrosine kinase receptor that has 2 subunits α and β. Upon the binding with insulin, the receptors oligomerize and trans-phosphorylate to trigger cell signalling. Specifically, the InsR trans-phosphorylation of tyrosine residues allow the docking proteins insulin receptor substrate 1 and 2 (IRS1, IRS2) to bind. Upon the binding of IRSs, these proteins are tyrosine phosphorylated and thus, other adaptor proteins such as the p85 regulatory subunit of phosphoinositide 3-kinase (PI3K) are recruited. PI3K is a lipid kinase constituted by the regulatory subunit p85 and the subunit p110 that catalyses the production of the secondary messenger phosphatidylinositol-3,4,5-triphosphate (PIP_3) in the membrane. In basal conditions, PI3K is found in the cytosol, while, upon insulin stimulation, it translocates to the membrane to engage IRSs and catalyses PIP_3 production.

This secondary messenger acts as an anchor point for proteins with pleckstrin domains such as AKT and 3-phosphoinositide-dependent protein kinase 1 (PDK1). Specifically, the PKBβ/AKT2 isoform is the most expressed in the insulin-sensitive tissues (*Hill et al., 1999*). Afterwards, PDK1 phosphorylates AKT in the threonine 308 and the atypical protein kinase C ζ/λ (PKCζ/λ). It has been described that the atypical PKCs participate in the fusion of the GSVs with the plasmatic membrane (*Liu et al., 2006*). Besides, active AKT induces GLUT4 translocation via activation of Rab proteins such as Rab4 and Rab11 by TBC1D4 phosphorylation (*Tanti et al., 1996; Cong, 1997; Bandyopadhyay et al., 2002*). Rabs are G-proteins that, in the GTP-bound form, regulate vesicle trafficking and fusion (*reviewed in Larance et al., 2001*). On the other hand, GTPase-activating proteins (GAPs) catalyse the conversion of the GTP-bound to the inactive GDP-bound form of the Rab. TBC1D4 has shown to have GAP activity for several Rabs (*Sano et al., 2007*). Importantly, TBC1D4 is bound to the GSV cargo protein IRAP and thus, in basal conditions, this protein inhibits the trafficking of GLUT4 (*Larance et al., 2005*). However, TBC1D4 GAP activity is inhibited by AKT phosphorylation, allowing Rab proteins to mediate the translocation of GSVs (*Mîinea et al., 2005*). In addition, TBC1D4 phosphorylation dissociates this protein with IRAP, allowing its release from GSVs and the GTP loading of Rabs (*Mafakheri et al., 2018*).

Furthermore, the inactivation of the Rab GAP function of TBC1D4 has been shown to accelerate GLUT4 protein degradation in mice (*Xie et al., 2016*), highlighting its importance in the retainment of GSVs in the cytosol in basal conditions. Besides TBC1D4, tether containing UBX domain for GLUT4-ubiquitin-like 1 (TUG) also regulates GSV sequestration and release (*Bogan et al., 2003*). This protein is present in the cytosol and membranes, tethering GLUT4 intracellularly in basal conditions. Upon insulin stimulation, the tethering between TUG and GLUT4 is broken down allowing the release of GSVs (*Yu et al., 2007*). However, the exact mechanism by which insulin releases GLUT4 from TUG remains unknown. Beyond the PI3K-dependent signalling, the recruitment of the Rho GTPase TC10 in caveolae is essential for GLUT4 translocation in adipocytes (*Watson et al., 2001*). Caveolae are a subtype of lipid rafts that are formed by the integral membrane protein CAV1 bound to cholesterol and glycosphingolipids. Importantly, CAV1 deficiency has been shown to reduce the protein stability of GLUT4 in adipocytes, highlighting the importance of these structures in the trafficking of GLUT4 (*González-Muñoz et al., 2009*). Mechanistically, the InsR phosphorylates Casitas B-lineage lymphoma (c-CBL), which ultimately activates TC10 (*Ribon et al., 1998; Ahmed et al., 2000*). Afterwards, TC10 localizes to caveolae allowing the GLUT4 in the cell membrane.

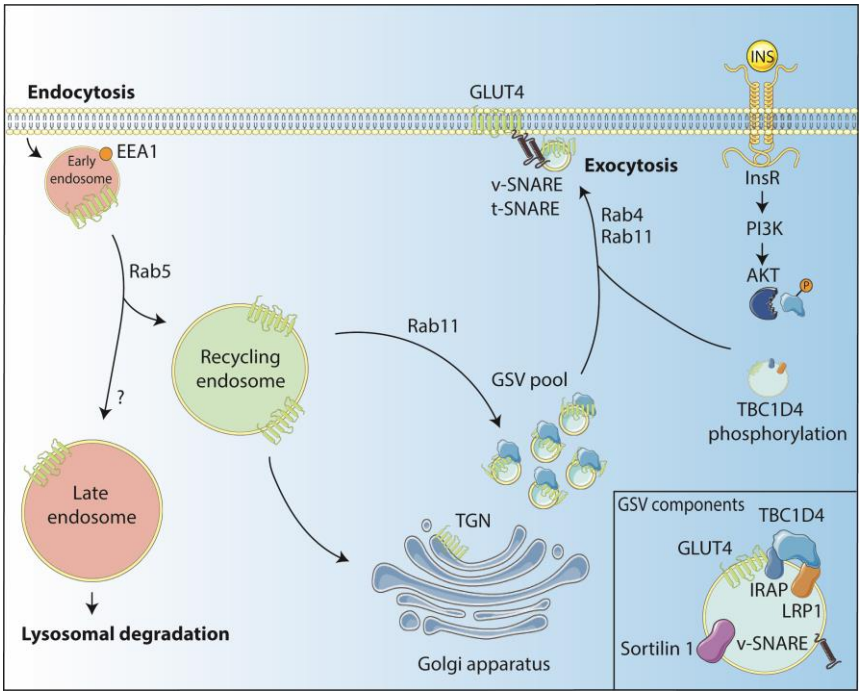


Figure 1. Scheme of the intracellular GLUT4 trafficking upon insulin stimulation. Schematic art pieces used in this figure are provided by Servier Medical Art under a Creative Commons Attribution 3.0 Unported License.

2.1.7. The Mammalian Target of Rapamycin

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that forms the catalytic subunit of 2 protein complexes, namely mechanistic target of rapamycin complex 1 and 2 (mTORc1 and mTORc2). The mTOR pathway is an important component in the signalling of insulin and growth factors. The complex mTORc1 regulates the balance between anabolism and catabolism in response to environmental stimuli, while mTORc2 (previously identified as PDK2) is important in the phosphorylation of the serine 473 of AKT (*Thomanetz et al., 2013*). MTORc1 is constituted by the mTOR kinase, and the regulatory proteins raptor and mammalian lethal with Sec13 protein 8 (mLST8) (*reviewed in Saxton et al., 2017*). Raptor facilitates the recruitment of the mTOR substrates, while mLST8 stabilizes the kinase activation loop of mTOR. In addition, mTORc1 also contains 2 inhibitory subunits, namely proline-rich AKT substrate of 40 kDa (PRAS40) and DEP domain-containing mTOR interacting protein (DEPTOR). On the other hand, mTORc2 is formed by the core components mTOR, rictor, mammalian stress-activated protein kinase-interacting protein 1 (mSIN1) and mLST8. Rictor interacts with the C-terminus of mTOR and binds to DEPTOR, whereas mSIN1 participates in the substrate recruitment for mTORc2. Besides their differences in the composition, the acute treatment with rapamycin disrupts mTORc1 function, while mTORc2 is only inhibited upon long-term treatments and thus, they are differentially regulated (*reviewed in Luo et al., 2018*).

Upstream the mTOR pathway, mTORc2 is induced only as an effector of the insulin/PI3K pathway, whereas mTORc1 is also regulated by growth factor signalling, energy levels, DNA damage and amino acid availability. Specifically, insulin can activate mTORc1 through the inhibition of tuberous sclerosis 1 and 2 (TSC1 and TSC2) protein complex (*reviewed in Montagne et al., 2001; Inoki et al., 2002*). This protein complex acts as a GTPase of the small G protein Ras homolog enriched in brain (RHEB) thereby driving RHEB into the inactive GDP-bound state. Upon insulin stimulation, AKT phosphorylates TSC2, disrupting its interaction with TSC1 and targeting TSC2 for proteasomal degradation. Afterwards, GTP-bound RHEB activates mTORc1. Contrarily, 5'AMP (adenosine monophosphate)-activated protein kinase (AMPK) can inhibit the mTORc1 activation by activating TSC2 (*Inoki et al., 2003*). Therefore, mTORc1 is only induced in conditions of energy sufficiency since this protein complex is key in the metabolic shifting towards the anabolism. Indeed, mTORc1 activates ribosomal S6 kinases 1 and 2 (S6K1 and S6K2), eukaryotic initiation factor E4-binding protein 1 (4E-BP1), transcription factor EB (TFEB) and Unc-51 like autophagy activating kinase 1 (ULK1) (*reviewed in Rabanal-Ruiz et al., 2017*). Ultimately, these are involved in ribosomal biogenesis, mRNA translation, autophagy, lipid and nucleotide synthesis and mitochondrial function.

The ribosomal kinases S6K1 and S6K2 have 2 isoforms, namely p70S6K-p85S6K for S6K1 and p56SK-p546K for S6K2. The isoform p70S6K is ubiquitously expressed, while the other isoforms of S6K1 and S6K2 are mainly localised in the nucleus of quiescent cells (*Koh et al., 1999*). The S6 kinases are activated mainly in response to growth factors and nutrients via mTORc1 (*Jones et al., 1999a; Jones et al., 1999b; Pinzani et al., 1991*). Specifically, p70S6K is directly phosphorylated by mTORc1 in its threonine 389, enabling its subsequent phosphorylation by PDK1. Afterwards, S6K1 promotes the phosphorylation of several substrates that promote the transcription of genes involved in protein synthesis and cell growth (*Hsieh et al., 2010*). In addition, S6K also phosphorylates the serine 2448 of the mTOR protein as a negative feedback mechanism (*Chiang et al., 2005*). Besides to S6K1 activation, mTORc1 also promotes cellular anabolism by activating 4E-BP1, allowing the transcription of eukaryotic translation initiation factor 4G (EIF4G) dependent genes that control cell proliferation (*reviewed in Qin et al., 2016*). In addition, mTORc1 also promotes cellular anabolism by inhibiting different catabolic pathways. Indeed, mTORc1 phosphorylates and inhibits the transcription factor TFEB, which is involved in the expression of lysosomal and autophagy-related genes (*Martina et al., 2012*). Likewise, mTORc1 inactivates ULK1, hence inhibiting autophagy. In addition, mTORc1 is critical in the regulation of adipogenesis (*reviewed in Cai et al., 2016*). Indeed, mTORc1 has been shown to promote clonal expansion through c/EBPβ and c/EBPδ induction (*Martin et al., 2015*). However, in contrast to mTORc1, the contribution of mTORc2 in adipogenesis remains unknown.

2.1.8. Autophagy regulation in adipocytes

Autophagy, also known as macroautophagy, is a genetically programmed catabolic process that degrades proteins, vesicles and damaged organelles such as mitochondria, peroxisomes, or endoplasmic reticulum. As mentioned previously, mTORc1 is one of the main regulators of the autophagic pathway. Specifically, mTORc1 inhibits autophagy initiation by ULK1 inactivation. In addition, mTORc1 mediates the down-regulation of several lysosomal and autophagic genes through the inactivation of the transcription factor TFEB (*reviewed in Rabanal-Ruiz et al., 2017*). Therefore, upon nutrient-rich conditions or by the action of growth factors, mTORc1 promotes cell growth and anabolism, while it represses the autophagic pathway. Contrarily, autophagy is activated upon low-energy levels or as a stress response through the activation of AMPK, which in turn inhibits mTORc1. For the sake of clarity, the autophagic pathway can be divided into different stages: induction and cargo recognition, phagophore formation and expansion, and autophagy resolution (*reviewed in Yu et al., 2018*). As previously mentioned, autophagy is initiated by the repression of mTORc1 by different stimuli. Upon mTORc1 inhibition, the ULK complex form by different autophagy-related (ATG) proteins and the serine-threonine kinases ULK1 and ULK2 are recruited to membranes to initiate autophagy (*Jung et al., 2009*). Afterwards, the ULK protein complex phosphorylates ATG13 and focal adhesion kinase family interacting protein of 200 kDa (FIP200), which are essential in the autophagy initiation (*Kim et al., 2013*). Then, a number of different ATG proteins are recruited to the membrane to initiate the phagophore formation and elongation.

Unlike the process of vesicle formation, double-membrane phagophores are usually generated by the addition of new membranes rather than by budding from the surface of other pre-existing organelles (*Andrejeva et al., 2020*). This process is normally initiated at the endoplasmic reticulum or mitochondria (*Axe et al., 2008*). Phagophore formation requires phosphatidylinositol 3-kinase (PtdIns3K) protein complex activation. Upon PtdIns3P activation, PIP_3 is formed in the initial phagophore and thus, several ATG proteins attach to complete the formation of the autophagosome (*reviewed in Mizushima et al., 2011*). In this process, the cytosolic protein light chain 3B form I (LC3-I) is conjugated to phosphatidylethanolamine to form the lipidated membrane-bound form LC3-II, which is found in the autophagosome membranes (*reviewed in Tanida et al., 2008*). Then, autophagosomes can fuse with lysosomes to form autolysosomes where the engulf content is degraded, or with endosomes to form amphisomes (*reviewed in Cadwell, 2016*). Afterwards, amphisomes can also fuse with lysosomes to undergo lysosomal degradation. This fusion requires lysosomal membrane protein 2 (LAMP2) and Rab7 (*Huynh et al., 2007*; *Eskelinen et al., 2002*).

After the fusion, the inner content of autophagosomes and amphisomes is degraded upon the action of the lysosomal acid hydrolases cathepsin B, D and L (*reviewed in Mrschtik et al., 2015*). In this process, LC3-II gets reverted to the non-lipidated LC3-I form. Finally, the resulting small molecules, particularly amino acids, are recycled back to the cytosol upon the action of ATG22 and other permeases. Despite autophagy was initially described as a non-selective nutrient recycling pathway, now we know that the cargo recognition is selective through the recognition of specific receptors (*reviewed in Gatica et al., 2018*).

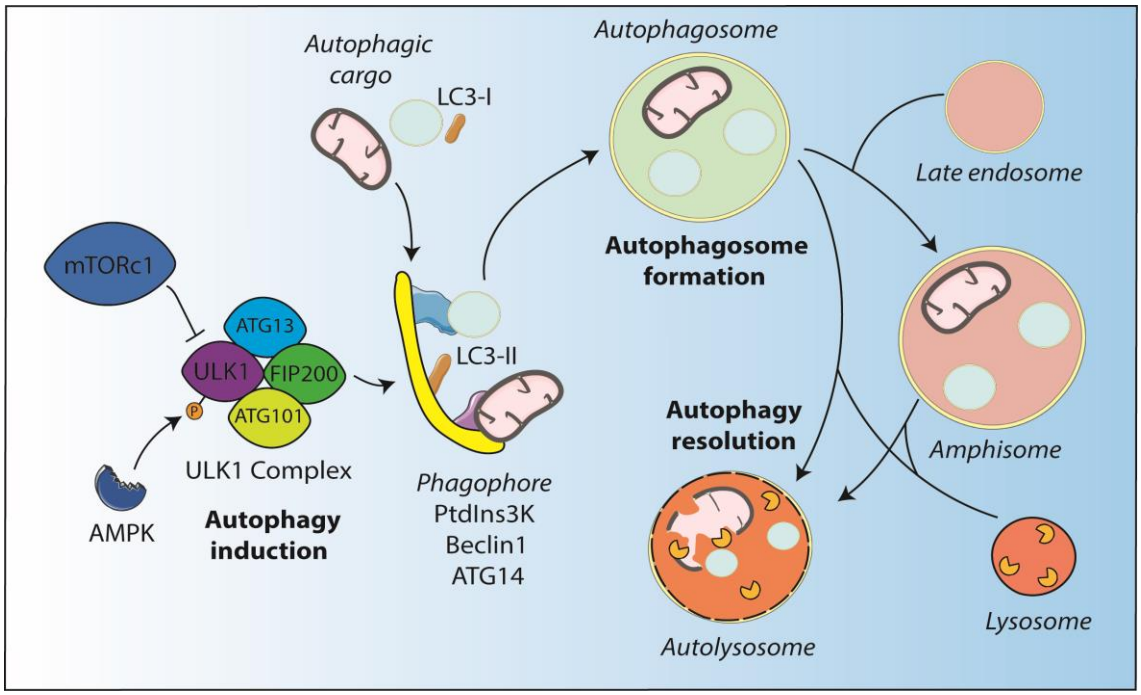


Figure 2. Scheme of the mammalian autophagy degradation. Autophagy is initiated by the formation and the expansion of phagophore, which engulfs autophagic cargo. Then, autophagosome is formed and fuses with a lysosome to form autolysosome to resolve autophagy. Schematic art pieces used in this figure are provided by Servier Medical Art under a Creative Commons Attribution 3.0 Unported License.

Autophagy is generally considered beneficial for the cell, but its deregulation has been linked to inflammation, mitochondrial stress and apoptosis (*Rodríguez-Nuevo et al., 2018*; *reviewed in Maiuri et al., 2019*). In adipocytes specifically, dysregulation in autophagy has been linked to obesity and T2D (*reviewed in Clemente-Postigo et al., 2020*). Indeed, adipocyte differentiation is dependent on autophagy. Mechanistically, some specific inhibitory adipogenic proteins such as *krüppel*-like factors 2 and 3 (KLF2/3), which are inhibitors of PPARγ, are removed through autophagy (*Zhang et al., 2013*). Besides that, autophagy can control adipogenesis by the selective removal of mitochondria, also known as mitophagy. Importantly, lipid droplets can also be targets of autophagy by a process known as lipophagy, which is especially relevant in the TAG catabolism of muscle and the liver (*reviewed in Kounakis et al., 2019*).

2.1.9. Mitophagy in adipocytes

The term mitophagy describes the selective degradation of mitochondria (*reviewed in Galluzzi et al., 2017*). This process controls the mitochondrial mass and quality and thus, it is required for the steady-state turnover of mitochondria to adjust the mitochondrial content to the changing metabolic requirements. Importantly, mitochondria are not isolated organelles but instead, they conform a dynamic network, the morphology of which determines mitochondrial metabolism, production of mitochondrial Reactive Oxygen Species (mtROS) and mitochondrial turn-over (*reviewed in Chan et al., 2006, reviewed in Zorzano et al., 2010*). Indeed, mitophagy is preceded by mitochondrial fission. Dynamin-related protein 1 (DRP1) is the master regulator of mitochondrial fission in most eukaryotic organisms. In this process, DRP1 is recruited from the cytosol to form polymeric spirals around mitochondria that constrict to sever the inner and outer membranes (*Ingerman et al., 2005*). In addition, mitochondrial fission is regulated by mitochondrial protein 18 kDa (MTP18) and fission protein 1 homolog (FIS1) (*reviewed in Liesa et al. 2009*). On the other hand, the proteins responsible for the regulation of mitochondrial fusion are mitofusin 1 and 2 (MFN1 and MFN2), and optic atrophy 1 (OPA1). Mitochondrial fusion is a 2-step process, where the outer and the inner mitochondrial membranes fuse through separate events (*Malka et al., 2005*). MFN1 and MFN2 are in the outer mitochondrial membrane and mediate the outer membrane fusion; while OPA1 is localised in the mitochondrial intermembrane space or attached to the inner mitochondrial membrane, where it mediates the inner membrane fusion.

Upon mitochondrial fission, mitophagy can be initiated by the mitochondrial kinase phosphatase tensin homolog-induced kinase 1 (PINK1) and the E3 ubiquitin ligase parkin (*Park et al., 2006; Clark et al., 2006*). These proteins are also involved in the formation of mitochondrial-derived vesicles, which participate in the trafficking of mitochondrial proteins and mitochondrial DNA (mtDNA) to the endosomal compartments (*McLelland et al., 2016*). Upon mitochondrial depolarization, PINK1 recruits parkin from the cytosol to damaged mitochondria and phosphorylates it. Afterwards, mitophagy receptors such as BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) binds to damaged mitochondria and thus, mitophagy is initiated. In addition, mitophagy can be PINK1/parkin independent and thus, independent of mitochondrial damage. As a matter of fact, other stimuli such as hypoxia can induce mitophagy through BNIP3 and BNIP3-like (BNIP3L, also known as NIX) proteins, which can interact with LC3 to initiate this process (*Zhu et al., 2013*). Besides, the expression of these genes is induced by hypoxia-inducible factor 1α (HIF1α) (*Hamacher-Brady et al., 2007*).

As mentioned previously, mitophagy is an essential process in adipogenesis (*reviewed in Clemente-Postigo et al., 2020*). As a matter of fact, adipogenesis is associated with an increase in the mitochondrial turn-over (*Goldman et al., 2011*). Therefore, the recycling of defective mitochondria is key in the maintenance of metabolic homeostasis in mature adipocytes. Furthermore, the control of the mitochondrial mass is critical in the differentiation of brown adipocytes and the *browning* of white adipocytes (*Taylor et al., 2017*). While the differentiation of white adipocytes to beige adipocytes requires mitochondrial biogenesis, the reversion to white adipocytes is dependent on mitophagy (*Lu et al., 2018*). In addition, it has been described that mitophagy is accelerated in senescent adipocytes (*Young et al., 2009*) and thus, it controls WAT senesce and ageing-induced inflammation. Indeed, cell-autonomous inflammation in non-immune cells has been linked to problems in the mitophagy resolution (*Rodríguez-Nuevo et al., 2018*). Therefore, we can speculate that mitophagy dysregulation could have a role in the development of chronic inflammation in WAT upon obesity.

2.1.10. Lipid metabolism in adipocytes

As mentioned previously, WAT is the most important tissue in the storage of energy in the form of TAGs, which can mobilize to meet the energy demands of the organism. The WAT mass is determined by the balance between the synthesis and breakdown of TAGs (*reviewed in Song et al., 2018*). Upon feeding, the liver synthesizes TAGs and packs them into chylomicrons or very-low-density lipoproteins (VLDL). Afterwards, the transported TAGs are hydrolysed upon LPL stimulation by insulin in the vascular endothelium of WAT. Released non-esterified fatty acids enter to the adipocyte through fatty acid transporters such as cluster of differentiation 36 (CD36) (*Endemann et al., 1993*) and fatty acid transport protein-1 (FATP1) (*Wu et al., 2006*). Afterwards, these FFAs are esterified with glycerol-3-phosphate into TAGs to form lipid droplets. This whole process is stimulated by insulin since glucose excess is converted into glycerol for lipid esterification (*Rotondo et al., 2017*). Contrarily, by inducing insulin resistance, TNFα and other proinflammatory cytokines can reduce the uptake of FFAs in adipocytes. In addition, TNFα reduces the expression of *Lpl* which is crucial in the hydrolysis of TAGs from lipoproteins. Besides fatty acid uptake, carbohydrates can also be converted into fatty acids to form TAGs through a process known as *de novo* lipogenesis. In principle, this anabolic pathway takes place in all cell types, although it is more active in metabolic tissues such as liver and WAT. In this process, upon insulin stimulation, glucose enters in the adipocyte upon GLUT4 translocation. Then, it undergoes glycolysis and tricarboxylic acid (TCA) cycle to produce energy. However, in adipocytes, the TCA cycle intermediate citrate is exported from the mitochondria to the cytosol for lipogenesis via citrate transport protein (CTP) (*reviewed in Costello et al., 2013*).

Afterwards, citrate is converted into acetyl-coenzyme A (CoA) by ATP-citrate lyase (ACLY). Then, this metabolite is converted to malonyl-CoA upon carboxylation from acetyl-CoA carboxylases 1 (ACC1). Finally, malonyl-CoA is converted into palmitate by fatty acid synthase (FASN), which undergoes elongation and desaturation reactions to generate different complex fatty acids such as palmitoleic or oleic acid. *De novo* lipogenesis is controlled by different hormones and the nutritional status of the organism. Upon fasting, AMP levels increase in adipocyte, which activates AMPK. Specifically, AMPK inhibits the transcription factor SREBP-1c, which regulates the expression of lipogenic genes (*Li et al., 2011; Kawaguchi et al., 2002*). In addition, protein kinase A (PKA) is activated by glucagon in this context, further inhibiting lipogenesis. Besides that, inflammation is associated with the down-regulation of lipogenic factors such as *Pparg* (*Poulain-Godefroy et al., 2008*). Contrarily, upon feeding, insulin stimulates the uptake of glucose, enhances the activity of lipogenic enzymes and increases the expression of lipogenic genes through SREBP1-c (*reviewed in Czech et al., 2013*).

The stored TAGs serve as an energy reservoir for the whole organism. Therefore, adipocytes are in charge of the mobilization of lipids through TAG hydrolysis via a process known as lipolysis. In this process, TAGs are sequentially hydrolysed to generate diacylglycerides (DAGs) and monoacylglycerides (MAGs) to release glycerol and FFAs. Three different lipases participate in this process, namely TAG lipase (ATGL), HSL and monoacylglyceride lipase (MGL). ATGL is the first lipase to act since it mediates the hydrolysis of TAGs. It is mainly expressed in WAT and it is associated in part with the lipid droplets (*Zimmermann et al., 2004*). The expression of this lipase is induced upon fasting in rats. In addition, its expression is highly induced upon dexamethasone treatment (*Villena et al., 2004*). Besides ATGL, triacylglycerol hydrolase also contributes to the TAG hydrolysis in adipocytes. However, this enzyme is mainly expressed in the liver (*Dolinsky et al., 2001*). On the other hand, HSL has lipase activity against a wide variety of substrates including TAGs, DAGs, cholesterol esters and retinol esters (*reviewed in Holm, 2003*). However, it has more activity against DAGs in comparison to TAGs and thus, it is fundamental in the hydrolysis of DAGs. This lipase is highly regulated since it can be induced by hormones such as catecholamines through cAMP and PKA dependent phosphorylation (*Samra et al., 1998*), whereas insulin inhibits it by the hydrolysis of cAMP (*reviewed in Holm et al., 2000*). Hence, HSL activation is the major regulatory factor that limits the kinetics of this catabolic pathway. Finally, MGL catalyses the hydrolysis of MAGs into glycerol and a fatty acid. Besides lipases, other proteins that are associated with the lipid droplets regulate lipolysis. Perilipin-1 is one of these proteins. In fact, it is the most abundant protein associated with lipid droplets (*Greenberg et al., 1991*).

The expression of *Plin1* is induced upon adipogenic differentiation and it has 2 different isoforms produced by alternative splicing: perilipin 1 and 2, being the first the most abundant in mature adipocytes. Functionally, perilipin acts as a barrier that separates the lipases and TAGs in basal conditions. Upon adrenergic stimulation via PKC, perilipin gets phosphorylated changing its conformation. Upon this conformational change, lipases gain access to the surface of the lipid droplets, allowing the lipolysis to occur (*Marcinkiewicz et al., 2006; Moore et al., 2005*). Besides, other proteins such as α/β hydrolase domain-containing protein 5 (ABHD5) (*Yamaguchi et al., 2004*), tail-interacting protein 47 (TIP47) (*Gao et al., 2006*), CAV1 (*Brasaemle et al., 2004*), fatty acid-binding protein (aFABP), adipocyte lipid-binding protein (ALBP) and aP2 (*Matarese et al., 1988*) interact with lipid droplets to regulate lipolysis.

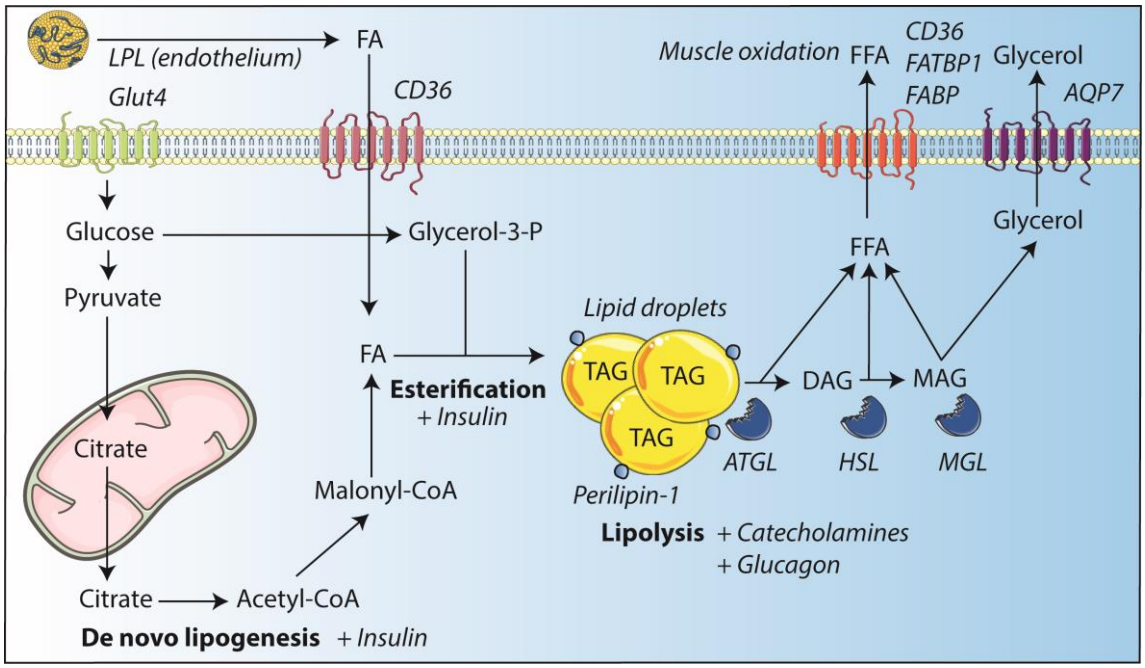


Figure 3. Schematic representation of lipid metabolism in adipocytes. Insulin signalling enhances the anabolic metabolism *de novo* lipogenesis, lipid esterification, and glucose and FFA uptake), whereas catecholamines and glucagon induce lipid mobilization (lipolysis) in adipocytes. Schematic art pieces used in this figure are provided by Servier Medical Art under a Creative Commons Attribution 3.0 Unported License.

Besides that, it has been described that TNFα and other cytokines can induce lipolysis activation in adipocytes (*reviewed in Cawthorn et al., 2008*). Although the exact mechanisms are unclear, it has been shown that TNFα can induce HSL phosphorylation (*Zhang et al., 2002*) and *Plin1* down-regulation (*Rydén et al., 2002; Tamori et al., 2002*), increasing the lipolytic rates. Nevertheless, it has been also described that TNFα reduces the expression of lipogenic genes such as *Atgl*, *Hsl* and *Mgl* (*Ruan et al., 2002*). Importantly, TNFα induced lipolysis could be important in the development of cachexic which is characterised by the loss of WAT and muscle mass, and it is driven by systemic inflammation (*reviewed in Cole et al., 2018*).

2.2. White adipose tissue inflammation drives metabolic syndrome and type II diabetes

2.2.1. NF-κB signalling in inflammation

Nuclear factor κ-light-chain-enhancer of activated B (NF- κB) represents a family of transcription factors that regulate the expression of genes involved in immunity and inflammation (*reviewed in Lawrence, 2009*). There are 5 members in this family, namely NF-κB1 (also known as p50), NF-κB2 (also known as p52), RelA (also known as p65), RelB and c-Rel. These transcription factors bind to the specific DNA regulatory elements κB enhancers in the form of dimers. Normally, the NF-κB transcription factors are found in the cytoplasm bound to IκB family proteins such as IκBα. NF-κB1 and NF-κB2 are produced from the precursor proteins p105 and p100 respectively, both of which can function as NF- κB inhibitor proteins. Regarding the activation of NF-κB transcription factors, they can be activated by 2 major signalling pathways: the canonical and noncanonical pathway. The canonical NF- κB pathway can be activated by different stimuli such as the activation of Pattern-Recognition Receptors (PRRs) by Damaged/Pathogen-Associated Molecular Patterns (DAMPs and PAMPs) or by specific immune cell receptors such as the T-cell receptor (TCR). Upon receptor activation, the degradation of IκB is triggered by the activation of the IκB kinase (IKK) complex. This complex is formed of 2 catalytic subunits, IKKα and IKKβ, and a regulatory subunit named NF-κB essential modulator (NEMO) or IKKγ. This kinase complex is phosphorylated and activated in response to the presence of DAMPs or PAMPs. Then, the IKK complex phosphorylates IκB proteins, triggering the ubiquitin-dependent degradation of these inhibitors by the proteosome. Afterwards, the NF-κB transcription factors translocate from the cytoplasm to the nucleus in the form of dimers, predominantly, p50/RelA and p50/c-Rel.

On the other hand, the noncanonical NF-κB pathway can be triggered in response to specific ligands of the TNFα receptor (TNFR) superfamily such as the receptors CD40, receptor activation of nuclear factor κB (RANK), B-cell activating factor receptor (BAFFR) and lymphotoxin β receptor (LTβR) (*reviewed in Sun, 2017*). In the noncanonical pathway, IκBα is not degraded. Instead, it depends on the processing of the NF-κB2 precursor protein p100 by NF-κB inducing kinase (NIK) and IKKα. Upon the phosphorylation induced by these 2 kinases, p100 is ubiquitinated and then processed, resulting in the generation of functional p52, which translocate to the nucleus in the form of p52/RelB dimers. Afterwards**,** the NF-κB transcription factors induce the expression of genes in non-immune and immune cells to regulate cell survival, immunity and inflammation. Importantly, upon metabolic stress, adipocytes activate the NF-κB pathway increasing the expression of several cytokines and chemokines, which drives the development of the metabolic syndrome and T2D, as it is described in section 2.2.2.

However, the induction of NF-κB target genes is just one part of the inflammatory response. Indeed, inflammation is orchestrated by the expression of NF-κB target genes and the assemble of the inflammasome. Inflammasomes are a group of intracellular protein complexes that are assembled upon the presence of PAMPs and DAMPs. Canonical inflammasomes are constituted of a ligand sensor receptor of the NOD-like receptors (NLRs) family or absent in melanoma 2 (AIM2), the adaptor protein apoptosis-associated speck-like protein containing CARD (ASC) and pro-caspase-1. The most studied inflammasome is the NOD-like receptor protein 3 (NLRP3). Upon inflammasome stimulation, it assembles and processes the conversion of pro-IL-1β to the functional cleaved-IL-1β (*reviewed in Liu et al., 2017*). In addition, inflammasome cleaves gasdermin-D to trigger pyroptosis by forming membrane pores (*Liu et al., 2016*). It is known that NF-κB is key in the priming of inflammasomes since interleukin-1β (*Il1b*) and *Nlrp3* are target genes of these transcription factors. After the priming signal, inflammasomes are assembled upon different stimuli such as ROS, K^+ efflux, cytosolic DNA among others (*reviewed in Liu et al., 2017*). Besides that, liposaccharide (LPS) can induce non-canonical activation of NLRP3. In this process, LPS is delivered to the cytosol and binds to caspase-11. Then, caspase-11 cleaves gasdermin-D to induce pyroptosis and activate the NLRP3 inflammasome (*reviewed in Man et al., 2015*).

2.2.2. Adipocyte inflammation in obesity

As mentioned above, upon homeostatic stressors, chronic inflammation emerges in adipocytes. This inflammation drives the development of the metabolic syndrome and T2D (*reviewed in Reilly et al., 2017*). In fact, many comorbidities associated with obesity such as T2D, nonalcoholic fatty liver disease, cancer or even cardiovascular diseases are related to low-chronic inflammation in WAT (*reviewed in Lumeng et al., 2011*). However, the specific factors that initiate inflammation in adipocytes are uncertain and thus, identifying such DAMPs may lead to the development of novel treatments to prevent the emergence of comorbidities associated with obesity. Upon obesity, different stressors generate DAMPs in the adipocyte and thus, acute inflammation is triggered in these cells. The initial inflammatory response is catabolic and alleviates the anabolic pressure to restore the homeostasis of the tissue. As a matter of fact, as it has been previously mentioned, inflammation induces lipolysis and reduces the expression of lipogenic and adipogenic genes (*reviewed in Cawthorn et al., 2008; Poulain-Godefroy et al., 2008*). However, in obesity, these stressors are chronically present and, as a result, there is a transition from the adaptative to maladaptive stress characterised by chronic inflammation and reduced expression of healthy adipokines.

As it has been mentioned, adipocytes act as energy storage. They can even reduce the food-intake via leptin through the sympathetic nervous system (*reviewed in Scherer et al., 2011*). In obesity, this brain-adipocyte crosstalk is deregulated. In addition, there is hypersecretion of insulin to compensate for the insulin resistance. As a result, adipocytes expand in number and size to increase lipid storage. Eventually, this situation induces stress on adipocytes and as a consequence, inflammation emerges. The DAMPs that initiate the initial inflammatory response in adipocytes remain unknown, yet some candidates have been proposed. Dietary or endogenous lipids may cause inflammation in adipocytes. As a matter of fact, FFAs can bind to toll-like receptors (TLRs) such as TLR4 or TLR2 to trigger NF-κB activation (*Weatherill et al., 2005*). On the other hand, some lipids have anti-inflammatory properties such as omega-3 essential fatty acids (*Cranmer-Byng et al., 2014*). In addition to lipids, gut-derived antigens have been shown to cause inflammation in adipocytes (*Reviewed in Saad et al., 2016*). Specifically, upon obesity, there is an increase in intestinal permeability, resulting in higher circulating levels of LPS produced by bacteria. Afterwards, LPS can bind to TLR4 in adipocytes to trigger inflammation (*Weatherill et al., 2005*).

Besides inflammation, hypoxia is developed in adipocytes as cells expand to increase the lipid storage. This expansion reduces the oxygen perfusion in WAT and leads to HIF1α activation in adipocytes (*Lee et al., 2014*). Afterwards, inflammation can be activated through hypoxia, although how this process induces inflammation is unclear (*reviewed in D'Ignazio et al., 2016*). On the other hand, inflammation can also up-regulate the expression of *Hif1a* (*Tacchini et al., 2004*) and thus, the causality of hypoxia in the emergence of adipocyte inflammation is difficult to establish. Nonetheless, WAT hypoxia constitutes a relevant pathophysiological change in the development of metabolic syndrome since HIF1α regulates the expression of VEGF and thus, is key in the vascularization of WAT.

Lastly, mitochondrial and oxidative stress upon obesity can trigger inflammation in adipocytes. Indeed, mitochondria are a source of numerous DAMPs including mtDNA and mtROS. Alterations in mitochondrial turnover, morphology or function can lead to inflammation in non-immune cells (*reviewed in Rodríguez-Nuevo et al., 2019*). Importantly, mtDNA can bind to TLR9 in amphisomal compartments to trigger TLR9 signalling in the muscle (*Rodríguez-Nuevo et al., 2018*). In addition, TLR9 can engage mtDNA in the cytosol upon mitochondrial damage to activate the DNA sensor cyclic Guanosine monophosphate (GMP)-AMP synthase (cGAS) to activate interferon regulatory factors (IRFs) and induce the interferon response (*Maekawa et al., 2019*). Besides mtDNA, mitochondrial stress appears upon obesity and causes oxidative stress, increasing the generation of mtROS in adipocytes (*reviewed in Bournat et al., 2010*).

Elevated mitochondrial substrate load increases the activity of the electron transport chain, increasing the production of mtROS. Afterwards, mtROS species such as H_2O_2 can activate the NF-κB transcription factors and induce inflammation *(reviewed in Morgan et al., 2011*). Although the exact mechanisms by which mtROS can activate NF-κB remain elusive, some mechanisms have been proposed. For instance, in response to H_2O_2, the spleen tyrosine kinase (SYK) (*Takada et al., 2003*) is phosphorylated, which in turn induces the phosphorylation of IκBα independently of IKK. Other studies indicate that H_2O_2 can activate the IKK complex by enhancing the association of IKKα and IKKβ with NEMO (*Herscovitch et al., 2008*). Besides, NIK has been shown to be positively regulated by mtROS through the inhibition of NIK phosphatases (*Li et al., 2006*). In addition, ROS can activate NLRP3 inflammasome to promote IL-1β cleavage and pyroptosis (*Heid et al., 2013*). Likewise, NLRP3 can induce ROS production to further increase inflammation. Therefore, mitochondrial oxidative stress can be one of the initial pathophysiological changes that initiate autonomous inflammation in adipocytes upon obesity.

2.2.3. Beyond the adipocyte, the role of immune cells in WAT inflammation

After the initial inflammatory response orchestrated by adipocytes, other immune cells are infiltrated in the tissue, exacerbating the initial inflammatory response. Indeed, upon NF-κB activation, adipocytes increase the expression of other cytokines or chemokines such as chemokine (C-C motif) ligand 3 (*Ccl2*) leading to the infiltration of monocytes in WAT. Besides, dying adipocytes due to the metabolic stress send out numerous signals that contribute to the recruitment of immune cells to WAT. Furthermore, local macrophages can polarize to inflammatory macrophages through the presence of proinflammatory cytokines (*reviewed in Appari et al., 2018*). WAT contains immune cells that contribute to the maintenance of adipocyte homeostasis. In lean animals, these immune cells are in the Type 2 state (T_H2) and thus, they contribute to the maintenance of the tissue by secreting T_H2 cytokines such as interleukin-33 (IL-33). As a matter of fact, it has been shown that IL-33 has protective effects in adipocyte inflammation in obese mice (*Miller et al., 2010*). In addition, IL-33 activates eosinophils to secrete interleukine-4 (IL-4), which induces the polarization of anti-inflammatory (M2) macrophages (*Molofsky et al., 2013*). M2 macrophages are essential to control adipocyte inflammation and insulin sensitivity in these cells. Indeed, M2 macrophages secrete interleukine-10 (IL-10), which has been shown to increase insulin sensitivity in adipocytes (*reviewed in Fujisaka et al., 2009*). Therefore, in energy balance conditions, immune cells contribute to the maintenance of the homeostasis of WAT. However, in obese animals, immune cells operate in the proinflammatory T_H1 state.

Hence, metabolic stressors induce inflammation in adipocytes, which increase the production of proinflammatory cytokines such as TNFα. The expression of these factors triggers inflammatory (M1) polarization of the tissue-resident macrophages (*Lumeng et al., 2007*). Also, inflammation increases the recruitment and the proliferation of inflammatory macrophages (*Haase et al., 2014*). These M1 macrophages further increase the expression of proinflammatory cytokines in WAT and thus, contribute to the development of insulin resistance and T2D. Besides macrophages, T $CD4^+$ and $CD8^+$ lymphocytes have been shown to regulate inflammation in WAT. Importantly, the number of T $CD8^+$ lymphocytes in WAT increase in obesity, which further contribute to the recruitment of blood monocytes to this tissue (*McLaughlin et al., 2014; Nishimura et al., 2009*). Contrarily, the number of T_{reg} Cells in WAT is reduced during obesity (*Bapat et al., 2015*). Finally, B cell recruitment to WAT has been shown to induce T cell trafficking to this tissue during obesity (*Duffaunt et al., 2009*). Due to the nature of the cytokine signalling, this inflammatory signal will further induce NF-κB signalling in adipocytes and immune cells. Thus, this positive inflammatory feedback fuels the chronic inflammatory response in obesity and leads to the development of metabolic pathologies and insulin resistance.

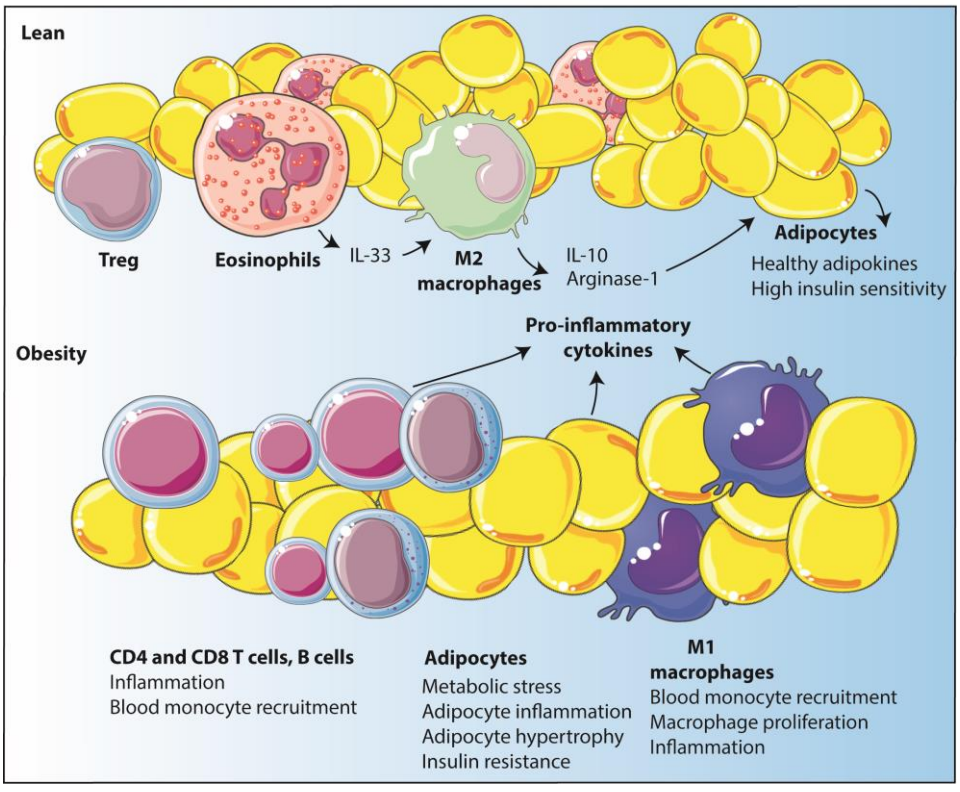


Figure 4. Scheme of WAT immunity. In normal conditions, the immune profile of WAT is T_H2, promoting the expression of healthy adipokines by adipocytes such as adiponectin. Upon obesity, the inflammatory profile switches to T_H1 and thus, the expression of proinflammatory cytokines increases by immune and non-immune cells, reducing the insulin sensitivity of the tissue. Schematic art pieces used in this figure are provided by Servier Medical Art under a Creative Commons Attribution 3.0 Unported License.

2.2.4. Inflammation induces insulin resistance

Insulin resistance is defined as the failure to respond to physiological insulin actions. This condition proceeds the development of T2D and it is directly associated to obesity-induced inflammation (*reviewed in Lumeng et al., 2011*). Indeed, inflammation disrupts insulin signalling at numerous levels. First, NF-κB activation increases the expression of proinflammatory cytokines such as *Il1b*, *Il6* and *Tnfa*. Afterwards, these cytokines enhance lipolysis and increase the serine/threonine phosphorylation of IRS-1 via c-Jun N-terminal kinase (JNK) and IKK stimulation (*reviewed in Kahn et al., 2006; Zhou et al., 2014*). IRS-1 serine/threonine phosphorylation block insulin signalling. Furthermore, it has been previously described that TNFα disrupts PPARγ transcriptional activity and thus, it reduces the expression of the *InsR*, *Irs1* and *Glut4* in adipocytes (*reviewed in Rehman et al., 2016*). IKK activation also reduces the expression of leptin and adiponectin (*Wang et al., 2011*). In addition, treatments with inhibitors of NF-κB such as curcumin-containing liposomes improve insulin sensitivity, highlighting the importance of this pathway in the development of insulin resistance (*Yekollu et al., 2011*).

It is thought that inflammation in adipose tissue constitutes a counter-regulatory mechanism to promote TAG mobilization to reduce adiposity. However, continued unresolved chronic inflammation leads to WAT hypoxia, insulin resistance, adipocyte death and fibrosis of the tissue, thus generating metabolic pathology. Therefore, the treatment against inflammation in obesity constitutes a therapeutical axis to prevent the development of comorbidities associated with obesity.

2.3. Neuregulins and ErbB receptors

2.3.1. Neuregulins, a subfamily of the epidermal growth factor family protein

Neuregulins constitute a subfamily of proteins that belongs to the epidermal growth factor (EGF) family protein given that they contain an EGF-like domain. This domain allows the binding of these factors to the avian erythroblastosis virus B (ErbB), which are tyrosine kinase receptors (*reviewed in Gumà et al., 2010*). Neuregulins are glycoproteins encoded by at least 4 different genes, namely *Nrg1-4*. Moreover, there are multiple isoforms of the protein neuregulin-1 (NRG1) due to alternative splicing of the transcript and the existence of tissue-specific promoters. Structurally, most of these factors contain a transmembrane domain and a bioactive *EGF-like* extracellularly localised. This domain is released upon proteolysis mediated by metalloproteases such as a disintegrin and metalloprotease domain 17 and 19 (ADAM17 and ADAM19) (*Yokozeki et al., 2007*). Then, the *EGF-like* domain can bind to ErbB3 or ErbB4 receptors. This domain is 45-55 amino acids long and is sufficient for the binding and the ErbB receptor activation. However, different neuregulin isoforms can bind with different affinities to ErbB receptors.

Specifically, NRG1 isoforms bind to ErbB3 and ErbB4 receptors with different affinities (*Jones et al., 1999a; Jones et al., 1999b*), whereas NRG4 can only bind to the ErbB4 receptor (*Harari et al., 1999*). Besides the EGF-like domain, the extracellular N-terminus sequence of neuregulins consists of different domains. Some of the NRG1 isoforms and neuregulin-2 (NRG2) contain immunoglobulin G (IgG)-like domains, which interacts with the proteoglycans of the extracellular matrix and thus, they allow the clustering of neuregulins in the extracellular space. Therefore, IgG-like domain-containing NRG1 isoforms and NRG2 are suitable candidates for autocrine and paracrine signalling. Other NRG1 isoforms contain cysteine-rich domains which partially enter to the plasma membrane as a secondary anchoring point. On the other hands, neuregulin-3 (NRG3) and NRG4 lack this domain and thus, one can speculate that these factors can be released and exert distal effects in addition to the local signalling. Indeed, studies in mice have shown that different isoforms of neuregulin that differ in their N-terminal sequence or in the *EGF-like* domain have different functions (*reviewed in Gumà et al., 2010*).

2.3.2. Neuregulin receptors: ErbB, Type I tyrosine kinase receptors

ErbB receptors, also known as human epidermal growth factor receptors (HERs) in humans, constitute a subfamily of the growth factor tyrosine kinase receptor family protein. This subfamily of proteins consists of 4 different receptors, namely ErbB1, ErbB2, ErbB3 and ErbB4. Structurally, these receptors contain an extracellular ligand-binding domain in the N-terminus, a transmembrane domain and an intracellular tyrosine kinase domain in the C terminal tail. Despite their common structure, these receptors bind different molecules of the EGF family members. ErbB1, also known as EGFR, can bind EGF and many different molecules, yet it fails to bind neuregulin (*reviewed in Gumà et al., 2010*). However, it can act as a coreceptor of ErbB3 and ErbB4, which can bind neuregulin. ErbB2, also known as Neu, has no known ligand and thus, it is an orphan receptor (*Klapper et al., 1999*). However, it shows an active tyrosine kinase domain and thus, it can signal upon receptor heterodimerization with ligand-bound ErbB3 and ErbB4. In fact, ErbB2 is the favoured receptor for the heterodimerization of ErbB3 and ErbB4 (*Graus-Porta et al., 1997*). Upon ligand binding, ErbB3 can heterodimerizes and activate ErbB2 or ErbB1 receptors, yet ErbB3 has impaired catalytic tyrosine kinase activity (*Guy et al., 1994*). Finally, ErbB4 can bind neuregulins and has catalytic activity, hence, upon ligand binding, it can preferentially heterodimerize with the co-receptors or even homodimerize to become active. Importantly, NRG4 can only bind to the ErbB4 receptor (*Harari et al., 1999*). Receptor oligomerization is needed to trigger signalling since these receptors exist in an autoinhibited form covering the dimerization domain. Upon ligand binding, these domains are exposed and thus, the receptors dimerize allowing the tyrosine trans-phosphorylation of the kinase activation loop (*reviewed in Linggi et al., 2006*). ErbB2 constitutively remains in the active conformation with the dimerization domain exposed since it lacks the ligand domain. ErbB phosphorylated tyrosine residues trigger cellular signalling allowing the binding of signalling and docking proteins that contain SH2 or PTB domains. Moreover, ErbB receptors also have non-tyrosine residues that can be phosphorylated and basic residues in the juxtamembrane region that modulate the interaction with other molecules such as PKC and the dimerization affinity of these receptors (*McLaughlin et al., 2005*). In addition to ligand-induced ErbB activation, these receptors are regulated by other ligand-independent mechanisms. The ErbB binding domain can be cleaved by the action of metalloproteases, which can lead to the formation of truncated or constitutively activated ErbB receptors (*Codony-Servat et al., 1999*). In addition, ErbB receptors can be internalised by endocytosis upon the binding of the ligand. Internalised receptors can be degraded via lysosomal degradation which results in protein receptor down-regulation to attenuate cell signalling (*reviewed in Sorkin et al., 2008*). Moreover, ErbB receptors can be translocated to the cellular nucleus, which can affect gene expression (*reviewed in Carpenter, 2003; Amit et al., 2007*).

Upon ErbB activation, they initiate several different downstream pathways, notably the MAPK cascade and AKT pathway (*reviewed in Katz et al., 2007*). Specifically, all the ErbB receptors can activate the MAPK cascade through the recruitment of adaptor proteins such as growth factor receptor bound 2 (GRB2) and GRB2-bound exchange factor son of sevenless (SOS). The MAPK pathway regulates cell cycle progression and cell proliferation. However, the AKT pathway can be differentially activated by different ErbB receptors. ErbB3 and one isoform of ErbB4 contain 1 and 6 direct binding sites for PI3K p85 respectively, whereas ErbB1 and ErbB2 can indirectly activate this pathway through the recruitment of adaptor proteins such as GRB-associated-binding protein 1 (GAB1) (*Soltoff et al., 1996*). The AKT pathway regulates cell metabolism, growth, proliferation and survival. Besides that, some ErbB receptors can activate the janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway to regulate cell proliferation and migration (*Olayioye et al., 1999*). Finally, some ErbB receptors can specifically recruit other signalling effectors such as phospholipase C-γ (PLC-γ) or focal adhesion kinase (FAK).

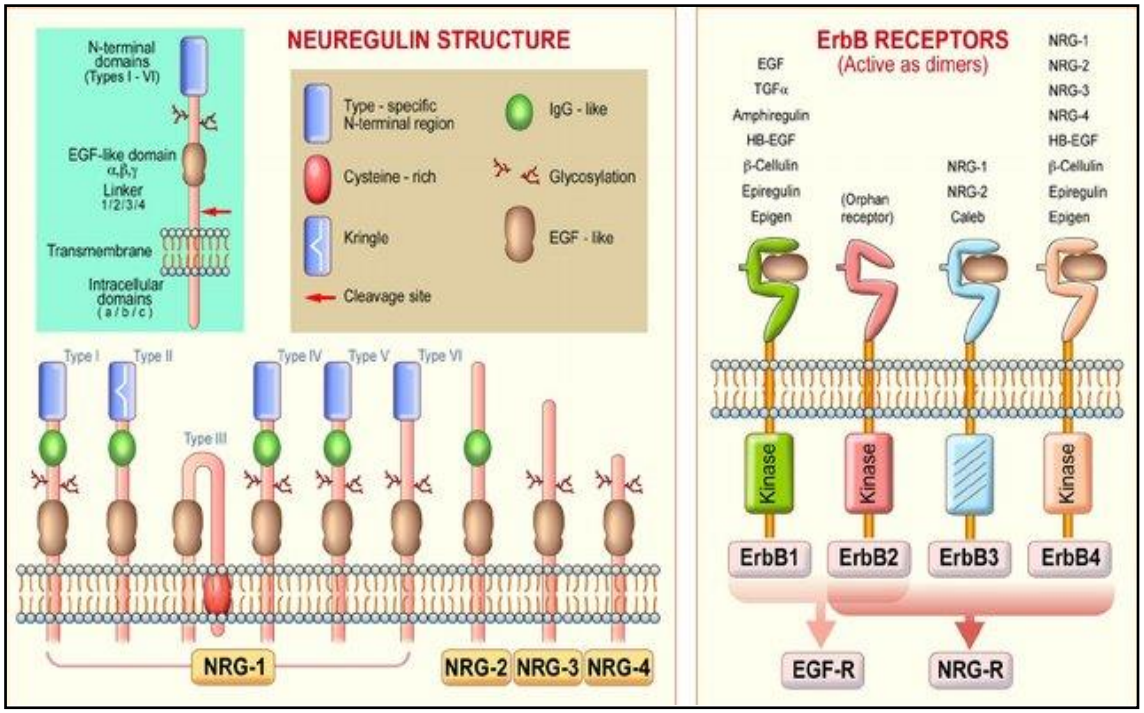


Figure 5. Structure of neuregulins and ErbB receptors. This scheme has been extracted from *Gumà et al., 2010* with permission from the main author.

2.3.3. ErbB1 receptor

As it has been previously mentioned, ErbB1 cannot bind neuregulins. Instead, this receptor binds EGF or other molecules such as transforming growth factor α (TGFα). Upon the binding of the ligand, this receptor dimerizes forming molecular clusters over clathrin-coated regions of the plasma membrane. Therefore, after ligand stimuli, ErbB1 receptors are internalized in endosomes that are eventually degraded through lysosomal degradation. ErbB1 plays a vital role in the regulation of cell proliferation and differentiation. As a matter of fact, *Erbb1*-deficient mice show defects in cell proliferation, differentiation and migration in multiple tissues including skin, central nervous system, lung, liver, kidneys among others (*Threadgill et al., 1995; Miettinen et al 1995*). In addition, EGFR overexpression drives cancer cell growth (*Kawamoto et al., 1983*).

2.3.4. ErbB2 receptor

The orphan receptor ErbB2 is constitutively active and therefore, it is the preferred partner for the heterodimerization of other ErbB receptors (*Worthylake et al., 1997*). Several factors can indirectly regulate ErbB2 tyrosine phosphorylation and its recycling such as IL-6 or EGF signalling (*Qiu et al., 1998*). ErbB2 is also over-expressed in several types of cancer such as lung and breast adenocarcinomas (*reviewed in Ross et al., 1998*) and thus, ErbB2-blocking antibodies (trastuzumab and herceptin) are used as a treatment (*reviewed in Yu et al., 2001*). Overexpression of *Erbb2* causes constitutive ErbB2 homo and heterodimerization in cancer cells (*Holbro et al., 2003*). In addition, overexpressed *Erbb2* can translocate to the mitochondria, where negatively regulates the mitochondrial oxidative metabolism (*Ding et al., 2012*). Besides these deleterious effects, ErbB2 is vital in heart development and has cardioprotective effects in adulthood (*Özcelik et al., 2002*). Knockout (KO) mice for *Nrg1, Erbb4* and *Erbb2* die at mid-embryo life due to altered heart ventricular trabeculation (*Gassmann et al., 1995; Lee et al., 1995*), highlighting the importance of this receptor in the development of the heart. As a matter of fact, some patients treated with anti-ErbB2 antibodies show cardiotoxic effects. It has been shown that ErbB2 is important in the expression of antioxidant enzymes to reduce mtROS levels (*Belmonte et al., 2015*). Therefore, oxidative stress upon ErbB2 loss-of-function has been hypothesised to be the source of the cardiotoxic effects upon anti-ErbB2 treatment. Besides, NRG1 signalling promotes cell survival via AKT activation mainly through ErbB3/ErbB2 heterodimers (*Fukazawa et al., 2003*).

2.3.5. ErbB3 receptor

As previously mentioned, unlike other ErbB receptors, ErbB3 lacks kinase activity due to a substitution in the catalytic domain of this receptor (*Plowman et al., 1990*). However, it mainly activates the AKT and AMPK pathways through the trans-phosphorylation in tyrosines by heterodimerization with other ErbB receptors upon the binding of a ligand. ErbB3/ErbB2 and ErbB3/ErbB4 can also activate the JAK/STAT and PKC pathway via NRG1 signalling (*reviewed in Mota et al., 2017*). ErbB3 signalling has been associated with cancer growth (*Holmes et al., 1992; Pietras et al., 1995*), muscle differentiation (*Bacus et al., 1992; Bacus et al., 1993; Peles et al., 1992; Suárez et al., 2001*), Schwann cell precursor differentiation (*Marchionni et al., 1993*) and keratinocytes proliferation (*Marikovsky et al., 1995*) *in vitro*. In addition, upon ErbB3 overexpression, it mainly heterodimerizes with ErbB2 and its signalling participates in the development of different types of cancer and metastasis (*Xue et al., 2006*).

2.3.6. ErbB4 receptor

The ErbB4 receptor can bind neuregulins and has a functional catalytic kinase domain. Upon the binding of the ligand, this receptor can activate different signalling pathway such as AMPK, PKC, JUN/STAT and AKT, although only some isoforms have one tyrosine residue able to bind the PI3K p85 regulatory subunit. This receptor is highly expressed in the central nervous system as in cardiac and skeletal muscle (*Plowman et al., 1993*). Besides, ErbB4 has 2 different isoforms with different juxtamembrane and C-terminus sequences, which conditionate the recruitment of the PI3K protein (*Elenius et al., 1999*). *In vitro*, ErbB4/ErbB2 heterodimers have been shown to promote cell survival via the PKB/AKT pathway (*Fukazawa et al., 2003; Bian et al., 2009*). Regarding its functions, it has been reported to have a role in neural and heart development (*Jones et al., 2003; Gassmann et al., 1995*). Other studies have highlighted the importance of this receptor in the development of schizophrenia and other neurological pathologies (*Corfas et al., 2004*). Besides, ErbB4 activation prevents cardiomyocyte apoptosis by reducing the ROS basal levels, as it is the case with ErbB2 (*Kuramochi et al., 2004*). As mentioned previously, ErbB4 is the only receptor that can bind NRG4, a growth factor that has emerged as a novel adipokine expressed by brown adipose tissue (BAT) and WAT. Interestingly, ErbB4 is one of the genes linked to obesity and diabetes, as shown by various International Consortiums such as the ADIPOGen and GENIE Consortium (*Gumà & Díaz-Sáez et al., 2020*). Indeed, heart-rescued *ErbB4* gene deletion in mice has been shown to cause obesity, dyslipidemia, hepatic steatosis, hyperglycaemia, hyperinsulinemia, insulin resistance and adipose tissue inflammation and thus, the NRG4-ErbB4 signalling axis may play a role in WAT homeostasis (*Zeng et al., 2018*).

Given the role of ErbB4 in the regulation of oxidative stress in the heart, one can speculate that ErbB4 signalling could regulate inflammation in metabolic tissues such as WAT by modulating the mtROS levels and mitochondrial function (*reviewed in Gumà & Díaz-Sáez et al. 2020*). This receptor locates in caveolar microdomains in cardiomyocytes (*Zhao et al., 1999*). Besides, upon NRG1 stimulation, ErbB4 gets recruited to the lipid rafts of neurons and regulates the synaptic plasticity of the brain (*Ma et al., 2003*). Therefore, the ErbB4 signalling in adipocytes may be initiated in these structures as well, which are abundant in this cell type. Besides ErbB4 actions in WAT, brain and heart, ErbB4 has been shown to regulate inflammation in macrophages (*Schumacher et al., 2017; Frey et al., 2009; Bernard et al., 2012; McElroy et al., 2014*). These pieces of evidence came from studies on inflammatory diseases such as Crohn's disease and ulcerative colitis. Those studies indicate that ErbB4 signalling promotes proinflammatory macrophage clearance. Thus, this function may be critical in the prevention of chronic inflammation since defects in this process can lead to autoinflammatory diseases and contribute to the development of metabolic syndrome in WAT.

2.3.7. Neuregulin-1 enhances insulin sensitivity in muscle and the liver

Neuregulins are released by different cell types of endothelial, mesenchymal and neural origin. As previously described, neuregulins regulate cellular differentiation and growth via ErbB signalling in different cell types. However, the role of neuregulins in the regulation of metabolism was described in the early 2000s. Suárez and her group discovered that NRG1 stimulates glucose uptake in the muscle (*Suárez et al., 2001*). Indeed, NRG1 induces GLUT4 translocation to the cellular membrane via PI3K/PDK1/PKCζ (*Cantó et al., 2004; Cantó et al., 2006*). Despite that this signalling axis is shared with the insulin pathway, GLUT4 translocation in muscle upon NRG1 challenge is additive with the insulin action. In muscle, the release of neuregulins is dependent on muscle contraction. Upon muscle contraction, Ca^{2+} is released from the sarcoplasmic reticulum, inducing calmodulin-dependent protein kinase II (CaMKII). In this context, the ATP levels drop and AMPK is activated. Both proteins promote GLUT4 protein translocation in muscle. In addition, the release of Ca^{2+} also activates metalloproteases that release neuregulins in muscle, which bind and activate ErbB4/ErbB2 heterodimers to induce GLUT4 translocation via PKCζ activation (*Cantó et al., 2006; review in Gumà et al., 2010*). Besides the actions of NRG1 in GLUT4 translocation, the chronic treatment with sub-myogenic concentrations of NRG1 induces mitochondrial biogenesis via proliferator-activated receptor γ coactivator 1-α (PGC1α) and PPARδ activation, the expression of both is increased upon exercise as an adaptative mechanism (*Cantó et al., 2007*). Therefore, NRG1 actions on muscle skeletal metabolism are similar to those of muscle training.

Similarly, NRG1 has a protective function in the heart by enhancing cell survival and increasing mitochondrial oxidative capacity (*Giraud et al., 2005*).

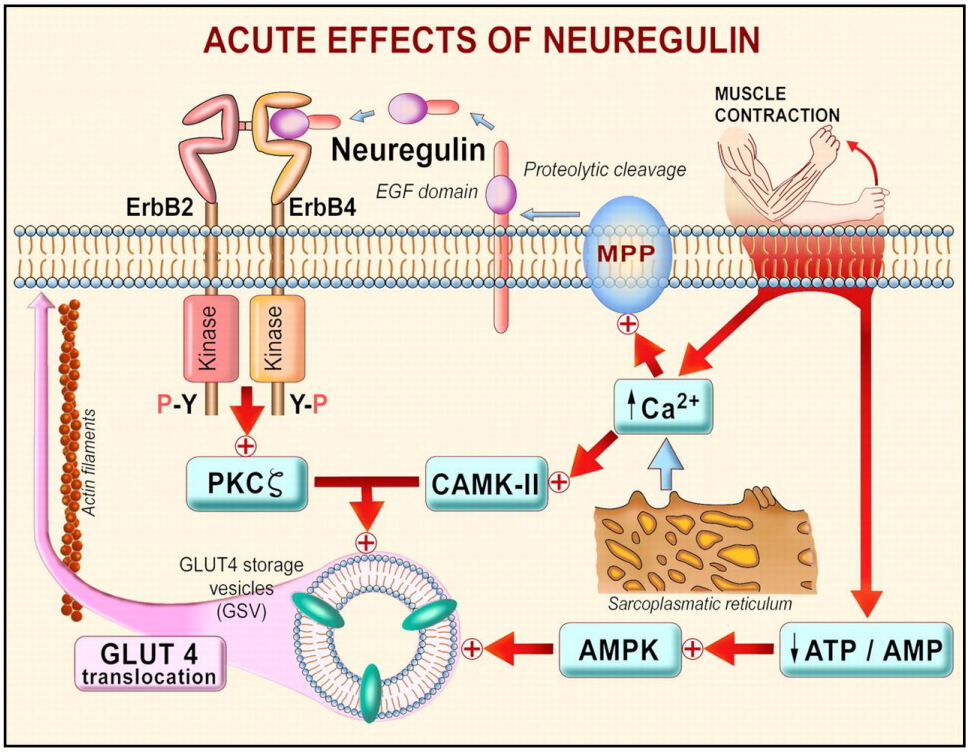


Figure 6. Neuregulin involvement in GLUT4 translocation upon muscle contraction. This scheme was extracted from *Gumà et al., 2010* with permission from the main author.

In addition to the local effects of NRG1 in muscle, *in vivo* treatments in rats with recombinant NRG1 show enhanced glucose tolerance without activation of the InsR in muscle and the liver (*López-Soldado et al., 2016*). Indeed, NRG1 has been detected in plasma and (*Ky et al., 2009*). Therefore, neuregulins could exert distal actions regulating the metabolism of different tissues. Interestingly, NRG1 treatment slightly increases the phosphorylation of PKCζ in muscle, while the AKT pathway is activated in the liver (*López-Soldado et al., 2016*). However, that study did not analyse the role of neuregulins in WAT metabolism and thus, the function of these factors in adipocyte physiology is largely obscured. Differences in the downstream signalling upon NRG1 treatment could be caused due to the different expression pattern of ErbB receptors in each tissue. Indeed, the expression of ErbB receptors is different between skeletal muscle, WAT and the liver, and it is regulated by different stimuli. In muscle, contractile activity (*Lebrasseur et al., 2003*), innervation (*Morano et al., 2018*) and myogenesis (*Shelton et al., 2019*) are some of the stimuli that regulate the expression of ErbB receptors. In 3T3-L1 adipocytes, adipogenesis down-regulates the expression of *Erbb1* and *Erbb2* (*Pagano et al., 2003*). Finally, the expression of ErbB receptors in the liver is modulated by the hepatic development and by the circadian rhythm (*Carver et al., 2002*).

2.3.8. Endocrine functions of the adipocyte-secreted neuregulin-4

In 2014 Wang and collaborators reported that *Nrg4* was mainly expressed in BAT and WAT (*Wang et al., 2014*). They generated *Nrg4*-gene-deleted mice and reported that the liver from these mice has increased expression of several lipogenic genes. In contrast, mice overexpressing *Nrg4* in adipose tissue attenuated liver lipogenesis, reduced plasma TAG levels and gained less weight upon High-Fat (HFD) diet feeding. Specifically, the authors described that NRG4 signalling in hepatocytes results in SREBP1c inhibition through the repression of liver X receptor (LXR) by STAT5 activation, thus attenuating hepatic lipogenesis. These results set a precedent and highlight the role of NRG4 signalling through hepatocytes. Afterwards, another study showed that *Nrg4* gene transfer inhibits lipogenesis and prevents HFD-induced obesity in mice, corroborating the results obtained by Wang and collaborators (*Ma et al., 2016*). In the same context, Chen and collaborators reported that NRG4 protects mice from diet-induced obesity by increasing energy expenditure and insulin sensitivity (*Chen et al., 2017*). The authors also corroborate the role of NRG4 regulating lipid metabolism in the liver, reducing hepatic steatosis upon HFD feeding in mice, promoting fatty acid β-oxidation and ketogenesis in the liver. In addition, the authors performed a microarray gene expression in WAT from Wild-Type (WT) and *Nrg4* transgenic mice. They observed that some genes related to mitochondrial function were upregulated in WAT from *Nrg4* transgenic mice. As mentioned, neuregulins increase mitochondrial oxidative capacity and mitochondrial biogenesis in myocytes (*Cantó et al., 2007*). Therefore, given the previous studies in muscle, one may think that NRG4 can also regulate fatty acid β-oxidation in the liver by increasing the mitochondrial content in this tissue. In other studies, it has been described that the hepatic NRG4 signalling constitutes a checkpoint for the progression of steatosis to non-alcoholic steatohepatitis (NASH) (*Guo et al, 2017*). This liver disease is strongly associated with obesity and metabolic syndrome and it is driven by liver inflammation and fibrosis. The authors demonstrated that NRG4 deficiency in mice accelerated liver injury, fibrosis, inflammation and cell death in a murine NASH model. Not only that, *Nrg4* transgenic mice reduced the phosphorylated levels of JNK in the liver, the signalling of which induces inflammation, insulin resistance and promotes hepatocyte apoptosis (*Reviewed in Seki et al., 2012*). In all, these studies highlight the importance of NRG4 endocrine signalling in the regulation of liver metabolism (*Wang et al., 2014; Ma et al., 2016; Chen et al., 2017, Guo et al., 2017*). However, the role of the novel adipokine NRG4 in the regulation of the physiology of muscle remains unknown. In addition to NRG4 impact in the liver, NRG4 has been detected in serum and its levels are associated with metabolic syndrome in patients diagnosed with T2D (*Yan et al., 2018; Yan et al., 2017; Wang et al., 2014*).

Hence, NRG4 might be not only important as a novel molecule to treat metabolic pathologies, but it may also constitute a new marker to diagnose metabolic syndrome. Besides NRG4 actions in liver metabolism, now we know that NRG4 is a white and brown adipokine that exerts a plethora of paracrine and endocrine effects in other cell types. For instance, some authors have explored the role of NRG4 in both innervation and vascularization of WAT. First, in 2014 Rosell *et al.* discovered that *Nrg4* expression was upregulated upon cold exposure in WAT (*Rosell. et al., 2014*). The authors demonstrated that NRG4 also promotes neurite growth by using conditioned media from brown adipocytes, highlighting the importance of this adipokine in the innervation of both adipose tissues. Hence, NRG4 might be a key factor for the acquisitions of BAT features of WAT depots. In 2018, Emoto and collaborators demonstrated that NRG4 enhances adipose tissue angiogenesis, counteracting the adipose tissue hypoxia in obese mice. (*Nugroho et al., 2018a; Nugroho et al., 2018b*). In this context, neuregulins have been identified as HIF1α suppressors in neurons (*Yoo et al., 2019*). Adipose tissue hypoxia is one of the first pathophysiological changes in WAT upon obesity that leads to HIF1α and NF-kB activation (*reviewed in Scherer et al., 2011*). Hence, this constitutes a therapeutical axis controlled by neuregulin signalling since NRG4 prevents hypoxia in WAT (*Yoo et al., 2019; Nugroho et al., 2018a; Nugroho et al., 2018b*). In spite of this novel results, the specific mechanism by which NRG4 regulates WAT vascularization and innervation are yet to be elucidated. Furthermore, the autocrine role of this adipokine in adipocyte biology is largely obscured.

2.3.9. Neuregulin-4 and white adipose tissue inflammation

Aside from the regulation of the metabolic homeostasis and vascularization of WAT, NRG4 regulates and is regulated by inflammation. Some authors showed that *Nrg4* expression is down-regulated by TNFα-induced NF-κB activation (*Wang et al., 2014*). Therefore, *Nrg4* expression in WAT could be repressed by the low grade chronic inflammatory signalling present in obesity. In contrast, the expression of the proinflammatory cytokines *Il1b* and *Tnfa* is up-regulated in *Nrg4* KO WAT from mice (*Chen et al., 2017*), although the authors did not assed the specific contribution of adipocytes to the overall WAT inflammation. On top of that, NRG4 can signal through macrophages as well (*Schumacher et al., 2017*) and thus, NRG4 can be considered to be an anti-inflammatory molecule. Specifically, Schumacher *et al.* reported that *in vitro* M1 macrophages upregulate the *Erbb4* expression. Upon NRG4 binding, ErbB4 is cleaved and one fragment translocates to mitochondria. This reduces the mitochondrial membrane potential, triggering apoptosis in M1 macrophages. Besides that, the authors analysed *in vivo* macrophages from Dextran Sodium Sulfate (DSS)-induced colitis in c57 black L/6 (c57BL/6) mice. In this model, exogenous treatment with NRG4 ameliorated inflammation by reducing the expression of

proinflammatory cytokines in colonic homogenates. These results showed for the first time the potential of NRG4 as an efficient clearance promoter of proinflammatory macrophages. This is critical to prevent chronic inflammation since defects in macrophage clearance could lead to autoinflammatory diseases. However, the role of the adipocyte-secreted NRG4 in the cellular crosstalk between adipocytes and WAT-infiltrated macrophages in the progression of metabolic syndrome is yet to be unveiled.

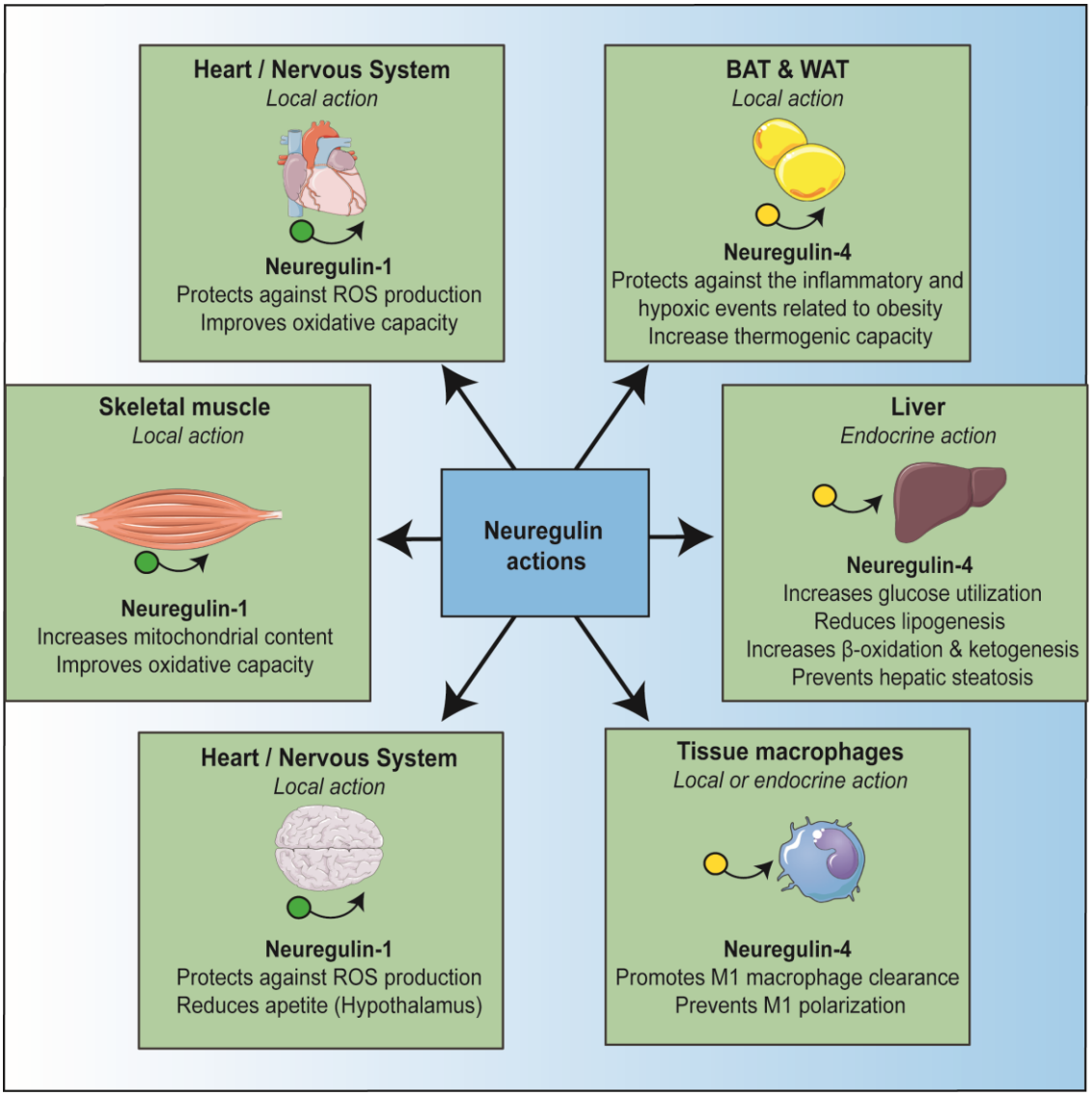


Figure 7. Actions of neuregulin in different tissues. Scheme extracted from *Gumà & Díaz-Sáez et al., 2020.* Schematic art pieces used in this figure are provided by Servier Medical Art under a Creative Commons Attribution 3.0 Unported License.

In all, neuregulins can act as anti-inflammatory molecules signalling through inflammatory macrophages. However, given that neuregulins regulate mitochondrial homeostasis, one may hypothesize that they also can regulate adipocyte inflammation by modulating mitochondrial function. As a matter of fact, there is increasing evidence that mitochondrial dysfunction is the primary cause of many autoinflammatory conditions (*reviewed in Dela Cruz et al., 2018*). As mentioned previously, mitochondria are a major source of DAMPs such as mtDNA (*Rodríguez-Nuevo et al., 2018*) and mtROS (*reviewed in Rimessi et al., 2016*). In fact, mitochondria are one of the primary sources of cellular ROS. MtROS are mainly produced in the complex I and complex III mitochondrial oxidative phosphorylation system (OXPHOS) proteins (*Quinlan et al., 2013*). This oxidative stress can initiate inflammation through the NF-κB pathway increasing the expression of several proinflammatory cytokines. However, the exact mechanisms by which ROS causes NF-κB activation are still a matter of discussion (*reviewed in Morgan et al., 2011*). In addition, ROS can activate NLRP3 inflammasome to promote IL-1β cleavage and pyroptosis. Likewise, NLRP3 can induce ROS production to further increase inflammation (*Heid et al., 2013*). This positive inflammatory feedback caused by oxidative stress feeds many inflammatory diseases such as metabolic syndrome and T2D, and constitute a novel therapeutical axis with its focus on mitochondrial homeostasis. As mentioned, neuregulins are key players of this inflammatory loop by preventing NF-kB activation in WAT (*Wang et al., 2014; Chen et al., 2017*) and promoting macrophage clearance (*Schumacher et al., 2017*), which in turn improves insulin sensitivity. However, further studies are needed to confirm the role of NRG4 in adipocyte inflammation and the development of insulin resistance.

3. Objectives

NRG4 has emerged as a novel adipokine that is downregulated in obese mice and humans. Its role in liver metabolism has been previously studied. Furthermore, other studies have shed some light on the function of this adipokine in WAT inflammation and vascularization, and macrophage polarization in a colitis murine model. However, the function of this adipokine on insulin action and sensitivity remains unknown in the peripheral insulin-targeted tissues, WAT and skeletal muscle. Given previous data, we hypothesised that NRG4 can regulate insulin sensitivity and inflammation in adipocytes. Importantly, NRG4 could have a paracrine action regulating the inflammatory state of WAT-infiltrated macrophages and thus, prevent the development of metabolic syndrome and T2D. Therefore, this thesis aims to describe the role of the adipocyte-secreted NRG4 in different *in vitro* and *in vivo* models, generating loss- or gain-of-function models of this adipokine and/or its receptor ErbB4. Specifically, the general objectives of this project are the following:

1- To study the local actions of NRG4 in 3T3-L1 adipocytes. To this end, adipogenesis, insulin sensitivity and the presence of inflammation will be assed in *Nrg4* and *Erbb4* knockdown (KD) 3T3-L1 adipocyte stable cell lines.

2- To generate primary immortalised white adipocytes as a novel cellular model to study the local actions of NRG4. To this end, the protocol to generate primary immortalised white adipocytes will be optimised. Afterwards, *Erbb2* and *Erbb4* receptors will be gene-deleted via Cre-LoxP recombination to study the local effects of NRG4 in adipocyte biology.

3- To evaluate the effects of the adipocyte-secreted NRG4 in macrophage inflammation and polarization. To this end, Bone Marrow-Derived Macrophages (BMDM) will be generated. In order to study NRG4 effects on the expression of proinflammatory and anti-inflammatory markers, conditioned media from *Nrg4* KD and control 3T3-L1 adipocytes will be supplemented to primary BMDM and to the secondary cell line RAW 264.7 macrophages before and after inducing macrophage polarization.

4- To analyse the distal effects of NRG4 in skeletal muscle from mice. To this end, we will characterise the phenotype of 2- and 6-months-old muscle-specific *Erbb4* KO mice upon normal diet (ND) and HFD feeding. Afterwards, protein and RNA extracts will be obtained from skeletal muscle, WAT and the liver to analyse the impact of the lack of NRG4 receptor signalling in muscle biology.

4. Results

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1. The role of neuregulin-4 and the ErbB receptors in the 3T3-L1 adipocyte cell line

4.1.1. Comparative expression of ErbB and neuregulins in 3T3-L1 cells and white adipose tissue fractions

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.2. *Nrg4* knockdown gene expression in 3T3-L1 cells

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.3. The expression of adipogenic genes is unchanged in NRG4-deficient adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.4. *Nrg4*-silenced adipocytes show insulin resistance

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.5. NRG4 deficiency triggers cell-autonomous inflammation in 3T3-L1 adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.6. NRG4 ablation induces *Hif1a* expression in 3T3-L1 adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.7. Treatment with recombinant human NRG4 or with endogenously expressed NRG4 from conditioned medium of 3T3-L1 adipocytes recover inflammation, insulin receptor and GLUT4 protein content in *Nrg4*-silenced adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.8. The anti-inflammatory agent dexamethasone recovers the insulin receptor protein and gene expression in NRG4-deficient adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.9. The anti-inflammatory agent sodium salicylate recovers the insulin receptor gene and protein expression in NRG4-deficient adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.10. The expression of several autophagy markers is enhanced in NRG4-deficient adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.11. Caveolin-1-deficient adipocytes have *Nrg4* gene down-regulation

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.12. The treatment with the lysosomal inhibitor bafilomycin A1 recovers the GLUT4 protein in *Nrg4*-silenced adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.13. The protein responsible for the GSV intracellular retention, TBC1D4, is reduced in *Nrg4*-silenced adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.14. The mitochondrial content is affected in *Nrg4*-silenced adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.15. The mitochondrial network morphology is altered in *Nrg4*-silenced adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.16. *Nrg4* knockdown adipocytes show higher oxidative stress

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.17. Lipid and glucose metabolism are altered in *Nrg4*-silenced adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.18. Inflammation is responsible of the higher lipolysis observed in *Nrg4*-silenced adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.19. Analysis of polar metabolites in *Nrg4*-silenced adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.20. Generation of *Erbb4* knockdown 3T3-L1 cells

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.21. The gene expression of neuregulins and ErbB receptors is altered in *Erbb4* silenced adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.22. The NRG4-ErbB4 signalling axis controls GLUT4 protein ablation and *InsR* down-regulation in adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.1.23. The absence of ErbB4 signalling triggers inflammation in 3T3-L1 adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.2. The role of neuregulin-4 and the ErbB receptors in primary immortalised adipocytes

4.2.1. Generation of primary immortalised preadipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.2.2. Characterisation of primary immortalised preadipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.2.3. Generation of knockout primary adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.2.4. Adipogenesis is impaired in *Erbb2* knockout adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.2.5. Cell-autonomous inflammation is triggered in *Erbb4* knockout adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.3. The role of ErbB4 and neuregulin-4 in macrophage polarization

4.3.1. Polarization of RAW 264.7 macrophages

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.3.2. The treatment with NRG4 derived from Scr adipocytes recovers inflammation in RAW 264.7 macrophages

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.3.3. The treatment with NRG4 derived from Scr adipocytes recovers inflammation in bone-marrow-derived macrophages from mice

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.3.4. The treatment with NRG4 derived from Scr adipocytes prevents inflammation in bone-marrow-derived macrophages from mice

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.3.5. RAW 264.7 have a low constitutive ASC gene and protein expression

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.4. Study of the distal effects of the adipokine neuregulin-4 on skeletal muscle

4.4.1. Phenotypic characterisation of 2-months-old muscle-specific *Erbb4* gene-deleted mice

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.4.2. Molecular characterisation of gastrocnemius from 2-months-old *Erbb4* knockout mice

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.4.3. Molecular characterisation of white adipose tissue from 2-months-old *Erbb4* knockout mice

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.4.4. Molecular characterisation of the liver from 2-months-old *Erbb4* knockout mice

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.4.5. Phenotypic characterisation of 6-months-old muscle-specific *Erbb4* knockout mice

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.4.6. Molecular characterisation of gastrocnemius from 6-months-old *Erbb4* knockout mice

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.4.7. Molecular characterisation of white adipose tissue from 6-months-old *Erbb4* knockout mice

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.4.8. Molecular characterisation of the liver from 6-months-old *Erbb4* knockout mice

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

4.4.9. Cachexia and expression of *Nrg4* in white adipose tissue

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

5. Discussion

5.1. NRG4 emerges as a novel adipokine that regulates insulin sensitivity in adipocytes

Here, we shed some light into the role of the adipocyte-secreted NRG4 in adipocyte physiology. We described that *Nrg4*-silenced 3T3-L1 adipocytes are affected by insulin resistance. This resistance is driven by the emergence of cell-autonomous inflammation and GSV protein degradation. As it was previously mentioned, the role of NRG4 in adipocytes remained largely obscured. While several studies shed some light on the role of this adipokine in the regulation of liver metabolism (*Wang et al., 2014; Ma et al., 2016; Chen et al., 2017, Guo et al., 2017*), none of them focused on the cell-autonomous effects of NRG4 in adipocytes. This is relevant since BAT and WAT are the main sources of NRG4 (*Wang et al., 2014*). We showed that 3T3-L1 adipocytes heavily induced the expression of this adipokine as early as D4 of differentiation. Likewise, the expression of its receptor, ErbB4, was also detected in 3T3-L1 adipocytes. Therefore, since differentiated 3T3-L1 adipocytes expressed both *Nrg4* and *Erbb4* one may think that NRG4 could have local effects in the regulation of adipocyte physiology. However, both 3T3-L1 adipocytes and the VA fraction from visceral WAT had lower *Erbb4* expression than the VS fraction from visceral WAT. Therefore, not only may NRG4 be able to signal through adipocytes, but other stromal cells from WAT may also be able to sense this factor. Given the high expression of *Nrg4* in adipocytes, we hypothesize that some NRG4 signals through adipocytes, however, given the low *Erbb4* expression in these cells, one part of the secreted NRG4 should bind to other cell types in a paracrine manner as well. This hypothesis is supported by the structure of NRG4 since it does not contain Ig-like or cysteine-rich domains to interact with the extracellular matrix, thus allowing the rapid release of the EGF-like domain of NRG4 upon metalloproteases cleavage (*reviewed in Gumà et al., 2010*). Therefore, upon NRG4 release, this factor can even signal through other distal tissues such as the liver, as previous studies have reported (*Wang et al., 2014*). Indeed, NRG4 has been found in blood and reduced levels of this factors are associated with metabolic syndrome in patients diagnosed with T2D (*Yan et al., 2018*). Besides NRG4, our results showed that the expression of *Nrg1* and *Nrg4* in adipocytes is mutually exclusive. While *Nrg4* is up-regulated during adipogenesis, *Nrg1* is down-regulated. Likewise, the expression of *Erbb1* and *Erbb2* is reduced during adipogenesis in 3T3-L1 adipocytes. Furthermore, our data show that gene-deleted-*Erbb2* and KD adipocytes could not undergo adipogenic differentiation. Therefore, NRG1 and ErbB2 may have a role in the induction of adipogenesis in pre-adipocytes. Indeed, NRG1 signalling can activate the MAPK and PKB pathways through ErbB signalling (*reviewed in Gumà et al., 2010*). These signalling pathways are critical in the induction of the clonal expansion during the differentiation process (*Smith et al., 1988*). Therefore, the NRG1-ErbB2 signalling axis may be important in the induction of the clonal expansion in a similar way to insulin in preadipocytes.

Although adipocytes expressed little *Erbb4*, we can rest assure that they are able to sense NRG4 and that this factor is critical in the maintenance of insulin sensitivity in adipocytes. While the expression of adipogenic genes was not affected in *Nrg4* KD adipocytes, these cells were resistant to the actions of insulin in the uptake of glucose. Specifically, *Nrg4* KD adipocytes had less InsR gene and protein expression as well as GLUT4 and GSV cargo protein degradation. Previous studies in adipocytes have shown that inflammation reduces the expression of PPARγ target genes such as *InsR, Irs* and *Glut4* in 3T3-L1 adipocytes (*Stephens et al., 1997; Rotter et al., 2003*). Thus, we next analysed the inflammatory state of *Nrg4*-silenced adipocytes. Our data show that these adipocytes were affected by cell-autonomous NF-κB activation. This autonomous inflammation increased the expression of several NF-κB target genes such as proinflammatory cytokines and *Hif1a*. Interestingly, the expression of the HIF1α target gene *Phd3* was chronically induced in *Nrg4*-silenced adipocytes, suggesting that HIF1α could be chronically activated in these cells as a consequence of inflammation. Indeed, non-hypoxic HIF1α stabilization and gene up-regulation has been shown to be directly regulated by NF-κB activation (*reviewed in Van Uden et al., 2008*). In addition, it is known that NF-κB signalling can impact the function of PPARγ, however, the increase in the expression of proinflammatory cytokines such as *Tnfa* was only detected at D7 of differentiation and thus, inflammation emerged as a late event in the differentiation process. Therefore, the presence of inflammation upon *Nrg4* silencing in adipocytes could explain the presence of insulin resistance and *InsR* down-regulation in differentiated adipocytes without affecting the expression of other adipogenic genes during the adipogenic differentiation.

Obesity is characterised by low-grade chronic inflammation, which contributes to the development of insulin resistance. Upon inflammation, the expression of *Nrg4* is reduced (*Wang et al., 2014*), however, we showed for the first time that upon *Nrg4* down-regulation cell-autonomous inflammation is triggered in adipocytes. In this context, it has been reported that *Nrg4* KO mice are affected by an obesity-related phenotype with increased expression of proinflammatory cytokines (*Chen et al., 2017*). To confirm that inflammation and insulin resistance was a direct consequence of the absence of NRG4 signalling in adipocytes, we treated these cells with exogenous rNRG4. As expected, the treatment with rNRG4 reduced the expression of proinflammatory cytokines and recovered the InsR and GLUT4 protein content. These findings allow us to propose the exitance of a negative feedback loop that involves NRG4 signalling and NF-κB in adipocytes, which can be relevant in the development and progression of metabolic syndrome. To confirm the link between inflammation and insulin resistance in *Nrg4*-silenced adipocytes we treated these cells with dexamethasone and sodium salicylate. Dexamethasone modulates adipogenesis through the activation of c/EBPβ, c/EBPδ and SREBP1, which induce the transcription of *Pparg* (*reviewed in Fajas, 2003; Farmer et al., 2005; Liu et al.,*

2001; Farmer et al., 2006). In addition, chronic treatment with dexamethasone can induce insulin resistance (*Tappy et al., 1994*). Therefore, it does not constitute a good anti-inflammatory agent to establish a relationship between inflammation and insulin resistance in our cellular model. Despite the problems of dexamethasone, the results that we obtained with both anti-inflammatory molecules were similar. The treatment with anti-inflammatory reagents recovered the expression of proinflammatory cytokines and the *InsR* expression. Therefore, we can establish a narrow relationship between inflammation and *InsR* down-regulation in *Nrg4*-silenced adipocytes. As previously mentioned, some studies have linked the action of TNFα and IL-6 in the down-regulation of the *InsR* and other adipogenic genes (*Stephens et al., 1997*). This down-regulation is dose- and time-dependent. Our studies with TNFα show that the action of this proinflammatory cytokine is sufficient to reduce the expression of *InsR* in control cells (*Rotter et al., 2003*). However, the expression of *Glut4* was only repressed in *Nrg4* KD cells treated with TNFα, suggesting that the combination of both exogenous TNFα and the cell-autonomous inflammation are needed to down-regulate the expression of this gene in adipocytes. Therefore, the *InsR* transcriptional regulation is more sensitive to inflammation than *Glut4* in 3T3-L1 cells. This result further confirms the link between inflammation and *InsR* down-regulation in *Nrg4* KD adipocytes.

Nevertheless, the treatment with anti-inflammatory molecules did not recover the GLUT4, IRAP or syntaxin-6 protein content. Thus, the GSV protein degradation and the *InsR* down-regulation upon *Nrg4* silencing are separated events. This indicates that not only does the lack of NRG4 trigger inflammation, but the degradation of GSVs is also induced through other mechanisms. It has been shown that ErbB receptors can regulate autophagy. For instance, EbB1 blockage increases autophagy and apoptosis in cancer cells (*reviewed in Henson et al., 2017*). Given that GLUT4 is found in the GSVs, we hypothesised that GLUT4 and other GSV proteins were degraded through autophagy in *Nrg4*-silenced adipocytes. Indeed, *Nrg4* KD adipocytes were unable to properly activate the mTORc1 pathway in basal conditions and upon insulin stimulus. Mechanistically, our data indicate that NRG4 can promote via ErbB4 the mTORc1 signalling pathway through the activation of AKT, thus regulating the autophagic flux. Indeed, the treatment with HRG induced AKT phosphorylation in adipocytes, hence supporting our view. In fact, it has been already described that ErbB4 signalling can induce mTORc1 activation in neurons (*Nie et al., 2018*). The increase in the LC3B-II protein content, which is a marker of autophagosomes, further confirms that autophagy is enhanced in *Nrg4* KD adipocytes. In addition, the cellular colocalization of GLUT4 and the lysosomal marker LAMP1 increased in *Nrg4*-silenced adipocytes. Therefore, NRG4 is playing an essential role in the control of the autophagy flux in adipocytes in basal conditions.

This opens a new field to investigate what are the exact mechanisms governed by the NRG4-ErbB4 signalling axis to control autophagy. In this context, our data indicate that the fall in GLUT4 protein is caused by the higher autophagic flux found in *Nrg4* KD adipocytes since bafilomycin A1 treatment recovered the content of this protein. Besides, GSV cargo proteins were equally affected in *Nrg4* KD adipocytes. Thus, it appears that the whole GLUT4 enriched endosomal compartment is affected in *Nrg4* KD adipocytes. As it has been previously described, GSVs are translocated upon insulin signalling into the plasma membrane to increase glucose uptake. In addition, GLUT4 vesicles have a continuous and low-rate traffic between the intracellular location and the surface membrane in basal conditions. After GSV translocation, they are recycled by endocytosis (*reviewed in Hou et al., 2007*). These early endosomes reach the recycling endosomes and from there, GLUT4 and other GSV cargo proteins are sorted to become functional again. However, we hypothesised that not all GSVs are recycled to become functional again but instead, the protein content of some GSVs could eventually be degraded by autophagy. As a matter of fact, it has been previously described that recycling endosomes contribute to the formation of autophagosomes (*reviewed in Puri, et al., 2018).* Therefore, reinternalized GSVs could be directed towards autophagic degradation in *Nrg4* KD adipocytes.

The TBC1D4 protein binds to IRAP in the GSVs and it has been proposed as the responsible for the intracellular retention of GLUT4 vesicles in basal conditions (*reviewed in Klip et al., 2019*). The fall in TBC1D4 protein observed in *Nrg4* KD adipocytes could be a consequence of the higher autophagy flux since the protein content of TBC1D4 is recovered by the treatment with bafilomycin. Therefore, we propose that, upon TBC1D4 degradation, GSVs lose retention in basal conditions, allowing them to undergo lysosomal degradation. Indeed, it has been previously described that TBC1D4 inactivation leads to GLUT4 protein degradation via autophagy in adipocytes (*Xie et al., 2016*). Mechanistically, the exocytosis of GSVs in basal conditions could be accelerated upon TBC1D4 deficiency and thus, they may get continuously endocytosed, increasing the number of early endosomes in basal conditions. This is consistent with the increase in clathrin and EEA1 protein content in *Nrg4* KD adipocytes since these proteins participate in the endocytosis of GLUT4. Alternatively, GLUT4 can be also reinternalized in caveolae (*Ros-Baró et al., 2001*). CAV1 is present on the surface membranes of adipocytes and it appears to have a role in the maintenance of GLUT4 protein stability since *Cav1* down-regulation reduces the protein content of GLUT4 (*González-Muñoz et al., 2009*). Interestingly, the CAV1 protein content is also reduced in *Nrg4*-silenced adipocytes, suggesting that the decrease in the CAV1/clathrin ration may signal GSVs for autophagosome engulfment. Additionally, Rab11 protein content is increased in *Nrg4*-silenced adipocytes, suggesting that the translocation of GSVs is enhanced in the absence of insulin.

Eventually, these re-internalised GSVs can be directed towards autophagy due to the lack of mTORc1 regulation in *Nrg4* KD adipocytes. However, this is just a hypothesis since we have not demonstrated that this is the exact route followed by GSVs towards autophagy degradation. Alternatively, upon TBC1D4 down-regulation, GSVs could be directly directed towards autophagy without membrane translocation. In all, further experiments are needed to elucidate the exact mechanism by which GSV proteins are degraded upon *Nrg4* silencing in adipocytes.

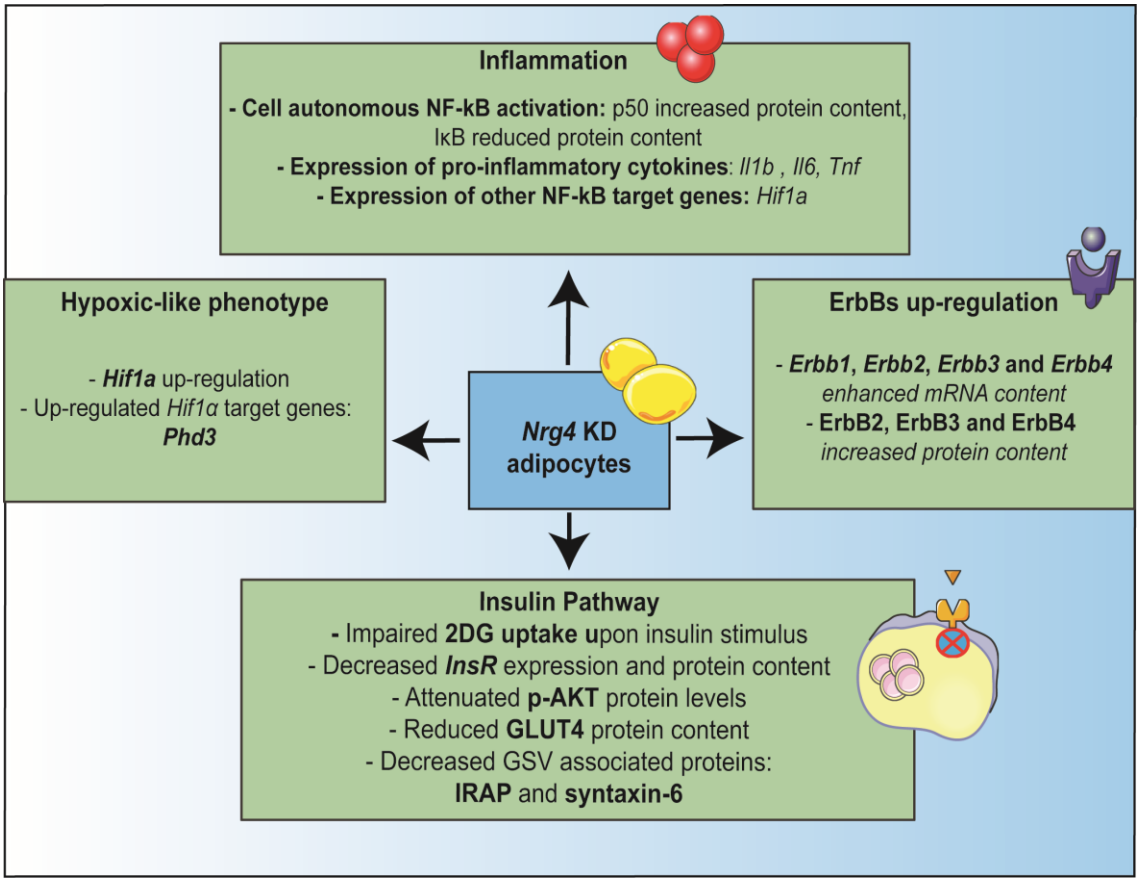


Figure 77. *Nrg4* silencing triggers a plethora of molecular changes in 3T3-L1 adipocytes. *Nrg4* KD adipocytes show insulin resistance, cell-autonomous inflammation, ErbB receptors up-regulation and hypoxia-like phenotype.

At this point, it is clear that NRG4 controls inflammation and autophagy in adipocytes. However, we generated *Erbb4* knockdown 3T3-L1 cells and primary immortalised *Erbb4* KO adipocytes to confirm that NRG4 signals through ErbB4 in adipocytes to regulate inflammation and GSV degradation. It is known that NRG4 can only bind to the ErbB4 receptor (*Harari et al., 1999*). However, the generation of *Nrg4*-silenced adipocytes was not enough to demonstrate that the resulting insulin-resistant phenotype was a consequence of the lack of NRG4 signalling through ErbB4. As it is the case with NRG4-deficient adipocytes, *Erbb4* KD 3T3-L1 adipocytes and *Erbb4* KO primary immortalised white adipocytes did not show impaired adipogenesis. However, the protein expression of the InsR and GLUT4 was reduced in *Erbb4* KD adipocytes, as it happens

with *Nrg4* KD cells. Furthermore, both *Erbb4* KD and *Erbb4* KO adipocytes increased the expression of proinflammatory cytokines, confirming the presence of cell-autonomous inflammation in these cells. Therefore, these data support the view that NRG4 controls GSV degradation and inflammation though ErbB4 signalling in adipocytes. In addition to the studies with gene-deleted-*Erbb4* adipocytes, the blockage of the ligand-binding domain of ErbB4 with a blocking antibody reduced the protein content of InsR and GLUT4 in control cells. Furthermore, the ErbB4 blockage also reduced the protein content of IκB in control cells, suggesting the presence of cell-autonomous NF-κB activation upon ErbB4 blockage. In all, as it was expected, we phenocopied the insulin-resistant phenotype of the *Nrg4* KD adipocytes in ErbB4-deficient adipocytes, highlighting the importance of the NRG4-ErbB4 signalling axis in the regulation of inflammation, autophagy and insulin sensitivity in adipocytes.

5.2. Inflammation and oxidative stress upon *Nrg4* silencing in adipocytes

Inflammation in adipocytes can be triggered by different stimuli. In the context of obesity, gut-derived antigens can trigger inflammation via LPS signalling through TLR4 (*reviewed in Saad et al., 2016*). In addition, hypoxia can induce inflammation upon adipocyte hypertrophy (*reviewed in D'Ignazio et al., 2016*), but, more importantly, inflammation can emerge as a consequence of oxidative stress upon metabolic dysfunction (*reviewed in Morgan et al., 2011*). In fact, mitochondria are a source of several DAMPs such as mtDNA and mtROS that, upon mitochondrial stress, can trigger inflammation (*reviewed in Rodríguez-Nuevo et al., 2019*). Given that *Nrg4* KD adipocytes have accelerated autophagy flux, we hypothesised that this could also affect the mitochondrial turnover and thus, generate oxidative stress. MtROS can trigger NF-κB, however, the exact mechanism by which mtROS can induce this pathway are elusive. It has been shown that IκB is degraded when the cellular levels of H_2O_2 are high (*Takada et al., 2003*), thus, leading to NF-κB activation.

Nrg4-silenced adipocytes have reduced mitochondrial mass. Specifically, the protein content of the mitochondrial protein TIM44 is reduced, while its gene expression remains unaffected. Importantly, the gene expression of other mitochondrial OXPHOS proteins remained unchanged in *Nrg4*-silenced adipocytes. Likewise, the protein content of PGC1α was unaffected in *Nrg4* KD adipocytes, supporting the view that mitochondrial biogenesis was not affected upon *Nrg4* silencing. Hence, we only detected TIM44 protein down-regulation, while the protein content of other mitochondrial markers remained unchanged. To confirm that the mitochondrial mass was really reduced upon NRG4 deficiency, we also analysed the mtDNA copy number and the mitochondrial mass by MitoTracker™ staining in these cells. Our data show a reduction in the mtDNA copy number as well as in the fluorometric intensity of the MitoTracker™ staining in

Nrg4-silenced adipocytes. Thus, all pieces of evidence indicate a reduction in the mitochondrial mass in NRG4-deficient adipocytes. We propose that autophagy regulation via NRG4-ErbB4 can also impact mitophagy and, hence, regulate the mitochondrial mass in adipocytes. Unlike NRG4 in adipocytes, chronic NRG1 treatment in muscle increases the total mitochondrial content through the induction of PPARβ/δ and PGC1α, which are involved in mitochondrial biogenesis (*Cantó et al., 2007*). Additionally, these factors can induce the expression of the mitochondrial fusion protein MFN2 (*Soriano et al., 2006*), which in turn increases the oxidative activity and insulin sensitivity (*Sebastian et al., 2012*). However, we were neither able to detect changes in the gene expression of mitochondrial genes nor in the PGC1α protein content in *Nrg4*-silenced adipocytes. Therefore, both NRG1 and NRG4 would regulate the mitochondrial mass in muscle and WAT, but through different mechanisms. Besides TIM44 protein, the protein content of the mitochondrial protein UCP1 was also reduced in *Nrg4* KD adipocytes, whereas its gene expression was induced, probably due to a genetic compensation. Importantly, *Nrg4* is highly expressed in BAT (*Wang et al., 2014*) and thus, NRG4 signalling in this tissue may be important in maintaining the protein stability of UCP1 in brown adipocytes. Therefore, we can speculate that NRG4 signalling could be important in the *browning* of white adipocytes by regulating UCP1 protein stability and mitophagy. Indeed, previous studies showed the importance of mitophagy in *browning* of white adipocytes. Specifically, when mitophagy is inhibited in mice, WAT shows an accumulation of mitochondria and white adipocytes acquire a beige or brown-like phenotype (*Lu et al., 2018*). In addition to TIM44 and UCP1 protein in *Nrg4* KD adipocytes, the protein expression of MFN2 was also reduced, while its gene expression remained unchanged. Contrarily, the protein and gene expression of the fusion protein OPA1 was not affected in *Nrg4*-silenced adipocytes. As it was mentioned previously, MFN2 stimulates the fusion of the outer mitochondrial membrane, along with MFN1, and it is critical in the maintenance of the mitochondrial oxidative activity. In fact, MFN2 ablation causes mitochondrial fission and doughnut-shaped mitochondrial network morphology, which has been linked to oxidative stress (*Jiang et al., 2018; Nie et al., 2014*). Indeed, the mitochondrial network morphology was profoundly affected in *Nrg4*-silenced adipocytes, with a higher abundance of condensed/doughnut-shaped mitochondria, which is consistent with higher oxidative stress (*Ahmad et al., 2013*).

In addition, MFN2 participates in PINK1 induced mitophagy (*Xiong et al., 2019*) and, at the same time, it can also be degraded through mitophagy (*Benischke et al., 2017*). To demonstrate that TIM44 and MFN2 proteins were degraded through lysosomal degradation, we treated NRG4-deficient cells with bafilomycin A1. As expected, the protein content of both proteins was recovered, confirming that at least these 2 mitochondrial markers were degraded through

lysosomal degradation upon *Nrg4* silencing in adipocytes. However, the exact mechanism by which mitophagy is induced in these adipocytes remains unknown and thus, further experiments are needed to shed some light on the matter. In summation, we propose that constitutive activation of mitophagy through mTORc1 dysfunctional regulation results in reduced mitochondrial mass and mitochondrial network morphology disruption, which can alter the mitochondrial metabolism and the oxidative state of the cell. Ultimately, the presence of oxidative stress due to impaired mitochondrial metabolism can explain the emergence of inflammation in these cells.

Hence, to confirm the presence of oxidative stress, we analysed the expression of *Sod2* since its expression is up-regulated upon oxidative stress and inflammation (*Shen et al., 2017*). Indeed, *Nrg4* KD adipocytes have increased *Sod2* gene expression and, likewise, increased H_2O_2 levels, confirming the presence of higher oxidative stress upon NRG4 ablation. Importantly, mitochondria in *Nrg4* KD adipocytes were hyperpolarised, which is consistent with an excessive mtROS production. These DAMPs are mostly produced in the OXPHOS complex I and complex III. Mechanistically, when the electron flux saturates the pool of coenzyme Q (CoQ), the electrons are pushed back from the reduced CoQ towards the Complex I to produce $O_2^{\cdot-}$. This process is known as reverse electron transport and is the most important mechanism of mtROS production under physiological conditions. Upon complex III inhibition, the mtROS production increases in this site as well (*reviewed in Murphy, 2009*). However, oxidative stress in *Nrg4*-silenced adipocytes may arise as a consequence of multiple factors. First, mTORc1 regulation is absent in basal conditions and upon insulin stimulation in these cells, promoting the acceleration of the autophagic flux, which can impact mitophagy as well. As a consequence, there is a reduction in the mitochondrial mass and mitochondrial network condensation, which leads to mitochondrial stress and loss of redox homeostasis (*reviewed in by Willems et al., 2015*). Specifically, MFN2 ablation triggers mitochondrial fragmentation and increases mtROS levels (*Muñoz et al., 2013*). Besides, OPA1 mutations that induce aberrations in the mitochondrial morphology also increase the mtROS levels (*Distelmaier et al., 2012*). Thus, mitochondrial fragmentation caused by the loss-of-function or ablation of mitochondrial fusion proteins is directly linked to the emergence of oxidative stress. Second, the ablation of UCP1 can lead to oxidative stress (*Stier et al., 2014*). In fact, UCP1 deficient mice suffer more oxidative stress upon cold exposure than control mice, highlighting the protective role of this protein against oxidative stress during BAT thermogenesis.

Given that the mitochondrial membrane potential increases upon *Nrg4* silencing, we can speculate that mitophagy would not be mainly induced through the PINK1-parkin pathway since it mediates the clearance of depolarised mitochondria. Mechanistically, upon mitochondrial depolarization, PINK1 stabilizes on the outer mitochondrial membrane and recruits parkin to initiate

mitochondrial degradation (*Narendra et al., 2010*). However, there are other signals that can trigger the elimination of polarised mitochondria that could be responsible for the mitochondrial protein degradation in *Nrg4*-silenced adipocytes. It has been described that excessive stimulation of mitochondrial OXPHOS increases mitochondrial turnover through NIX and BNIP3 induced mitophagy by several potential mechanisms. First, NIX and BNIP3 can cause mitochondrial dysfunction increasing the production of ROS, which in turn activates autophagy by the repression of mTORc1 (*reviewed in Scherz-Shouval et al., 2011*). Second, both proteins can interact with Rheb and thus, inhibiting mTORc1 and promoting autophagy (*Melser et al., 2013; Li et al., 2007*). Importantly, BNIP3 is induced upon hypoxia and it has been proposed to cause hypoxia-induced mitophagy (*Bellot et al., 2009*). Therefore, given that NRG4-deficient adipocytes have mTORc1 inhibition, mitochondrial hyperpolarization and *Hif1a* gene up-regulation, we speculate that mitochondrial protein degradation may be promoted through BNIP3-induced mitophagy, yet further studies are needed to confirm this hypothesis.

To link the emergence of oxidative stress with the cell-autonomous inflammation in *Nrg4*-silenced adipocytes, we treated these cells with the antioxidant agent NAC. Upon NAC treatment, *Nrg4* KD adipocytes reduced the expression of proinflammatory cytokines and recovered the IκB protein content, thereby reverting the cell-autonomous inflammation. In addition, NAC treatment also recovered some GLUT4 protein content, which indicates that oxidative stress may also contribute to the mTORc1 inhibition in *Nrg4* KD adipocytes (*reviewed in Scherz-Shouval et al., 2011*). Therefore, we can rest assure that mtROS are the main DAMPs that trigger inflammation upon *Nrg4* silencing in adipocytes, however, we cannot rule out the possibility that other DAMPs may also contribute to the activation of NF-κB pathway in our model. As a matter of fact, despite that NAC treatment completely reverted the expression of *Tnfa* it did not completely recover the IκB protein expression, hence supporting the view that other DAMPs may be also implicated in the autoinflammatory response. As mentioned previously, besides mtROS, it has been shown that mtDNA can engage TLR9 in amphisomal compartments to trigger NF-κB activation and cell-autonomous inflammation in skeletal muscle (*Rodríguez-Nuevo et al., 2018*). Therefore, alterations in the mitophagy flux can generate autonomous inflammation via mtDNA-TLR9 signalling. In addition, PINK1 deficient cells increase the leakage of mtDNA into the cytosol where it engages the DNA sensor cGAS to induce inflammation (*reviewed in Newman et al., 2018*). Thus, alterations in the mitophagy upon *Nrg4* silencing could generate inflammation through other DAMPs besides mtROS, such as mtDNA. Importantly, NRG4-deficient adipocytes have reduced mtDNA copy number. This reduction is also observed in *Opa1* KD myocytes as a result of mtDNA instability, which promotes the binding of mtDNA to TLR9 upon mitophagy induction (*Rodíguez-Nuevo et al., 2018*). However, the role of mtDNA in the cell-autonomous

inflammatory response in *Nrg4* KD adipocytes has not been explored yet. In summation, we propose that autophagy deregulation through deficient mTORc1 activation in *Nrg4* KD adipocytes leads to the degradation of some mitochondrial proteins, presumably via mitophagy, leading to a reduction in the mitochondrial mass and alterations in the mitochondrial network morphology. These alterations in the mitochondrial homeostasis cause oxidative stress, which generates mtROS and ultimately promotes cell-autonomous NF-κB activation in NRG4-deficient adipocytes.

5.3. Adipocyte metabolism is altered upon *Nrg4* silencing

Nrg4 KD adipocytes have several molecular alterations that ultimately affect their cellular metabolism, namely autonomous inflammation, mitochondrial dysfunction and insulin resistance. To shed some light into the lipid metabolism of these cells, we first analysed the TAG content during the differentiation of *Nrg4*-silenced adipocytes. NRG4-deficient adipocytes had a reduction in the TAG content at D7 of differentiation. This result is consistent with the emergence of inflammation at D7 of differentiation. The reduction in the TAG content is caused by alterations in the basal lipolysis and *de novo* lipogenic rates in differentiated *Nrg4* KD adipocytes. Our data show an increase in the basal lipolytic rates in *Nrg4* KD adipocytes at D7 of differentiation, which is consistent with the appearance of inflammation and insulin resistance. Indeed, it has been described that TNFα can induce lipolysis (*reviewed in Cole et al., 2018*). This inflammation is characterised by reduced expression of lipogenic genes such as *Plin1, Atgl, Hsl,* and *Mgl* (*Ruan et al., 2002*) and HSL phosphorylation (*Zhang et al., 2002*). On the other hand, insulin inhibits lipolysis promoting the hydrolysis of cAMP and thus, inactivating HSL (*reviewed in Holm et al., 2000*). TNFα-induced lipolysis is also characterised by increased basal HSL phosphorylation, reduction in the gene expression of the lipases and perilipin-1 protein down-regulation. Besides that, the treatment with the anti-inflammatory reagent dexamethasone recovered the perilipin-1 protein content and induced the ATGL protein expression, confirming the link between inflammation and TNFα-induced lipolysis in *Nrg4* KD adipocytes.

Besides lipolysis induction, *Nrg4* KD adipocytes have also reduced *de novo* lipogenic rates in basal conditions and upon insulin stimulation. As it was previously mentioned, *Nrg4*-silenced adipocytes were unable to increase glucose uptake upon insulin stimulation given the reduction in the GLUT4 and InsR protein content. Insulin promotes the lipogenic pathway by enhancing the activity and the expression of lipogenic genes, and by increasing the uptake of glucose as a raw material to synthesize new fatty acids (*reviewed in Czech et al., 2013*). Therefore, the presence of insulin resistance is consistent with the reduction in the lipogenic rates upon insulin stimulation. In basal conditions, however, *Nrg4* KD adipocytes also showed a reduction in the

lipogenic rates. This could be explained due to the presence of inflammation in these cells. In fact, it has been described that inflammation is associated with down-regulation of lipogenic factors (*Poulain-Godefroy et al., 2008*). Many lipogenic genes are expressed through PPARγ and the NF-κB activation disrupts the transcriptional activity of this factor (*Wang et al., 2013*). Although inflammation appears as a late event in the differentiation program, it can directly impact the expression and function of lipogenic genes thus affecting the lipogenic metabolism. More importantly, the dexamethasone treatment recovered the normal TAG content in *Nrg4*-silenced adipocytes, confirming that inflammation disrupts lipid metabolic homeostasis by reducing the accumulation of TAGs in adipocytes. Hence, inflammation promotes a metabolic switch towards the catabolism of lipids. This may constitute a compensatory mechanism to counteract the excessive lipid accumulation in the context of adipocyte hypertrophy upon obesity. However, chronic inflammation drives the development of inflammatory syndromes such as metabolic syndrome in the context of obesity or even cachexia in the context of other inflammatory-based pathologies such as cancer, which is characterised by the loss of WAT and muscle mass (*Han et al., 2018*). Given the link between NRG4, inflammation and lipolysis in adipocytes, this factor may have a role in the pathophysiology of metabolic syndrome and cachexia.

Given that adipocytes are affected by mitochondrial and oxidative stress, we also analysed the glucose oxidation and the anaerobic glycolysis to further characterise the flux of glucose in NRG4-deficient adipocytes in basal conditions. *Nrg4* KD adipocytes have reduced mitochondrial mass and mitochondrial network condensation and thus, it was expected that the glucose oxidation would be diminished in these cells. As a matter of fact, MFN2 deficient cells have reduced glucose oxidation (*Sebastián et al., 2012*). Surprisingly, it was not the case in *Nrg4* KD adipocytes, which maintained the same glucose oxidation rate of control cells. This result is consistent with the appearance of oxidative stress in these adipocytes since they oxidize glucose at the same rate as control adipocytes, while the mitochondrial content is reduced. This observation allows us to propose that *Nrg4* KD adipocytes oxidize more glucose per mitochondria, which may explain the mitochondrial hyperpolarization. Ultimately, inefficient glucose oxidation leads to the production of mtROS and causes oxidative stress (*reviewed in Bonnefont-Rousselot, 2002*). Alternatively, the production of CO_2 from glucose through other cytosolic enzymes could be enhanced in *Nrg4* KD adipocytes, increasing the production rate of radioactive CO_2 beyond the electron transport chain.

Regarding the anaerobic glycolysis in *Nrg4* KD adipocytes, the release of lactate was diminished in basal conditions. However, the basal glucose uptake and the glucose oxidation in *Nrg4*-silenced adipocytes remained unchanged and thus, the intracellular levels of the metabolites glucose and pyruvate were also unchanged. Given that the release of lactate is reduced in *Nrg4* KD adipocytes, the consumption of glucose could be increased by other pathways in these cells. Specifically, glucose could be used to increase glycine production. Metabolomic data revealed that the levels of glycine were reduced in *Nrg4* KD adipocytes and thus, the consumption of glycine may be enhanced to increase the synthesis of the anti-oxidant molecule glutathione to counteract the oxidative stress (*El-Hafidi et al., 2018*). Alternatively, more lactate could be consumed by *Nrg4* KD adipocytes as a redox substrate.

Besides, the levels of branched-chain amino acids were increased in *Nrg4*-silenced adipocytes. Interestingly, elevated intracellular concentrations of branched-chain amino acids have been shown to promote the activation of NF-κB and mTORc1 (*reviewed in Neishabouri et al., 2015*). Furthermore, it has been observed that the supplementation with high concentrations of branched-chain amino acids promotes the generation of ROS in cells, activating the NF-κB pathway (*Zhenyukh et al., 2017*). Indeed, the activity of branched-chain amino acid aminotransferase (BCAT), which catalyses the supply of TCA intermediates from branched-chain amino acids, is an important source of cellular ROS (*Richard et al., 2018*). Thus, the presence of oxidative stress may prevent the consumption of branched-chain amino acids by *Nrg4* KD adipocytes as an anti-oxidant mechanism, causing their accumulation in these cells. Interestingly, high concentrations of branched-chain amino acids in plasma are associate with insulin resistance and T2D (*Flores-Guerrero et al., 2018*).

Besides branched-chain amino acids, the excretion of citrate was doubled in *Nrg4* KD adipocytes. High citrate levels in the cytoplasm have been linked to the emergence oxidative stress via NADPH accumulation (*Williams et al., 2018*). Citrate is vital in the sustainment of *de novo* lipogenesis; therefore, since *Nrg4* KD adipocytes had reduced *de novo* lipogenic rates, the excretion of cytosolic citrate could constitute a compensatory mechanism to ameliorate the oxidative stress in *Nrg4* KD adipocytes (*reviewed in Costello et al., 2013*). Indeed, we also detected reduced cytosolic citrate levels in these cells. In addition to citrate, we detected reduced cellular levels of malate and fumarate, which is consistent with the reduced mitochondrial content reported in *Nrg4*-silenced adipocytes. In all, the loss of NRG4 signalling disrupts the metabolic homeostasis of adipocytes.

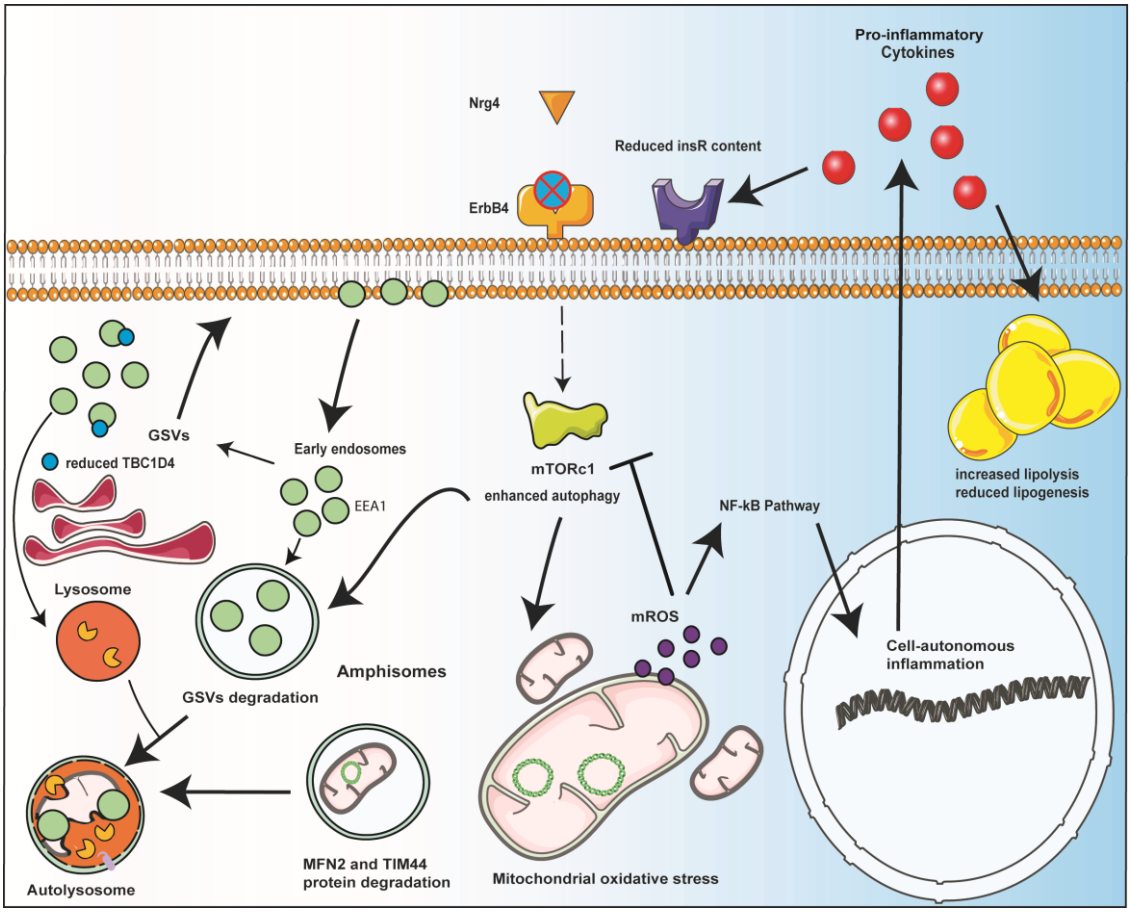


Figure 78. Schematic representation of the cellular alterations upon NRG4 deficiency in adipocytes. Our data suggest that, upon the disruption of the NRG4-ErbB4 signalling axis, the basal levels of p-AKT are reduced thereby leading to a loss of mTORc1 basal activation. This enhances the autophagy flux. Upon uncontrolled autophagy induction, TBC1D4 protein is degraded. We propose that TBC1D4 protein degradation in basal conditions allows GSVs to undergo lysosomal degradation. Mechanistically, GSVs could translocate to the plasma membrane and then, GLUT4 and other GSV associated proteins are internalised. Afterwards, early endosomes enriched with GSV associated proteins can be directed towards autophagy given the lack of mTORc1 induction upon *Nrg4* silencing. Alternatively, GSVs could be directly directed towards the autophagic pathway. GSV protein degradation reduces the protein content of GLUT4 in adipocytes, which profoundly affects the metabolic homeostasis of adipocytes. In addition to GSV protein degradation, the degradation of mitochondrial proteins such as TIM44, UCP1 and MFN2 via lysosomal degradation is enhanced. This uncompleted mitophagy is still not well characterised, yet the total mitochondrial mass is reduced in *Nrg4* KD adipocytes. Additionally, the mitochondrial elongation is disrupted and thus, mitochondrial dysfunction emerges. Mitochondrial dysfunction leads to increased mtROS production and oxidative stress. We linked the emergence of oxidative stress with the appearance of cell-autonomous inflammation via NF-κB activation. Inflammation emerges as a late event in the differentiation of adipocytes. This autonomous inflammation increases the expression of proinflammatory cytokines such as *Tnfa*, *Il1b* and *Il6*, which further fuels inflammation. Ultimately, inflammation impacts the expression of PPARγ target genes such as the *InsR*, causing insulin resistance. In addition, inflammation and insulin resistance profoundly affect the cellular metabolism of adipocytes. As a consequence, basal lipolysis is induced, whereas *de novo* lipogenesis is repressed, reducing the TAG content in these adipocytes at D7 of differentiation.

5.4. The ErbB expression pattern is altered in *Nrg4*-silenced adipocytes

Besides the metabolic alterations, *Nrg4* KD adipocytes have altered ErbB receptor expression. Specifically, the expression of *Erbb1*, *Erbb2*, *Erbb3* and *Erbb4* was induced in *Nrg4*-silenced adipocytes at D7 of differentiation. Moreover, *Erbb4* KD 3T3-L1 adipocytes and *Erbb4* KO primary immortalised white adipocytes had increased expression of *Erbb2* and *Erbb3*. The physiological role of this ErbB up-regulation remains unknown. However, it seems to be caused by the emergence of inflammation since the treatment with TNFα also induced the expression of ErbB receptors in control cells. Besides, the treatment with anti-inflammatory molecules reduced the expression of these receptors in *Nrg4* KD cells. As a matter of fact, previous studies with macrophages show that *Erbb4* expression is indeed up-regulated upon inflammation in these cells (*Schumacher et al., 2017*). Therefore, we propose that *Erbb4* up-regulation constitutes a compensatory mechanism that promotes the resolution of inflammation through NRG4 signalling.

5.5. The role of the adipocyte-secreted NRG4 in macrophage inflammation

Macrophages are vital in the maintenance of adipocyte and WAT homeostasis. In homeostatic conditions, macrophages display a M2 phenotype, enhancing the insulin sensitivity in adipocytes via IL-10 release. However, upon obesity or inflammatory stimuli, macrophages display a M1 phenotype and participate in the recruitment of other immune cells to promote adipocyte and WAT inflammation (*reviewed in Lauterbach et al., 2017*). As it has been mentioned, previous studies in an inflammatory colitis mice model revealed that NRG4 promotes M1 macrophage clearance (*Schumacher et al., 2017; Frey et al., 2009; Bernard et al., 2012; McElroy et al., 2014*). In this study, it has been described that M1 macrophages induce the expression of *Erbb4* and, upon NRG4 treatment, the expression of proinflammatory cytokines is reduced. Specifically, we first analysed the impact of NRG4 in the resolution of inflammation. Already-polarised M1 BMDM and RAW 264.7 macrophages treated with conditioned media from Scr adipocytes reduced the expression of proinflammatory cytokines. Furthermore, non-polarised RAW and BMDM macrophages treated with conditioned media from *Nrg4* KD adipocytes increased the expression of proinflammatory cytokines and *Erbb4*. Thus, the presence of proinflammatory cytokines in that media may cause the proinflammatory polarization of non-polarised macrophages since TNFα and other cytokines can induce macrophage polarization (*Wu et al., 2015; Degboé et al., 2019*). This result further confirms the presence of inflammation in *Nrg4* KD adipocytes. On the other hand, already polarised M2 BMDM reduced the expression of *Il10* upon treatment with conditioned media from *Nrg4* KD cells, which further supports the view that this media promotes macrophage polarization towards a more inflammatory profile.

These results highlight the importance of the adipocyte-secreted NRG4 in the resolution of macrophage inflammation. Likewise, NRG4 was also able to prevent inflammation since it prevented M1 macrophage polarization. Indeed, the expression of proinflammatory cytokines was diminished in BMDM treated at the same time with LPS and conditioned media from Scr adipocytes. Similarly, the treatment with this media further induced the expression of *Arg1* as an early induced anti-inflammatory gene in BMDM treated with IL-4. Therefore, we can speculate that NRG4 could have synergic effects with IL-4 in the M2 macrophage polarization by promoting the expression of *Arg1*. Therefore, our data show that the NRG4 derived from adipocytes is able to prevent and resolve macrophage inflammation, highlighting the importance of this adipokine in the prevention of WAT inflammation (*Chen et al., 2017*). As it was mentioned previously, macrophages play a vital role in the development of WAT inflammation upon obesity, which leads to insulin resistance and metabolic syndrome. In light of our results, we propose that NRG4 constitutes an adipokine that mediates adipocyte-macrophage crosstalk.

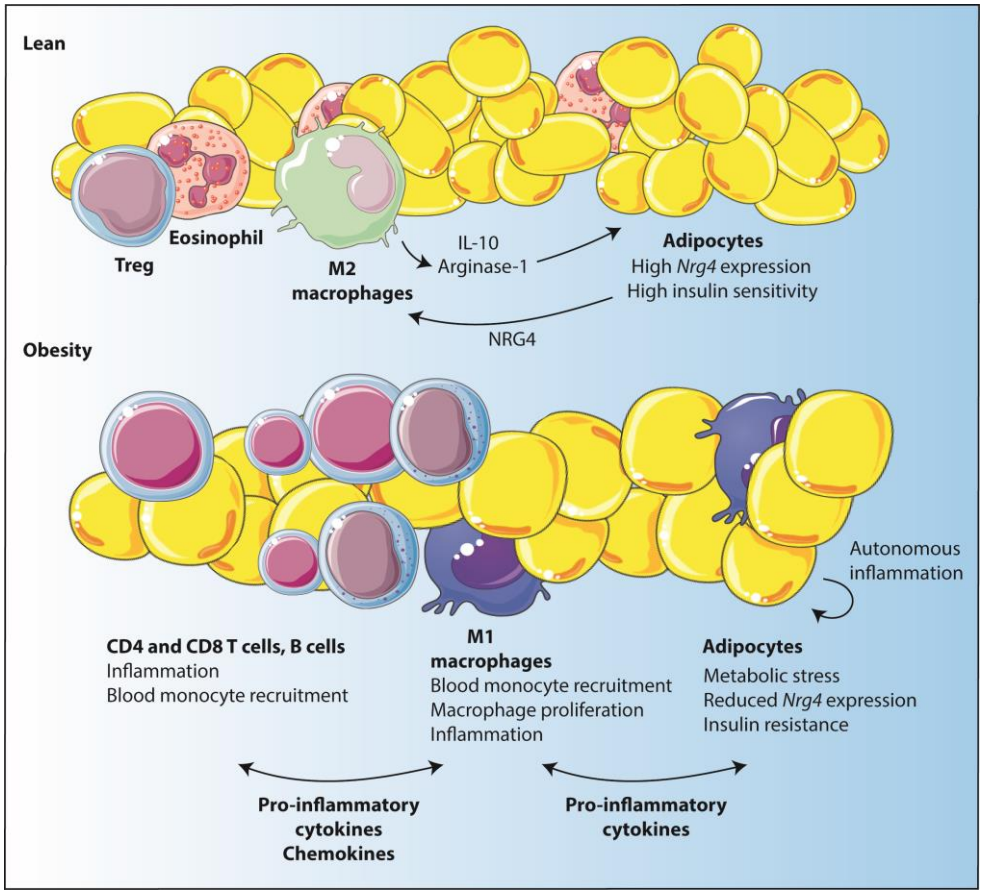


Figure 79. WAT immunity and NRG4. The ErbB4-NRG4 signalling constitutes a protective axis against autonomous adipocyte inflammation. Besides that, adipocyte-secreted NRG4 prevents and resolves inflammation in BMDM. Upon inflammation, the production of NRG4 by adipocytes is reduced and this protective mechanism is lost, thus contributing to adipocyte and macrophage inflammation upon obesity. Schematic art pieces used in this figure are provided by Servier Medical Art under a Creative Commons Attribution 3.0 Unported License.

Thus, in the absence of inflammation, *Nrg4* expression is not affected in adipocytes. Consequently, the NRG4-ErbB4 signalling axis prevents oxidative stress-induced inflammation in adipocytes and, at the same time, prevents M1 macrophage polarization upon inflammatory stimuli in WAT. This equilibrium can be disrupted by the emergence of chronic autonomous inflammation in adipocytes upon obesity due to metabolic stress. In these pathological conditions, *Nrg4* expression is reduced by inflammation, which in turn further induces inflammation in adipocytes. In the absence of NRG4, this protective mechanism against inflammation is lost, and macrophages can polarize towards a more inflammatory phenotype in this condition. Furthermore, the exacerbated production of proinflammatory cytokines by adipocytes can initially increase macrophage inflammation. Afterwards, M1 macrophages secrete proinflammatory cytokines and chemokines that guide the recruitment and proliferation of other immune cells in WAT. Therefore, we propose that NRG4 has a role in the prevention of WAT inflammation by preventing adipocyte and macrophage inflammation.

5.6. Distal effects of NRG4 in mice glycaemia and muscle physiology

As previously mentioned, several studies have analysed the distal effects of the adipokine NRG4 in liver metabolism. It is known, however, that NRG1 regulates a plethora of molecular aspects in skeletal muscle. NRG1 is released by neuronal axons inducing the formation of the postsynaptic plate (*Altiok et al., 1995*). In addition, upon contraction, NRG1 is released from muscle and exerts several autocrine effects in this tissue. First, NRG1 is essential for the myogenesis (*Suárez et al., 2001*), acting in an additive way with the IFG1 (*Florini et al., 1996*). Our data also suggest that NRG1 and ErbB2 are critical in the induction of the adipogenic differentiation. Second, NRG1 promotes the translocation of GLUT4 in muscle in an additive manner to the insulin action (*Cantó et al., 2004*). Third, chronic NRG1 treatment also improves oxidative metabolism and induces mitochondrial biogenesis in myocytes (*Cantó et al., 2007*). However, the function of the adipokine NRG4 in skeletal muscle biology remains unknown. Therefore, given the role of NRG4 in the regulation of inflammation and preservation of metabolic homeostasis in other tissues, we shed some light on the distal effects of this adipokine in muscle.

To this end, we first analysed the phenotype of muscle-specific *Erbb4* KO mice at 2 and 6 months upon ND feeding. At 2 months, we did not detect any significant changes in the body weight as well as fasting and feeding glycaemia in these mice. Likewise, GTT and ITT analyses confirmed a similar glucose and insulin tolerance in *Erbb4* KO mice compared to control mice. Therefore, at 2 months, the absence of NRG4 signalling via ErbB4 in skeletal muscle does not have an impact on the regulation of the blood glucose levels in mice. However, upon HRG challenge, glycaemia of *Erbb4* KO animals was more reduced in comparison to control mice during the GTT analyses.

Indeed, NRG1 has systemic effects in the regulation of the blood glucose levels since it induces GLUT4 translocation in skeletal muscle (*Cantó et al., 2004*). In addition, NRG1 regulates the glycaemia by enhancing the hepatic glucose utilization without promoting the activation of the InsR in the liver (*López-Soldado et al., 2016*). Interestingly, *Erbb4* KO mice are more sensitive to the actions of HRG in the regulation of glycaemia. This could constitute a compensatory mechanism to the lack of NRG4 signalling in muscle. Likewise, at 6 months, the phenotype of the muscle-specific *Erbb4* KO mice was similar than in younger mice. The animals tended to have similar body weights as well as glucose and insulin tolerance. Upon HRG stimulus, as it happened at 2 months, these animals also tended to reduce the glycaemia more than control mice during the GTT analyses. Overall, the only phenotypical change that we detected was the enhanced HRG sensitivity in *Erbb4* KO mice during the GTT analyses. Thus, these animals were not insulin resistant, unlike *Nrg4*- and *Erbb4*-silenced adipocytes.

However, upon HFD feeding, these animals tended to have lower body weights and fasting glycaemia than control mice. In addition, they tended to have more glucose and insulin sensitivity, although the data was not conclusive. Regarding HRG sensitivity, these mice loss the tendency towards a reduction in the glycaemia during the GTT analysis. Importantly, *Erbb4* KO mice had lower perigonadal WAT weight and less TAG content in gastrocnemius upon HFD feeding. Thus, we speculate that *Erbb4* gene-deleted mice compensate the extra caloric intake at this point and thus, the resulting phenotype is not the typical obese insulin-resistant phenotype but rather, it displays some inflammatory-like features. As mentioned, inflammation can induce lipolysis in WAT (*reviewed in Czech et al., 2013*). In addition, inflammation can disrupt adipogenesis (*reviewed in Ye, 2008*) and *de novo* lipogenesis (*Poulain-Godefroy et al., 2008*), which ultimately can reduce WAT mass. Given the role of NRG4 in autophagy in adipocytes, TAG reduction in muscle could be a result of accelerated lipophagy in *Erbb4* gene-deleted muscle through mTORc1 inhibition. Alternatively, TAG lipolysis in muscle could be enhanced upon *Erbb4* gene deletion. It is known that skeletal muscle has a marked lipolytic activity (*Jacob et al., 1999*), although it is differentially regulated in comparison to WAT. For instance, upon oral glucose feeding, lipolysis is repressed in WAT by insulin, yet it remains unaltered in skeletal muscle (*Bolinder et al., 2000*). However, the regulation of lipolysis in muscle has not been fully explored, although it is known that inflammation can promote lipolysis also in this tissue (*Wolsk et al., 2010*; *Li et al., 2011*). Thus, it has been proposed that the IL-6 release by this tissue during exercise promotes the lipolysis of ectopic TAG in muscle. This constitutes a compensatory mechanism to promote the mobilization of ectopic lipid droplets in muscle. Therefore, inflammation could be present in *Erbb4* gene-deleted muscle, as it happens in adipocytes, and this may cause the reduction in the TAG content in muscle in HFD-fed *Erbb4* KO mice.

Indeed, our data confirm the emergence of inflammation in skeletal muscle from *Erbb4* KD mice at 2 months by increasing the expression of proinflammatory cytokines and reducing the protein content of IκB. This inflammation is also present at 6 months and it does not get worse upon HFD feeding. Therefore, the inflammation induced by *Erbb4* gene deletion in muscle is not additive with the HFD-induced inflammation. We propose that not only does the NRG4-ErbB4 signalling axis protect against WAT inflammation, but it may also have a role in the regulation of skeletal muscle inflammation. Chronic inflammation in muscle has deleterious effects promoting insulin resistance and myopathy (*reviewed in Mandel et al., 2017*). Therefore, NRG4 could have a broader spectrum protecting against the deleterious effects of HFD by signalling through other distal tissues such as the liver (*Wang et al., 2014*) and muscle. However, inflammation in *Erbb4* gene-deleted muscle did not affect mice glycaemia nor insulin tolerance during the ITT analyses. Therefore, other metabolic tissues could be compensating muscle dysfunction and inflammation upon Erbb4 gene deletion in mice.

The gene and protein expression of InsR was affected in muscle from *Erbb4* KO mice, which suggest the presence of insulin resistance in muscle. This down-regulation can be caused due to the action of proinflammatory cytokines in this tissue (*Šestan et al., 2018*). Indeed, control mice upon HFD feeding reduced the expression of *InsR*, whilst the expression of proinflammatory cytokines was induced. However, as it is the case with WAT, the gene expression of *Glut4* remained unchanged in the muscle from these mice upon ND feeding. Likewise, HFD feeding-induced inflammation did not reduced the expression of *Glut4* in muscle from control mice. However, unlike *Nrg4* KD adipocytes, the GLUT4 protein content was unaffected in *Erbb4* KO mice upon ND feeding. This result suggests that the NRG4-ErbB4 signalling axis, although it modulates inflammation in muscle and WAT, may have different effects in each tissue and thus, autophagy may not be increased in muscle upon *Erbb4* gene silencing. However, as it happens in WAT, NRG4 may also regulate mitochondrial function in muscle via ErbB4. As it was mentioned, it is known that NRG1 promotes mitochondrial biogenesis through the activation of PPARβ/δ and PGC1α in muscle. Similarly, NRG4 has been shown to regulate mitochondrial protein degradation through the lysosomal pathway and thus, NRG4-deficient adipocytes have reduced mitochondrial mass. Therefore, neuregulins have a role in the maintenance of mitochondrial homeostasis in different tissues through different mechanisms (*Gumà & Díaz-Sáez et al., 2020*). Our data show an increase in the expression of *Fgf21* in muscle from *Erbb4* KO mice, which can be caused by mitochondrial dysfunction (*Ost et al., 2016*). Indeed, some studies have linked mitochondrial stress with increased *Fgf21* expression in muscle. For instance, it has been shown that OPA1 deficiency in muscle causes mitochondrial dysfunction and consequently increases the *Fgf21* expression in tissue (*Rodríguez-Nuevo et al., 2018; Tezze et al., 2017*). Although more

studies are needed to confirm the presence of mitochondrial dysfunction in muscle upon *Erbb4* gene deletion, this could also explain the presence of inflammation in these mice. Likewise, control mice fed with HFD also induced the *Fgf21* expression in muscle, further supporting the view that mitochondrial dysfunction is linked with inflammation in this tissue. Besides that, the expression of neuregulins and ErbB receptors was also affected in muscle from *Erbb4* KO mice. Upon ND feeding, 2-months-old mice had reduced ErbB2 protein and gene expression, while the gene expression of all ErbB receptors was normalised at 6 months. Hence, our data indicate that muscle-specific *Erbb4* gene-deleted mice do not compensate the lack of ErbB4 by increasing the expression of other ErbB receptors. Upon HFD feeding, the expression of *Erbb1*, *Erbb2* and *Erbb3* tend to decrease in control and *ErbB4* KO mice, whereas the expression *Erbb4* increases in control mice. This result further supports the view that *Erbb4* expression is induced upon inflammatory stimuli to promote the resolution of inflammation, as it happens in macrophages (*Schumacher et al., 2017*) and adipocytes.

Regarding neuregulins, *Nrg1* expression remained unchanged between genotypes and diets, however, *Nrg4* expression in muscle was reduced in *Erbb4* KO mice upon ND feeding and in all genotypes upon HFD feeding at 6 months. Therefore, muscle *Nrg4* expression is negatively regulated by HFD-induced inflammation and, presumably, by the emergence of autonomous inflammation in muscle-specific *Erbb4* KO mice upon ND feeding. As mentioned previously, inflammation reduces *Nrg4* expression in WAT (*Wang et al., 2014*). Interestingly, the absolute expression of *Nrg4* is comparatively higher than *Nrg1* expression in gastrocnemius, although this expression is negligible in comparison to *Nrg4* expression in WAT. Therefore, we speculate that the main source of NRG4 that the muscle sense comes from the BAT and WAT production via endocrine signalling, yet we cannot rule out the possibility that local NRG4 may have autocrine effects in muscle. Regarding NRG1, it is important to note that these studies were performed in the absence of muscle contraction and it is this process that governs the local release of the EGF-like domain of this factor in skeletal muscle (*Cantó et al., 2006; reviewed in Gumà et al., 2010*). Thus, NRG1 release upon muscle contraction may be the limiting step in the regulation of the local actions of this factor in muscle and not its gene expression. Hence, although the expression of *Nrg1* was lower than *Nrg4* in this tissue, this does not negate the physiological importance of NRG1 local signalling in muscle physiology.

5.7. Tissue crosstalk between *Erbb4* gene-deleted muscle and other metabolic tissues

As it is the case with WAT, skeletal muscle acts as an endocrine organ by producing hundreds of different myokines that exert autocrine, paracrine and endocrine effects (*reviewed in Severinsen et al., 2020*). Thus, muscle myokines have been shown to mediate muscle crosstalk with different organs, tissues and cells, namely brain, liver, pancreas, bone, WAT, kidney, immune cells among others. Therefore, to evaluate how the absence of NRG4 signalling in skeletal muscle affects this tissue crosstalk, we analysed the expression of proinflammatory cytokines, neuregulins, ErbB receptors and metabolic genes in WAT and the liver from muscle-specific *Erbb4* KO mice. Regarding muscle-WAT crosstalk, myokines have been shown to regulate lipid metabolism upon exercise in WAT through the production of IL-6. In addition, the production of myokines is important in the *browning* of white fat inducing the expression of UCP1 by IL-6 and meteorin-like factor (*Rao et al., 2014; Knudsen et al., 2014*). Upon ND feeding, the expression of proinflammatory cytokines in WAT was neither induced in 2-months-old nor 6-months-old *Erbb4* KO mice. Besides, the WAT expression of *Nrg1, Nrg4*, ErbB receptors as well as the metabolic markers *Glut4*, *InsR* and *Fgf21* remained unchanged in *Erbb4* gene-deleted mice upon ND feeding. Thus, muscle to WAT crosstalk was not altered upon ND feeding in *Erbb4* KO mice. Therefore, we can speculate that the emergence of inflammation in skeletal from *Erbb4* gene-deleted muscle is not enough to impact WAT metabolism or the inflammatory state of this tissue upon ND feeding.

However, upon HFD, the expression of proinflammatory cytokines increased in all genotypes since obesity and HFD causes low-grade chronic inflammation in WAT (*reviewed in Lee et al., 2013*). Moreover, the expression of *Glut4* and *InsR* was reduced in all genotypes, suggesting that the HFD-induced inflammation is enough to down-regulate the gene expression of these genes in WAT. Likewise, the expression of *Nrg4* was also reduced in all the genotypes, probably due to the same reason since WAT inflammation reduces the expression of this adipokine (*Wang et al., 2014*). Similarly, the expression of the receptor *Erbb4* was also induced upon HFD in all genotypes, hence supporting the view that upon inflammatory stimuli *Erbb4* expression is up-regulated in WAT, as it happens in *Nrg4* KD adipocytes. Finally, the expression of *Fgf21* was also upregulated in all the genotypes, which is consistent with the emergence of mitochondrial stress upon HFD feeding. Importantly, neither the WAT expression of proinflammatory cytokines nor the down-regulation of adipogenic genes was worsened in *Erbb4* KO mice fed with HFD. However, upon HFD, as it was previously mentioned, the perigonadal WAT weight was reduced in *Erbb4* gene-deleted muscle, which is consistent with muscle to WAT tissue crosstalk mediated by proinflammatory cytokines. Indeed, muscle produced IL-6 increases the lipolytic rates and fat

oxidation in WAT from mice (*Pedersen et al., 2003*). Therefore, this supports the view that the reduction in WAT mass upon HFD feeding in 6-months-old *Erbb4* KO mice is caused by the production of proinflammatory cytokines by *Erbb4* gene-deleted skeletal muscle. Hence, we can speculate that muscle inflammation upon *Erbb4* gene deletion in this tissue, despite not triggering WAT inflammation, it impacts lipid metabolism in WAT. This effect is only visible upon HFD feeding since in this situation we favour lipid anabolism in WAT and thus, the effects of lipid catabolism in this tissue are more evident. However, this constitutes a hypothesis for the time being, since we have not demonstrated the existence of systemic inflammation in muscle-specific *Erbb4* KO mice that could mediate this tissue crosstalk.

Regarding muscle to liver crosstalk, it is known that muscle-produced IL-6 regulates hepatic glucose production upon exercise (*Febbraio et al., 2004*). Moreover, it has been described that muscle production of IL-6 enhances AKT signalling in the liver and reduces the expression of gluconeogenic genes, having beneficial effects on glucose and insulin homeostasis (*Peppler et al., 2019*). However, sustained inflammatory signalling in the liver is also deleterious since promotes hepatic fibrosis and NASH progression (*reviewed in Schuppan et al., 2018*). Upon ND feeding, our findings show that the expression of *InsR*, ErbB receptors, proinflammatory cytokines and *Fgf21* remained unchanged in muscle-specific *Erbb4* KO mice at 2 and 6 months. However, these mice showed a dramatic reduction in the hepatic *Nrg4* expression. As it was previously mentioned, the role of NRG4 in the liver has been previously described (*Wang et al., 2014; Ma et al., 2016; Chen et al., 2017, Guo et al., 2017*). Given that *Nrg4* expression is strongly down-regulated by inflammation, we propose that the emergence of muscle inflammation upon *Erbb4* deletion in this tissue can impact the expression of this factor in the liver through muscle to liver crosstalk. Hence, the higher HRG sensitivity showed during the GTT analyses in *Erbb4* KO mice upon ND feeding could be caused by NRG4 desensitization due to the lower hepatic *Nrg4* expression.

Besides that, upon HFD feeding, the hepatic expression of *Il6* was induced in all the genotypes. Furthermore, the hepatic expression of *Nrg4* was down-regulated, supporting the view that the expression of this factor can be down-regulated by liver inflammation upon HFD feeding. Likewise, the expression of *Erbb4* was up-regulated in all the genotypes, further supporting the view that *Erbb4* is induced upon inflammatory stimuli in the liver. Finally, the hepatic expression of *Fgf21* was also induced upon HFD in all genotypes. The liver is the major source of FGF21 and, although it has beneficial effects improving insulin sensitivity, long term HFD feeding increases hepatic FGF21 production, causing FGF21 resistance in other tissues such as WAT (*Geng et al., 2019; reviewed in Markan, 2018*).

Regarding the absolute expression of neuregulins and ErbB receptors, as it was previously described in WAT total homogenates, the comparative expression of *Erbb1* and *Erbb2* was higher than *Erbb3* and *Erbb4*. However, the WAT expression of *Nrg4* was higher than *Nrg1*. Hence, despite the role of NRG4 in the regulation of adipocyte physiology, given the low expression of *Erbb4* in adipocytes, NRG4 can signal through other tissues in an endocrine manner. Contrarily, in the liver, the absolute expression of *Erbb3* was higher than the rest of ErbB receptors. In addition, the expression of *Nrg1* was higher than *Nrg4*. This highlights the importance of the NRG1-ErbB3 signalling in the regulation of liver metabolism, as it has been previously described (*López-Soldado et al., 2016*). However, it is also known that NRG4 regulates liver metabolism, despite the low absolute expression of *Erbb4* in this tissue (*Wang et al., 2014*). Finally, in skeletal muscle, the absolute expression of *Erbb3* and *Erbb4* receptors were similar, whereas the expression of *Nrg4* was higher than *Nrg1*. On the one hand, NRG1 regulates muscle metabolism upon its cleavage by muscle contraction (*Cantó et al., 2006; reviewed in Gumà et al., 2010*). Then, NRG1 can bind to ErbB3 and ErbB4 to regulate muscle metabolism. On the other hand, skeletal muscle may be able to sense endogenous and, especially, systemic NRG4 through ErbB4 to regulate muscle physiology. Thus, different neuregulin isoforms regulate various aspects of the cellular metabolism and physiology of different organs and tissues given the differential endogenous expression of these factors and their receptors.

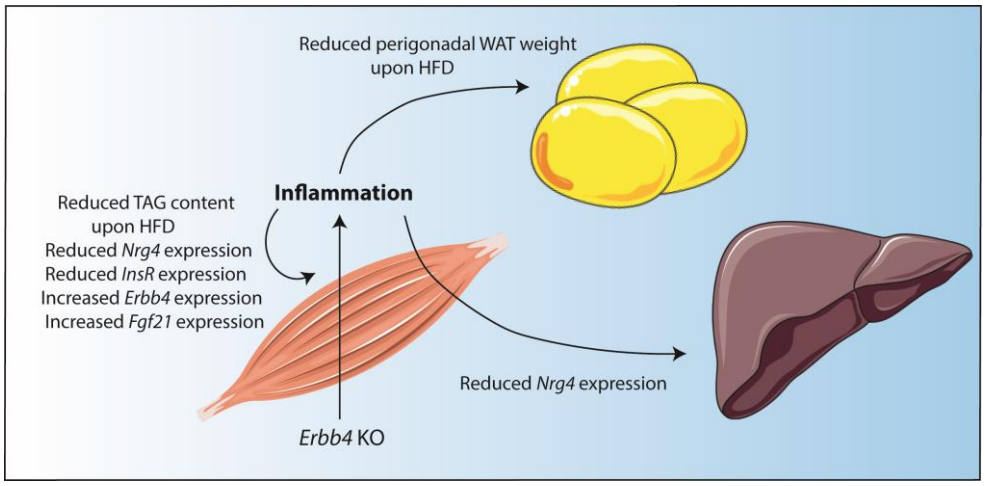


Figure 80. Schematic representation of the possible mechanisms that mediate muscle to WAT and liver crosstalk upon *Erbb4* gene deletion in muscle. In basal conditions, inflammation emerges in muscle upon *Erbb4* KO. This may contribute to reduce the expression of *Nrg4* in muscle and the liver. In addition, the emergence of inflammation in skeletal muscle is consistent with the down-regulation of the *InsR* and the up-regulation of *Erbb4* and *Fgf21*. Upon HFD, inflammation may induce lipolysis or lipophagy in muscle, reducing the TAG accumulation as a compensatory mechanism to the ectopic lipid accumulation. On the other hand, muscle inflammation may be responsible for the loss of perigonadal WAT weight upon HFD feeding by inducing lipolysis and repressing lipogenesis and adipogenesis in this tissue. However, the existence of systemic inflammation in muscle-specific Erbb4 KO mice have not been demonstrated and hence, this model is just hypothetical. Schematic art pieces used in this figure are provided by Servier Medical Art under a Creative Commons Attribution 3.0 Unported License.

5.8. Future prospects

NRG4 emerges as a novel adipokine that controls a plethora of aspects in adipocyte physiology. However, many questions are still remaining. For instance, it is unclear the exact route followed by the GSVs in adipocytes towards the autophagic degradation. We hypothesize that upon TBC1D4 down-regulation, GSVs can undergo lysosomal degradation, however, it is still unclear whether GSVs are translocated to the plasma membrane before undergoing autophagy. Thus, experiments with GLUT4 fluorescent constructs should be performed to shed some light on the matter. In addition, the generation of TBC1D4 loss-of-function and gain-of-function cellular models will be helpful to fully comprehend the role of this protein in the GSV degradation and trafficking.

In addition, the exact mechanism by which some mitochondrial proteins are degraded in *Nrg4* KD adipocytes remains unknown. We only observed TIM44, UCP1 and MFN2 protein degradation, while the protein content of other mitochondrial proteins such as OPA1 remained unchanged in these adipocytes. Therefore, further experiments are needed to clarify the exact mechanism by which some mitochondrial proteins are directed towards lysosomal degradation upon *Nrg4* silencing in adipocytes.

On the other hand, we demonstrated that mtROS cause NF-κB activation upon *Nrg4* silencing in adipocytes. However, we cannot rule out the possibility that other DAMPs contribute to the emergence of inflammation in our model. Specifically, mtDNA could have a role in inflammation upon mitophagy deregulation in adipocytes since it can engage TLR9 in the amphisomal compartments (*Rodríguez-Nuevo et al., 2018*). Therefore, experiments with mtDNA depleted cells by ethidium bromide treatment should be carried out to shed some light on the role of this DAMP in the inflammatory response upon NRG4 deficiency in adipocytes. These mitochondrial alterations, along with the insulin resistance, caused metabolic alterations in *Nrg4* KD adipocytes. To fully characterise these alterations, mitochondrial metabolism should be assed via Seahorse Respirometry in *Nrg4* KD adipocytes.

Beyond the role of NRG4 in adipocytes, this factor has paracrine and endocrine effects that are important in the regulation of macrophage inflammation. Thus, to fully demonstrate the role of NRG4 in adipocyte-macrophage crosstalk, co-culture experiments should be performed. In addition, the NRG4-ErbB4 signalling axis has been shown to be protective against muscle inflammation in mice. However, it remains unclear the exact mechanism by which muscle inflammation is triggered upon *Erbb4* gene deletion. Our data suggest that mitochondrial dysfunction could cause inflammation in skeletal muscle, however, the protein and gene expression of mitochondrial proteins should be analysed to confirm this hypothesis.

In addition, we explored muscle to WAT and liver crosstalk upon *Erbb4* KO in muscle. We observed reduced perigonadal WAT mass and muscle TAG content upon HFD as well as reduced hepatic *Nrg4* expression in these tissues. These alterations are consistent with the presence of systemic inflammation in mice upon *Erbb4* gene deletion in muscle, however, this has not been analysed. Thus, ELISA analyses of proinflammatory cytokines should be performed with plasma from these mice to confirm this hypothesis. Alternatively, recovery experiments by treating muscle-specific *Erbb4* KO mice with anti-inflammatory molecules could be performed to analyse the impact of muscle inflammation in the muscle to tissue crosstalk and the hepatic *Nrg4* down-regulation in *Erbb4* KO mice.

In summation, NRG4 plays an essential function protecting against inflammation in adipocytes, macrophages and skeletal muscle through ErbB4 signalling. Furthermore, NRG4 regulates the autophagic flux and thus, the degradation of GSVs in 3T3-L1 adipocytes. Hence, the novel adipokine neuregulin-4 emerges as an anti-inflammatory factor with local and systemic effects that contribute to the preservation of insulin sensitivity in the whole organism.

6. Conclusions

1. *Nrg4*-silenced 3T3-L1 adipocytes are insulin resistant according to the lack of insulin action on 2DG uptake. Both, the expression of the insulin receptor and the GLUT4 protein content are downregulated in *Nrg4* KD adipocytes. Furthermore, *Nrg4* KD adipocytes displayed a reduced content of proteins located in the GLUT4 storage vesicles, namely IRAP and syntaxin-6.

2. Cell-autonomous inflammation emerges as a consequence of the silencing of *Nrg4* in 3T3-L1 adipocytes. This inflammation is characterised by NF-κB activation and enhanced proinflammatory cytokine gene expression. Contrary, the treatment with TNFα reduces the expression of *Nrg4* in adipocytes.

3. Anti-inflammatory agents such as dexamethasone and sodium salicylate recover the expression and the protein content of the insulin receptor, but not the GLUT4 content, in *Nrg4*-silenced adipocytes.

4. NRG4 regulates mTORc1 activation in both, basal conditions and upon insulin stimulus in 3T3-L1 adipocytes. As a consequence, NRG4-deficient adipocytes have accelerated autophagic flux. Disruption of the lysosomal activity with bafilomycin A1 recovers the protein content of GLUT4 and the other markers of the GLUT4 storage vesicles, IRAP and syntaxin-6, suggesting that they are degraded via autophagy in *Nrg4* KD adipocytes.

5. *Nrg4* KD adipocytes have reduced mitochondrial mass and condensed mitochondrial network morphology. Mitochondrial protein markers such as MFN2 and TIM44 are reduced in Nrg4 KD adipocytes and there are pieces of evidence supporting the view that they are degraded via lysosomal degradation. Ultimately, mitochondrial disruption triggers mitochondrial oxidative stress in *Nrg4* KD adipocytes. The treatment with the antioxidant NAC reverted the cell-autonomous inflammation in these adipocytes. Therefore, mtROS are the main DAMPs that cause the cell-autonomous NF-κB activation upon *Nrg4* silencing in adipocytes.

6. *Erbb4*-silenced 3T3-L1 adipocytes reduce the GLUT4, IRAP and syntaxin-6 protein content. The blockage of the ErbB4 protein receptor in 3T3-L1 adipocytes with a blocking antibody also reduced the GLUT4 and IRAP protein content. Likewise, ErbB4 disruption in 3T3-L1 adipocytes triggers cell-autonomous inflammation. Furthermore, *Erbb4* KO primary immortalised white adipocytes also show cell-autonomous inflammation and *InsR* down-regulation. Therefore, the NRG4-ErbB4 cell signalling axis directly regulates autonomous inflammation and GSV protein degradation in adipocytes.

7. *Erbb2* gene-deleted primary adipocytes and *Erbb2* KD 3T3-L1 adipocytes cannot undergo adipogenic differentiation. Likewise, the gene expression of *Nrg1* and *Erbb2* in preadipocytes is higher than in differentiated adipocytes. Hence, both proteins could have a role in adipocyte differentiation.

8. M1-polarised bone marrow-derived macrophages induce the expression of the receptor of NRG4, *Erbb4*. NRG4 supplementation via conditioned media from 3T3-L1 adipocytes prevents and recovers the M1 polarization in RAW 264.7 macrophages and BMDM upon LPS treatment. Contrarily, supplementation with conditioned media from NRG4 KD adipocytes promotes inflammation in non-polarised BMDM. Thus, NRG4 prevents and promotes the resolution of inflammation in macrophages.

9. Glucose and insulin tolerance are not affected in muscle-specific *Erbb4* KO mice. Instead, upon HFD feeding, these mice tend to have increased glucose and insulin sensitivity. Therefore, the absence of *Erbb4* in muscle does not cause insulin resistance in mice.

10. ErbB4 protects against muscle inflammation in mice since the gene deletion of *Erbb4* in muscle causes inflammation and *Fgf21* up-regulation in this tissue. In physiological conditions, the hepatic *Nrg4* expression is reduced upon *Erbb4* gene deletion in muscle, suggesting the existence of muscle to liver crosstalk that regulates hepatic *Nrg4* expression.

11. Upon HFD, inflammation is not worsened in *Erbb4* gene-deleted muscle. Likewise, HFD-induced inflammation remains unchanged in WAT and liver between control and *Erbb4* KO mice. However, in these conditions, the muscle TAG content and the perigonadal WAT weight are reduced in *Erbb4* KO mice, which is consistent with the emergence of muscle inflammation upon *Erbb4* deletion in muscle. Hence, muscle inflammation upon *Erbb4* gene deletion may induce lipid catabolism in WAT from mice.

12. *Nrg4* expression is reduced in WAT from cancer cachectic rats. Therefore, this factor could constitute a central adipokine that regulates the pathophysiology of the cachexic syndrome in WAT.

7. Methods

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

7.1. Reagent references

Table 1. Information of commonly used reagents.

Product	Vendor	Reference
10000 U/mL Penicillin-Streptomycin	Gibco	15140122
2-Deoxy-D-[2,6-^{3}H]glucose	American Radiolabeled	ART-103A
2-Deoxy-D-glucose (2DG)	Sigma-Aldrich	D8375
2-Mercaptoethanol	Sigma-Aldrich	M6250
37% Hydrochloric acid	Sigma-Aldrich	320331
3-Isobutyl-1-methylxanthine (IBMX)	Sigma-Aldrich	I5879
Adenosine 5'-Triphosphate (ATP)	Sigma-Aldrich	FLAAS
Ammonium persulfate (APS)	Sigma-Aldrich	A3678
Amonium chloride	Sigma-Aldrich	2413330
Ampicillin	Sigma-Aldrich	A1593
Amplex® Red Hydrogen Peroxide / Peroxidase Assay	Invitrogen	A22188
100x Antibiotic-Antimycotic	Gibco	15240062
Ascorbic acid	Sigma-Aldrich	A4034
Bafilomycin A1	Santa Cruz Biotech	sc-201550
Biotin	Sigma-Aldrich	B4639
Bis-Acrylamide 30%	Sigma-Aldrich	A3574
Bovine Serum Albumin (BSA) – Fatty acid free	Sigma-Aldrich	6002
Bromophenol blue	Sigma-Aldrich	B0126
Calcium chloride hydrate	Sigma-Aldrich	202940
Calf Serum (CS)	Gibco	16170078
Chloroform	Sigma-Aldrich	C2432
Collagenase type II	Worthington	LS004176
D-(+)-glucose	Sigma-Aldrich	G8270
D-[U-C^{14}]glucose	Amersham	CFB96
DAPI	Sigma-Aldrich	D9542
Dexamethasone	Sigma-Aldrich	D2915
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D8418
DMEM 1g glucose/L	Gibco	11885084
DMEM 4,5 g/l Glucose; w/L-Gln; w/Sodium pyruvate	Biowest	L0104-500
DMEM F-12	Gibco	11320033
DMOG	Sigma-Aldrich	D3695
DNeasy® Blood & Tissue Kit columns	Qiagen	69504
dNTPs mix	Invitrogen	R0181
EBSS media	Gibco	24010043
EcoLite™ Scintillation liquid	MP Biomedicals	882475
Glycerol Phosphate Oxidase/Peroxidase kit	Bio Systems	12812
Ethanol absolute pure	Panreac	141086.1212
Ethylenedinitrilotetraacetic acid (EDTA)	Sigma-Aldrich	03620
Fluoromount™	Sigma-Aldrich	F4680
Foetal Bovine Serum (FBS)	Gibco	10270106
Glycerol	Sigma-Aldrich	G5516
Glycerol Dehydrogenase (GDH)	Sigma-Aldrich	G3512
Glycerol kinase (GK)	Sigma-Aldrich	G6278
Glycine	Roche	03117251001
Glycol ether diamine tetraacetic acid (EGTA)	Sigma-Aldrich	E3889
HEPES	Sigma-Aldrich	H3375
Heptane	Sigma-Aldrich	246654
Human recombinant insulin	Sigma-Aldrich	I5500
Hydrazinium hydroxide	Sigma-Aldrich	8046080250
HyperPAGE Prestained Protein Marker	Meridian Bioscience	BIO-33066
IFNγ	Invitrogen	PMC4031
Igepal® CA630	Sigma-Aldrich	I8896

Isopropanol	Panreac	131090.1611
Lactate Assay Kit EnzyChrom™	BioAssay Systems	ECLC-100
LB broth with agar	Sigma-Aldrich	L2897
LB broth	Sigma-Aldrich	L3022
LPS	Sigma-Aldrich	L4516
Magnesium chloride	Sigma-Aldrich	M8266
Magnesium sulfate heptahydrate	Sigma-Aldrich	230391
Methanol	Panreac	141091.1212
MitoTracker™ Green FM	Invitrogen	M7514
MitoTracker™ Red CMXRos	Invitrogen	M7512
Mouse Erytrocyte Lysing Kit	R&D Systems	WL2000
NAC	Sigma-Aldrich	A7250
NAD	Sigma-Aldrich	NAD100-RO
Oil Red O	Sigma-Aldrich	O-0625
Oligo(dT)20 primers	Invitrogen	18418020
Pantothenic acid	Sigma-Aldrich	P5155
Paraformaldehyde 16% aqueous solution	Polyscience	23966
Perchloric acid	Sigma-Aldrich	244252
Phosphatase inhibitors	Roche	04906845001
Pierce® BCA Protein assay kit	Thermo Scientific	23225
Polybrene	Sigma-Aldrich	H9268
Polyethyleneimine-2500 (PEI)	Polyscience	24313
Polyvinylidene fluoride (PVDF) membrane	MERCK Millipore	IPVH00010
Porcine insulin	Eli Lilly and Company	U-100
Potassium chloride	Sigma-Aldrich	P9541
Potassium hydroxide	Sigma-Aldrich	P5958
Potassium phosphate monobasic	Sigma-Aldrich	P0662
Protease inhibitors	Thermo Scientific	78430
PureLink™ HiPure Plasmid Midiprep Kit	Invitrogen	K210005
PureLink™ DNase Set	Thermo Scientific	12185010
Purelink™ RNA Mini Kit columns	Invitrogen	12183018A
Puromycin	Sigma-Aldrich	P8833
Recombinant Human Heregulin-β1 (HRG)	PreproTech	100-03
Recombinant human NRG4 (rNRG4)	Reprokine	RKQ8WWG
Recombinant murine IL-4	Gibco	PMC0046
RNaseOUT™	Invitrogen	10777019
Rosiglitazone	Sigma-Aldrich	R2408
Sodium azide	Sigma-Aldrich	S2002
Sodium chloride	Panreac	A2942
Sodium deoxycholate	Sigma-Aldrich	D6750
Sodium dodecyl sulfate	Panreac	142363
Sodium hydrogen phosphate	Sigma-Aldrich	255793
Sodium hydroxide	Sigma-Aldrich	221465
Sodium pyruvate	Sigma-Aldrich	P2256
Sodium salicylate	Sigma-Aldrich	S3007
Sucrose	Sigma-Aldrich	S8501
Sulfuric acid	Sigma-Aldrich	1090721000
SuperScript™ II Reverse Transcriptase	Invitrogen	18064022
SYBR™ Green PCR Master Mix	Applied Biosystems	4367659
TEMED	Sigma-Aldrich	T22500
ECL™ Kit	Amersham	15387655
TMRM Assay Kit	AbCam	ab228569
TNFα	PreproTech	300-01A
Tris-Base	Sigma-Aldrich	T1503
Tris-HCl	Sigma-Aldrich	T3253
TRIzol™	Sigma-Aldrich	11667157001
Trypsin-EDTA (0.05%)	Gibco	25300-062
Tween®20	Sigma-Aldrich	P1379

Table 2. Primary antibody information. Western Blot (WB), immunolocalization (IL), Molecular Weight (MW) (kDa).

Antibody	Source	Vendor	Reference	MW	Dilution
39 kDa Complex I	Mouse	Invitrogen	PAS39270	39	1/1000 WB
AKT	Rabbit	Cell Signalling	9272	60	1/2000 WB
AMPK α-Subunit	Rabbit	Cell Signaling	2532	62	1/1000 WB
ASC	Rabbit	Santa Cruz	Sc-22514	21	1/1000 WB
ATGL	Rabbit	Cell Signaling	21385	55	1/1000 WB
CAV1	Mouse	BD Transduction	610406	22	1/1000 WB 1/100 IL
Clathrin	Rabbit	Cell Signaling	5796	40	1/1000 WB
EEA1	Rabbit	Cell Signaling	3288S	180	1/1000 WB
ErbB1	Rabbit	Cell Signaling	4267	-	1/100 IL
ErbB1	Rabbit	Santa Cruz	Sc-03	180	1/500 WB
ErbB2	Rabbit	Cell Signaling	4290	-	1/100 IL
ErbB2	Rabbit	Santa Cruz	Sc-245	180	1/1000 WB
ErbB3	Rabbit	Cell Signaling	12708	-	1/100 IL
ErbB4	Rabbit	Abcam	Ab2995	180	1/500 WB
ErbB4 (Ab3)	Mouse	Neomarkers	MS-304	-	10 μg/ml for blocking
GAPDH	Rabbit	Cell Signaling	5174	36	1/9000 WB
GLUT4	Rabbit	Generated in our lab	OSCRX	55	1/2000 WB 1/400 IL
HSL	Rabbit	Cell Signaling	18381	82	1/1000 WB
IL-1β	Rabbit	Abcam	Ab9722	31	1/1000 WB
InsR β chain	Rabbit	BD Transduction	611277	95	1/500 WB
IRAP	Rabbit	Cell Signaling	3808	165	1/500 WB
IκB-α	Rabbit	Santa Cruz	Sc-203	36	1/1000 WB
LAMP1	Mouse	Abcam	Ab233567	-	1/400 IL
LC3B	Rabbit	Cell Signaling	2775	16	1/1000 WB
MFN2	Mouse	Abcam	Ab56889	50	1/1000 WB
mTOR1	Rabbit	Cell Signaling	2972	290	1/1000 WB
NF-κB p-50	Rabbit	Cell Signaling	Sc-114	50	1/1000 WB
OPA1	Mouse	Abcam	Ab42364	90/100	1/1000 WB
Perilipin-1	Rabbit	Santa Cruz	Sc-67164	56/65	1/1000 WB 1/100 IL
PGC1α	Rabbit	Abcam	Ab54481	90	1/500 WB
p-S(660)HSL	Rabbit	Cell Signalling	4126	82	1/1000 WB
PPARγ	Rabbit	Santa Cruz	Sc-7196	57	1/500 WB
p-S(2448) mTOR1	Rabbit	Cell Signaling	2971	290	1/1000 WB
p-S(473)AKT	Rabbit	Cell Signaling	9271	60	1/1000 WB
p-T(172) AMPK	Rabbit	Cell Signaling	9275	62	1/1000 WB
p-T(308)AKT	Rabbit	Cell Signaling	2531	60	1/500 WB
Rab11	Rabbit	Cell Signaling	2413	24	1/1000 WB
Syntaxin-6	Rabbit	Cell Signaling	2869	41	1/1000 WB
TBC1D4	Rabbit	Cell Signaling	2670	160	1/1000 WB
TIM44	Rabbit	Abcam	Ab244466	44	1/1000 WB
TUG	Rabbit	Cell Signaling	2049	75	1/1000 WB
UCP1	Rabbit	Abcam	Ab10983	33	1/1000 WB
α-Complex V	Mouse	Invitrogen	PAS25799	50	1/1000 WB

Table 3. Secondary antibody information.

Antibody	Target	Vendor	Reference	Dilution
Alexa Fluor® 488	Mouse	Invitrogen	A11029	1/800 IL
Alexa Fluor® 647	Rabbit	Abcam	Ab150075	1/800 IL
HRP	Mouse	Jackson ImmunoResearch	715-035-150	1/20000 WB
HRP	Rabbit	Jackson ImmunoResearch	711-035-152	1/20000 WB

Table 4. Primer sequences for qPCR.

Gene	Sequence Forward	Sequence Reverse
16s	ATACCGCGGCCGTTAAA	CACTGCCTGCCCAGTGA
Adipoq	CGGCAGCACTGGCAAGTT	CCGTGATGTGGTAAGAGAAGTAGTAGA
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
Arp	AAGCGCGTCCTGGCATTGTCT	CCGCAGGGGCAGCAGTGGT
Asc	GAAGCTGCTGACAGTGCAAC	GCCACAGCTCCAGACTCTTC
Atgl	GGATGGCGGCATTTCAGACA	CAAAGGGTTGGGTTGGTTCAG
Atp5l	GAGAAGGCACCGTCGATGG	ACACTCTGAATAGCTGTAGGGAT
Cav1	ATGTCTGGGGGCAAATACGTG	CGCGTCATACACTTGCTTCT
Cytb	GCTTTCCACTTCATCTTACCATTTA	TGTTGGGTTGTTTGATCCTG
Dloop1	AATCTACCATCCTCCGTGA	TCAGTTTAGCTACCCCCAA
Dloop2	CCCTTCCCCATTTGGTCT	TGGTTTCACGGAGGATGG
Dloop3	TCCTCCGTGAAACCAACAA	AGCGAGAAGAGGGGCATT
Erbb1	GCCATCTGGGCCAAAGATACC	GTCTTCGCATGAATAGGCCAAT
Erbb2	GCTGCTGGACATTGATGAGA	GGGATCCCATCGTAAGGTTT
Erbb3	CGCCAGATGACAAGCAGTTA	AGGTCATCAACTCCCAAACG
Erbb4	TCCCCCAGGCTTTCAACATAC	GCACCCTGAGCTACTGGAG
Fgf21	CTGCTGGGGGTCTACCAAG	CTGCGCCTACCACTGTTCC
Glut4	GTGACTGGAACACTGGTCCTA	CCAGCCACGTTGCATTGTAG
Hif1a	ACCTTCATCGGAAACTCCAAAG	CTGTTAGGCTGGGAAAAGTTAGG
Hsl	CCAGCCTGAGGGCTTACTG	CTCCATTGACTGTGACATCTCG
Ifnb	CCCTATGGAGATGACGGAGA	CCCAGTGCTGGAGAAATTGT
Il10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
Il1b	GCACTACAGGCTCCGAGATGAAC	TTGTCGTTGCTTGGTTCTCCTTGT
Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
InsR	ATGGGCTTCGGGAGAGGAT	GGATGTCCATACCAGGGCAC
Irap	TTTACCAATGATCGGCTTCAGC	TCGAACCTCGGGGCTCATATT
Lpl	GGGAGTTTGGCTCCAGAGTTT	TGTGTCTTCAGGGGTCCTTAG
Mfn2	AGAACTGGACCCGGTTACCA	CACTTCGCTGATACCCCTGA
Mgl	CGGACTTCCAAGTTTTTGTCAGA	GCAGCCACTAGGATGGAGATG
Ndufv1	TTTCTCGGCGGGTTGGTTC	GGTTGGTAAAGATCCGGTCTTC
Nrg1	TTCCCATTCTGGCTTGTCTAGT	CCAGGGTCAAGGTGGGTAG
Nrg2	TCGACCCTAACGGCAAAAACA	AACCAGCGATAGGAGGGCT
Nrg3	TTACGCTGTAGCGACTGCATC	GCCTACCACGATCCATTTAAGC
Nrg4	CACGCTGCGAAGAGGTTTTTC	CGCGATGGTAAGAGTGAGGA
Opa1	TGGAAAATGGTTCGAGAGTCAG	CATTCCGTCTCTAGGTTAAAGCG
Phd3	AGGCAATGGTGGCTTGCTATC	GCGTCCCAATTCTTATTCAGGT
Plin1	CTGTGTGCAATGCCTATGAGA	CTGGAGGGTATTGAAGAGCCG
Pparg	CCAGAGCATGGTGCCTTCGCT	CAGCAACCATTGGGTCAGCTC
Sod1	AACCAGTTGTGTTGTCAGGAC	CCACCATGTTTCTTAGAGTGAGG
Sod2	CAGACCTGCCTTACGACTATGG	CTCGGTGGCGTTGAGATTGTT
Stx6	ACAGGCCGTCATGCTAGATG	GGATGGCTATGGCACACCAC
Tbc1d4	GCATTCAGGATGAGCCTTTCC	CTCCCACGTACCATAGCCG
Tert	CTAGCTCATGTGTCAAGACCCTCTT	GCCAGCACGTTTCTCTCGTT
Tim44	CTAGGCAGCGGAATCCAATTT	GCAAGCCTGACAAAAACCCTTT
Tnfa	TGATGAGCTACTACTGGTCAGC	GATCTCTTAGCACAAGGATGGC
Ucp1	AGGCTTCCAGTACCATTAGGT	CTGAGTGAGGCAAAGCTGATTT
Uqcrh	GTGGACCCCCTAACAACAGTG	CGGGAAGACACGCGATTATCA

7.2. General solutions recipes

Table 5. Western blot general solutions.

4x Laemmli Sample Buffer (LSB)	
Reagents	**Final concentration**
Tris H-Cl, Ph=6.8	0.125 M
Sodium Dodecyl Sulphate (SDS)	8% (w/v)
Glycerol	40% (v/v)
Bromophenol blue	0.04% (w/v)
1x Electrophoresis buffer	
Reagents	**Final concentration**
Tris-base	25 nM
Sodium Dodecyl Sulphate (SDS)	0.1 (w/v) SDS
Glycine	190 mM
1x Blotting buffer	
Reagents	**Final concentration**
Tris-base	25 nM
Glycine	0.1 (w/v) SDS
Methanol	20 (v/v) mM
1x Tris Buffer Saline (TBS)	
Reagents	**Final concentration**
NaCl	150 mM
Tris-HCl	50 mM
Adjust pH=7.4	
1x Tris Buffer Saline with Tween®20 (TBST)	
Reagents	**Final concentration**
TBS	1x
Tween®20	0.1% (v/v)
1x Phosphate Buffer Saline (PBS)	
Reagents	**Final concentration**
NaCl	137 mM
KCl	2.7 mM
Na_2HPO_4	4.3 mM
Adjust pH=7.4	
1x Blocking solution	
Reagents	**Final concentration**
TBST	137 mM
non-fat dry milk or Bovine Serum Albumin (BSA)	5% (Milk) 3% (BSA)

Table 6. Polyacrylamide gels recipe.

Running gel (8%)	
Reagents	**Final concentration**
Acrylamide	8% (v/v)
1.5 M Tris-HCl solution pH=8.8	0.375 M
Sodium Dodecyl Sulphate (SDS)	0.1% (w/v)
Ammonium persulfate (APS)	0.1% (w/v)
TEMED	6.6 mM (v/v)
Running gel (10%)	
Reagents	**Final concentration**
Acrylamide	10% (v/v)
1.5 M Tris-HCl solution pH=8.8	0.375 M
Sodium Dodecyl Sulphate (SDS)	0.1% (w/v)
Ammonium persulfate (APS)	0.1% (w/v)
TEMED	6.6 mM (v/v)
Running gel (12.5%)	
Reagents	**Final concentration**
Acrylamide	12.5% (v/v)
1.5 M Tris-HCl solution pH=8.8	0.375 M
Sodium Dodecyl Sulphate (SDS)	0.1% (w/v)
Ammonium persulfate (APS)	0.1% (w/v)
TEMED	6.6 mM (v/v)
Stacking gel (3.3%)	
Reagents	**Final concentration**
Acrylamide	3.3% (v/v)
0.5 M Tris-HCl solution pH=6.8	0.125 M
Sodium Dodecyl Sulphate (SDS)	0.1% (w/v)
Ammonium persulfate (APS)	0.1% (v/v)
TEMED	6.6 % (w/v)

Table 7 Recipes for other commonly used solutions.

1x Krebs-Ringer-HEPES Buffer (KRHB)	
Reagents	**Final concentration**
NaCl	137 mM
KCl	4.7 mM
$MgSO_4.7H_2O$	1.18 mM
KH2PO4	1.18 mM
$CaCl_2.H_2O$	2.5 mM
HEPES	20 mM
Bovine Serum Albumin (BSA)	0.2% (w/v)
1x Krebs-Ringer-HEPES Buffer (KRHB) with sodium pyruvate	
Reagents	**Final concentration**
KRHB	1x
Sodium pyruvate	2 mM
1x HEPES-EDTA-Sucrose (HES) buffer	
Reagents	**Volume**
HEPES	20 mM
EDTA	1 mM
Sucrose	255 mM
1x Cell lysis buffer	
Reagents	**Volume**
1 M Tris-base pH=7.5	20 mM
NaCl	150 mM
Igepal® CA630 (NP40)	1% (v/v)
EDTA	1 mM
EGTA	1 mM
Sodium deoxycholate	0.5% (w/v)
Sodium Dodecyl Sulphate (SDS)	0.1% (m/v)

7.3. Cell cultures

7.3.1. General cell culture protocols

7.3.1.1. Cell thawing

Thaw the cell vials in the water batch at 37ºC until ice starts melting. This process has to be fast since the cryopreserving agent dimethyl sulfoxide (DMSO) is cytotoxic at Room Temperature (RT). Then, the cell suspension is diluted in 5 mL of warm growth media. The cells are then centrifuged at 1200 rpm for 4 min to pellet cells. After that, the DMSO containing media is discarded and the cells are resuspended with fresh growth media. Finally, the cells are transferred to T-75 T-flasks or 10 cm Ø culture dish. Alternatively, if the cell count is low, cells can be transferred to a smaller culture dish to increase cell survival. After 24h, the cell media was changed with fresh growth media. Cells were sub-cultured if needed.

7.3.1.2. Cell cryopreservation

Cells were grown to 80% of confluence. Afterwards, cells are rinsed with fresh Phosphate Buffer Saline (PBS). Then, the PBS is discarded and trypsin is added. The amount of trypsin to add depends on the surface of the cell container, as a reference, 1 mL of trypsin is used for 10 cm Ø culture dish, while 3 mL is used for T-75 T-flasks. Afterwards, the cells covered in trypsin are placed in the cell incubator at 37 ºC for 1 minute. Then, the trypsin is diluted with 5 mL of fresh growth media to stop trypsin protease activity. This medium is also used to detach the remaining cells from the surface of the cell container. Then, the medium is recovered to centrifuge at 1200 rpm for 4 minutes. Then, the supernatant is discarded and the pellet is resuspended in cold freezing media consisting of 90% FBS and 10% DMSO. The pellet is resuspended in this media. Afterwards, 1 mL this medium is placed into vials inside a StrataCooler® cryopreservation module (Agilent) at -80 ºC. Finally, commonly used cells are stored at -80 ºC or in the liquid nitrogen tank for better preservation.

7.3.1.3. Cell growth and propagation

In general, cells are maintained between 30-70% confluence during propagation to allow cell proliferation. To sub-culture them, cells are rinsed once with PBS. Afterwards, trypsin is added as mentioned in section 8.3.1.2. Once the cells are resuspended in warm growth media, they are transferred to new containers at the desired split ratio. Afterwards, cellular media are changed every 2 days, unless cells were split.

7.3.2. Secondary cell lines

7.3.2.1. 3T3-L1

This cell line constitutes the main secondary cell line used in this study. These cells were generated from *Swiss albino Mus musculus* embryo 3T3 cells between day 17-19 of development (*Todaro et al., 1963*) and were purchased from American Type Culture Collection (ATCC) (ATCC CL-173^{TM}). These fibroblast-like cells undergo a preadipocyte to adipocyte differentiation as they progress from the proliferation state to a confluent and contact-inhibited state. In the proliferation phase, cell media are changed every 2 days avoiding confluency during this phase. These cells were cultured at 37 ºC, 95% humidity and 8% CO_2. Cells were manipulated as stated by ATCC.

7.3.2.2. HEK 293T

These cells were generated from clone 293 of the transformed HEK cell line. This transformed cell line was generated by the infection with the adenovirus 5 (Ad5), which is widely used in gene transfer experiments. These cells were used in this study to produce adenoviral and lentiviral particles. HEK cells contain the early expression region 1 sequence from Ad5, thus allowing the growth of adenovirus lacking this sequence. Specifically, HEK 293T cells are a sub-line generated from HEK 293 cells by the stable expression of the SV40 large T antigen, which allows the replication of plasmids that contain an origin of replication SV40. This allows HEK 293T cells to produce large amounts of lentiviral and adenoviral particles. HEK 293 T cells were cultures at 37 ºC, 95% humidity and 5% CO_2 and were given by Dr Muñoz J. P. from IRB.

7.3.2.3. TEFLY-MO

To obtain MLV retroviral particles, the FLY packaging cell line was used. This cell line comes from the TEGF-human fibrosarcoma cells. Specifically, TEFLY cells contain the gene sequences of *gag*, *pol* and *env*, which allow the packaging of retroviral particles. Thus, TEFLY-MO cells have constitutive production of MLV retroviral particles. These cells were maintained at 37ºC, 95% humidity and 5% CO_2 and were given by Dr Romero M. from IRB.

7.3.2.4. RAW 264.7

The RAW 264.7 cells are a secondary cell line of murine macrophages. Cells were cultured in 6-well plates until they reached 90% of confluence to induce macrophage polarization. Cells were maintained in the proliferative avoiding cell confluency and changing media every 2 days. Cells were cultured at 37 ºC, 95% humidity and 5% CO_2. These cells were given by Dr Campderrós L. from the biochemistry and molecular biomedicine department, UB.

7.3.3. Protocols with secondary cell lines

7.3.3.1. 3T3-L1 differentiation

After preadipocytes reached 100% cell confluence, 3T3-L1 cells were differentiated by changing growth media with differentiation media 1 for 72h. Afterwards, differentiation media 1 was substituted with differentiation media 2 for 24h. Finally, differentiation media 2 was removed and it was changed with differentiation media 3 for 72h. The overall procedure lasted 7 days. The composition of the used media can be consulted in Table 8.

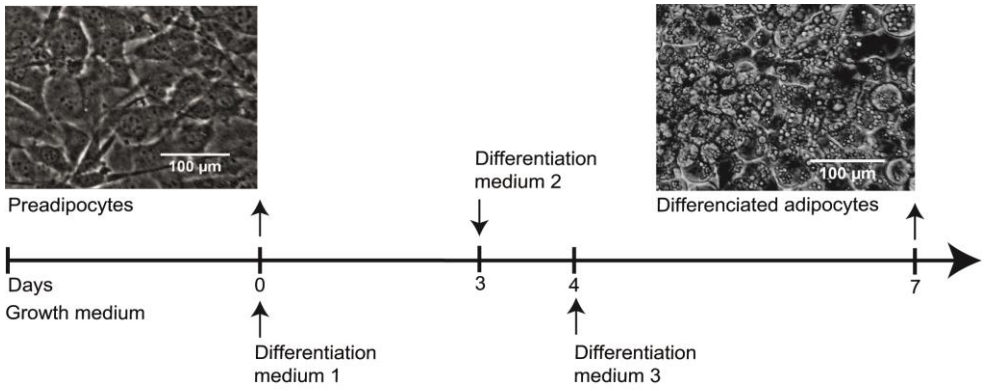


Figure 81. 3T3-L1 differentiation protocol.

7.3.3.2. RAW 264.7 M1 polarization

After cellular confluency, RAW 264.7 cells were serum-starved for 16h. Then, the M1 polarization was done by 60 ng/mL LPS treatment for 24h in polarization media. Non-polarised cells were maintained in polarization media in the absence of LPS. After that, macrophages were collected to obtain protein and RNA extracts. The composition of the used media can be consulted in Table 9.

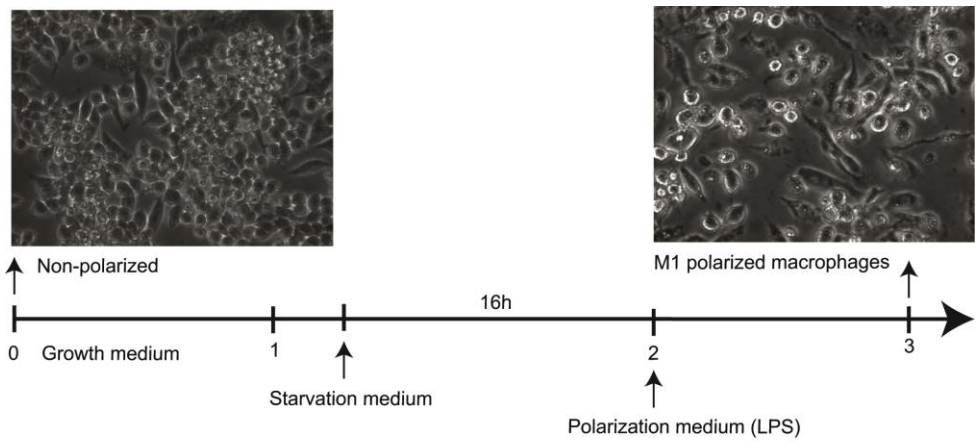


Figure 82. RAW 264.7 polarization protocol.

7.3.4. Cell media composition of secondary cell lines

Table 8. Cell culture media composition for 3T3-L1 cells.

3T3-L1 Growth media	
Reagents	**Final concentration**
DMEM 4,5 g/l Glucose; w/L-Gln; w/Sodium pyruvate	89% (v/v)
Activated Calf Serum (CS)	10% (v/v)
Penicillin-Streptomycin	100 U/ml – 1% (v/v)
3T3-L1 Differentiation media 1	
Reagents	**Final concentration**
DMEM 4,5 g/l Glucose; w/L-Gln; w/Sodium pyruvate	89% (v/v)
Activated Foetal Bovine Serum (FBS)	10% (v/v)
Penicillin-Streptomycin	100 U/ml – 1% (v/v)
Human recombinant insulin	1 μg/mL
Dexamethasone	0.25 μM
3-Isobutyl-1-methylxanthine (IBMX)	0.11 mg/mL
Rosiglitazone	1 μM
3T3-L1 Differentiation media 2	
Reagents	**Final concentration**
DMEM 4,5 g/l Glucose; w/L-Gln; w/Sodium pyruvate	89% (v/v)
Activated Foetal Bovine Serum (FBS)	10% (v/v)
Penicillin-Streptomycin	100 U/ml – 1% (v/v)
Human recombinant insulin	1 μg/mL
3T3-L1 Differentiation media 3	
Reagents	**Final concentration**
DMEM 4,5 g/l Glucose; w/L-Gln; w/Sodium pyruvate	89% (v/v)
Activated Foetal Bovine Serum (FBS)	10% (v/v)
Penicillin-Streptomycin	100 U/ml – 1% (v/v)
3T3-L1 Serum-starvation media	
Reagents	**Final concentration**
DMEM 4,5 g/l Glucose; w/L-Gln; w/Sodium pyruvate	89% (v/v)
Bovine Serum Albumin (BSA)	0.2% (w/v)
Penicillin-Streptomycin	100 U/ml – 1% (v/v)

Table 9. Cell culture media composition for RAW 264.7.

RAW 264.7 Growth media	
Reagents	**Final concentration**
DMEM 4,5 g/l Glucose; w/L-Gln; w/Sodium pyruvate	89% (v/v)
Foetal Bovine Serum (FBS)	10% (v/v)
Penicillin-Streptomycin	100 U/ml – 1% (v/v)
HEPES	0.02 M
RAW 264.7 Serum starvation media	
Reagents	**Final concentration**
DMEM 4,5 g/l Glucose; w/L-Gln; w/Sodium pyruvate	99% (v/v)
Foetal Bovine Serum (FBS)	1% (v/v)
Penicillin-Streptomycin	100 U/ml – 1% (v/v)
HEPES	0.02 M
RAW 264.7 Polarization media	
Reagents	**Final concentration**
DMEM 4,5 g/l Glucose; w/L-Gln; w/Sodium pyruvate	89% (v/v)
Bovine Serum Albumin (BSA)	0.2% (w/v)
Penicillin-Streptomycin	100 U/ml – 1% (v/v)
HEPES	1 μg/mL
LPS (to induce M1 polarization)	60 ng/mL

Table 10. Cell culture media composition for other immortalised cell lines.

HEK 293T Growth media	
Reagents	**Final concentration**
DMEM 4,5 g/l Glucose; w/L-Gln; w/Sodium pyruvate	89% (v/v)
Foetal Bovine Serum (FBS)	10% (v/v)
Penicillin-Streptomycin	100 U/ml – 1% (v/v)
HEPES	0.02 M
TEFLY-MO Growth media	
Reagents	**Final concentration**
DMEM F-12	99% (v/v)
Foetal Bovine Serum (FBS)	1% (v/v)

7.3.5. Primary cell lines

7.3.5.1. Bone marrow-derived macrophages

BMDM were generated from the bone marrow of c57BL/6 mice. Primary macrophages were isolated and maintained in media obtained from L929 helper cells. After 7 days, BMDM are considered to be differentiated and thus, they were polarised. These cells were maintained at 37ºC, 95% humidity and 3% CO_2 and were generated in collaboration with Dr Bodoy S. from IRB.

7.3.5.2. Primary immortalised preadipocytes

In this thesis, we generated primary immortalised preadipocytes as a physiological model to study the role of NRG4 and ErbB4 in the physiology of adipocytes. Primary preadipocytes were isolated from WT, *Erbb2* and *Erbb4* Flox c57BL/6 mice. Afterwards, preadipocytes were immortalised upon retroviral transduction using the MLV retroviral particles, which contain the gene sequence of the T antigen SV40. Immortalised preadipocytes were cultured in 10 cm Ø culture dish at 33 ºC, 95% humidity and 7% CO_2 with the presence of IFNγ to maintain the cells in the immortalised proliferator state. Media were changed every 2 days avoiding cell confluency.

7.3.6. Primary cell protocols

7.3.6.1. BMBD generation and polarization

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

7.3.6.2. Isolation and maintenance of primary preadipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

7.3.6.3. Immortalization of primary preadipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

7.3.6.4. Primary immortalised preadipocytes differentiation

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

7.3.7. Cell media of primary cell lines

Table 11. Cell culture media composition for primary preadipocytes.

Primary preadipocytes growth media	
Reagents	**Final concentration**
DMEM F-12	89% (v/v)
Activated Calf Serum (CS)	10% (v/v)
Biotin	16 μM
Pantothenic acid	18 μM
Ascorbic acid	100 μM
Antibiotic-antimycotic	1% (v/v)
Digestion solution for the isolation of primary preadipocytes	
Reagents	**Final concentration**
DMEM F-12	98% (v/v)
Biotin	16 μM
Pantothenic acid	18 μM
Ascorbic acid	100 μM
Antibiotic-antimycotic	2%
Collagenase type II	2 mg/mL

Table 12. Cell culture media composition for primary immortalised preadipocytes.

Primary immortalised preadipocytes growth media	
Reagents	**Final concentration**
DMEM F-12	89% (v/v)
Activated Foetal Bovine Serum (FBS)	10% (v/v)
Antibiotic-antimycotic	1% (v/v)
IFNγ	20 U/mL
Primary immortalised preadipocytes transduction media	
Reagents	**Final concentration**
TEFLY-MO conditioned media	100% (v/v)
Polybrene	2.5 μg/mL

Table 13. Differentiation media for primary immortalised white adipocytes.

Primary immortalised adipocyte differentiation media 1	
Reagents	**Final concentration**
DMEM F-12	89% (v/v)
Activated Foetal Bovine Serum (FBS)	10% (v/v)
Human recombinant insulin	1 μg/mL
IBMX	0.5 mM
Dexamethasone	0.25 μM
Rosiglitazone	2.5 μM
Penicillin-streptomycin	100 U/ml - 1% (v/v)
Primary immortalised adipocyte differentiation media 2	
Reagents	**Final concentration**
DMEM F-12	89% (v/v)
Activated Foetal Bovine Serum (FBS)	10% (v/v)
Human recombinant insulin	1 μg/mL
Penicillin-streptomycin	100 U/ml - 1% (v/v)
Primary immortalised adipocyte differentiation media 3	
Reagents	**Final concentration**
DMEM F-12	89% (v/v)
Activated Foetal Bovine Serum (FBS)	10% (v/v)
Human recombinant insulin	0.5 μg/mL
Penicillin-streptomycin	100 U/ml - 1% (v/v)

Table 14. Cell culture media composition for BMDM.

BMDM differentiation media	
Reagents	**Final concentration**
DMEM 1g glucose/L	55% (v/v)
Foetal Bovine Serum (FBS)	10% (v/v)
Penicillin-streptomycin	5% (v/v)
Conditioned media from the L929 helper cells (with MCSF)	30% (v/v)
BMDM polarization media	
Reagents	**Final concentration**
DMEM 1g glucose/L	55% (v/v)
Foetal Bovine Serum (FBS)	10% (v/v)
Penicillin-streptomycin	5% (v/v)
Conditioned media from the L929 helper cells (with MCSF)	30% (v/v)
LPS	100 ng/mL
Recombinant murine IL-4	10 ng/mL
BMDM maintenance media for non-polarised macrophages	
Reagents	**Final concentration**
DMEM 1g glucose/L	85% (v/v)
Foetal Bovine Serum (FBS)	10% (v/v)
Penicillin-streptomycin	5% (v/v)

7.3.8. Cell treatments

7.3.8.1. Treatments in 3T3-L1 adipocytes

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

7.3.8.2. Treatments in macrophages

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

7.4. Gene expression

7.4.1. Transient gene expression

7.4.1.1. Amplification of adenoviral particles

To amplify Cre-GFP and Scr-GFP containing adenoviral particles, 100 µL of cell media containing Cre-GFP and Scr adenoviruses was used to transduce HEK 293T cells. To this end, HEK 293T were growth to 90% confluency in a 10 cm Ø culture dish and then, the adenoviral containing media was added. These media were given by Dr Muñoz J. P. from IRB. After 72h of the adenoviral transduction, HEK 293T begun to detach from the culture dish. The cellular media was collected with the HEK 293T cells in suspension. Afterwards, the infected HEK 293T were lysed upon 3 cycles of freezing and thawing, thus releasing the adenoviral particles from the cell cytosol to the cellular media. Then, the obtained cellular media was spun at 1400 rpm for 5 minutes to eliminate the cellular debris. The supernatant was collected and kept at -80 ºC. Afterwards, a second adenoviral amplification was done to increase the viral title.

To this end, 400 µL of the initial amplification media was added to a new culture of HEK 293T in 15 cm Ø culture dish. The HEK 293T cells were then collected after 30h of the adenoviral transduction. The cell culture was spun and the pellet containing HEK 293T cells was kept. The infected cells were then lysed with cycles of freezing and thawing in 2 mL of growth media to concentrate the viral particles. Afterwards, this solution was spun at 1400 rpm for 5 minutes and the pellet was discarded to eliminate the cellular debris. This adenoviral solution was stored at – 80 ºC. Afterwards, the adenoviral title was determined by transducing HEK 293T with 100 µL of the obtained solutions. After 48h, GFP containing cells were quantified to determine the viral title. The obtained title of the adenoviral solutions was $6x10^{10}$ ifu/mL for Scr-GFP and $1.5x10^{10}$ for Cre-GFP.

7.4.1.2. Adenoviral transduction and preadipocyte knockout generation

Erbb2 Flox (iFlox *Erbb2*) and *Erbb4* Flox (iFlox *Erbb4*) primary immortalised preadipocytes were transduced with Scr-GFP and Cre-GFP adenoviral particles to remove the *Erbb2* and *Erbb4* gene respectively. To this end, primary immortalised preadipocytes were transduced with 300 µL of serum-free growth media containing the adenoviral particles supplemented with 0.2 % (w/v) BSA and 1% antibiotic-antimycotic. The MOI used to delete the gene expression of *Erbb2* and *Erbb4* was optimised in this study as it can be seen in Figures 45, 46. It was determined to use 250 MOI to delete the expression of these genes and preserve the adipogenic capabilities of the preadipocytes. The transduction had a length of 3h and it was done at 37 ºC. Afterwards, the transduction media was replaced with primary immortalised preadipocyte growth media for 72h. Finally, the transduced KO primary immortalised preadipocytes could be propagated, frozen or differentiated into adipocytes.

7.4.2. Stable gene expression

7.4.2.1. Obtention of transformed bacteria

Transformed *Escherichia coli* containing shRNA and Scr sequences to generate lentiviral particles were obtained from the Functional Genomics facility of the IRB. All bacteria contained pLKO.1-Puro with different shRNA or Scr sequences against specific target genes. The pLKO.1-Puro plasmids were purchased from Mission® RNA_i (Sigma-Aldrich Inc). In this thesis, the following shRNA sequences against *Nrg4*, *Erbb2* and *Erbb4* have been used (Table 15).

Table 15. ShRNA sequences used in this study.

Nrg4	
ShRNA target sequence	**Clone number**
GCCTGGTAGAGACAAACAATA	15
CAGCATCCCAAGCGAAAGTAA	16
Erbb4	
ShRNA target sequence	**Clone number**
CCAGAACAAATTCCTATGTTA	1
GCTTTGACATTTGCTGTCTAT	2
GCAACCTGTGTTATTACCATA	3
Erbb2	
ShRNA target sequence	**Clone number**
CCCACTACAAGGACTCATCTT	1
GCTGGCTGCAAGAAGATCTTT	2
CGTCCTGTTGTTCCTGATCAT	3
CCTCCGTAAGAATAACCAGCT	4
Scr	
Scr sequence	**Clone name**
CCAACCCAACCACAAAGTCAT	Scr

7.4.2.2. Bacterial growth

To obtain the shRNA vectors from the transformed bacteria, midipreps were performed. To this end, bacteria were grown in 3 mL of LB broth with ampicillin at 37 ºC under shacking (225 rpm) for 7-8h to create the starter culture. Afterwards, 500 µL of the starter culture was inoculated to 50 mL of LB broth with the selection antibiotic in sterile Erlenmeyer flasks. Then, the Erlenmeyer flasks were placed into a shaker and were incubated at 225 rpm and 37 ºC for 15-16h. After the bacterial growth, the cultures were ready for plasmid purification via midiprep.

7.4.2.3. Plasmid purification

Plasmid purification of pLKO.1-Puro containing bacteria was done using the PureLink® HiPure Plasmid Midiprep Kit from Invitrogen™ following the manufacturer's instructions except for the elution step. In that step, DNase-free water was used instead of the kit's elution buffer. Afterwards, DNA was quantified using the Nanodrop™ spectrophotometer 2000/2000c (Thermo Scientific™) with the ND1000 software (Thermo Scienfitic™). The lentiviral packaging plasmids pCMV-dR8.2 and pMD2.G were provided by Dr Trono D. (*École Polytechnique Federal de Lausanne*, Switzerland).

7.4.2.4. 3T3-L1 stable knockdown generation

ShRNA encoding sequences were delivered to 3T3-L1 preadipocytes by lentiviral transduction (*Muñoz et al., 2015*). Thus, the stable expression of shRNA against the *Nrg4*, *Erbb4* and *Erbb2* mRNA generated stable KD cells. In parallel to the generation of KD cells, 3T3-L1 expressing Scr shRNA were generated in all the experiments. Lentiviral particles were generated by the transfection of the packaging plasmids pMD2.G (3 μg)., pCMV-dR8.2 (7 μg) and the target shRNA pLKO.1-puro or Scr lentiviral constructs (10 μg) into HEK 293T at 70% confluence. These plasmids were mixed in 1560 μL of filtered 150 mM NaCl solution. The mixture was equilibrated for 5 minutes. Afterwards, 78 μL of 1 mg/mL PEI (pH=7, filtered) was added to the mixture and incubated for 20 minutes at RT to allow the formation of DNA-PEI complexes. Then, this solution was added to the growth media of HEK 293T cells without antibiotics. After 6h of PEI transfection, the growth media was changed for fresh growth media with antibiotics. Transfected cells were then placed in a 33 ºC incubator to improve lentiviral particle stability and production. Lentiviral production was allowed for 48h after the transfection. After 24h of the initial transfection, 3T3-L1 preadipocytes were plated at a low confluence (200.000 cells per 10 cm Ø culture dish). In addition, 1 extra plate was always plated and not transduced with lentiviral particles as a control. Afterwards, 2 subsequent lentiviral transductions were performed. To this end, the media from the transfected HEK 29T cells was retrieved and filtered with a 0.45 μm filter. Then, 2.5 μg/mL polybrene was added to the media to facilitate the lentiviral transduction. Finally, the infection medium was added to 3T3-L1 adipocytes at 30% confluence. These steps were repeated an additional time with new fresh transduction media from HEK 293T. Finally, 48h after the second transduction, the transduced 3T3-L1 adipocytes were split and selected with 2.5 μg/mL puromycin. The puromycin treatment was maintained in the growth media of silenced and Scr adipocytes and was removed before the induction of adipocyte differentiation. The protocol of stable KD generation is depicted in Figure 10.

7.5. Animal studies

7.5.1. Animal models and animal care

The inbred c57BL/6 strain of *Mus musculus* was used in this study. The control WT and Cre mice were previously bought from *Harlan Ibérica S.A.* The muscle-specific *Erbb2* and *Erbb4* floxed mice kindly provided by Dr Rudolph Brener H. from Pharmazentrum of the University of Basel, Sweden. All animals were maintained in the Animal Facility of the Faculty of Biology from UB in an environmentally controlled room at 22 ºC and with 12h of light/dark cycle. The animals were supplied *ad libitum* with 10% kcal fat rodent diet (#D12450B, Research Diets Inc.) and water. HFD mice were fed *ad libitum* with 60% kcal fat rodent diet (#D12492, Research Diets Inc.) and water. All animal procedures were performed with the approval of the ethical committee of the UB.

7.5.2. Generation of muscle-specific Erbb2 and Erbb3 knockout mice

The muscle-specific *Erbb2* and *Erbb4* floxed mice had these genes flanked with LoxP sequences. Specifically, the exon 1 of *Erbb2* and exon 2 of *Erbb4* are flanked with these sequences. Thus, upon the activity of the recombinase Cre, these genomic sequences can be selectively removed. Cre animals expressed the Cre recombinase of the P1 bacteriophage, which expression was controlled by the human skeletal actin (HSA). Thus, *Cre* gene was only expressed in the skeletal muscle of these mice. In this study, we analysed the impact of the absence of *Erbb4* expression in skeletal muscle from these mice. To generate muscle-specific *Erbb4* gene-deleted mice, Cre animals were crossed with *Erbb4* floxed mice. Therefore, we defined 3 different experimental groups: WT mice ($Erbb4^{+/+}$, $Cre^{-/-}$), the genome of which was not modified; *Erbb4* Flox mice ($Erbb4^{flox/flox}$), which *Erbb4* gene was flanked with LoxP regions and muscle-specific *Erbb4* KO mice ($Erbb4^{flox/flox}$, $Cre^{+/-}$). Thus, upon the expression of *Cre* under the HAS promotor, the gene sequence of the exon 2 of *Erbb4* is removed in the skeletal muscle of the *Erbb4* KO mice. This truncated form of the *Erbb4* gene does not encode for a functional product.

7.5.3. Glucose tolerance test analysis

GTT analyses were performed in fasted mice. To this end, animals were placed in special cages with *ad libitum* access to water without food for 14h. Then, 20% (w/v) glucose solution in saline (0.9% NaCl) was prepared. HRG was also diluted in saline (445 µg/mL HRG) and stored at 4 ºC for a maximum of 2 days. Before the experiment, animals were weighed and the fasted glycaemia was determined. At time point 0, HRG (50 ng HRG/g of body weight) or saline was administrated intraperitoneally. After 15 minutes, the animals were intraperitoneally injected with glucose (2 g glucose/Kg of body weight).

Blood samples were collected from the tail of these animals at different times (0, 7.5, 15, 30, 60, 120 minutes) for the glucose measurements using a glucometer. After the last measurement, the animals were permitted access to food again. The obtained blood samples were then centrifuged at 3000g for 20 minutes at 4 ºC. Then, the plasma was collected and frozen at -20 ºC.

7.5.4. Insulin tolerance test analysis

ITT analyses were performed in fed animals to avoid the acute induction of hypoglycaemia upon insulin challenge in mice. The insulin solution was prepared with insulin diluted in 5 mM HCL solution. Before the experiment, animals were weighed and the fed glycaemia was measured. Afterwards, 0.5 UI - 1UI insulin/Kg body weight was administered for animals fed with ND and HFD respectively. Then, blood samples were collected from the tail at different times (0, 7.5, 15, 30, 60, 120 minutes) and the glucose levels were measured. The animals were kept isolated in individual cages, covered from light to reduce the stress levels since catecholamines promote glucose mobilization and thus, they increase the blood glucose levels.

7.5.5. Mice Anaesthesia and tissue dissection

All mice were dissected in the Animal Facility of the Faculty of Biology from UB. To this end, anaesthesia was induced via isoflurane inhalation. Animals were placed in an isolated chamber saturated with isoflurane. Upon the loss of consciousness of the animals, they were placed in a horizontal surface with isoflurane influx to keep the animals under the effects of the anaesthesia during the dissection. Thus, animals were alive during the dissection process to preserve tissue homeostasis as much as possible. Upon the absence of animal reflexes, the dissection was initiated. The following tissues and organs were extracted: WAT pads, BAT, pancreas, liver, heart, brain, kidneys among others. Regarding skeletal muscle, gastrocnemius, soleus, Extensor Digitorum Longus muscle (EDL) and quadriceps were extracted. All the obtained tissues were weighted. Specifically, the liver, gastrocnemius and gonadal WAT was used to obtain RNA and protein extracts. The dissected tissues were stored at -80 ºC.

7.5.6. White adipose tissue fractionation

3 mice, 2 males and 1 female, were anesthetised with isofluorane to extract the peri-gonadal WAT pads. VA and VS fractions from visceral WAT were isolated by mechanical lysis with scissors followed by enzymatic digestion with type 2 collagenase. The obtained homogenates were then centrifuged at 1600 rpm for 10 minutes. The supernatants contained the adipocyte-enriched fractions, while the pellets contained the stromal fractions. Then, the RNA was extracted from the generated fractions as described in section 8.7.3.

7.5.7. Yoshida ascites hepatoma cells inoculation in Fischer rats

Here, in collaboration with Dr Busquets S. from the Biochemistry and Molecular Biomedicine from the UB, we intraperitoneally inoculated Yoshida ascites hepatoma cells (AH-130) in Fischer rats to generate a cachexic-like animal model. For the tumour inoculation, AH-130 cells were collected from the peritoneal liquid of a donor bearing-tumour rat though peritoneal aspiration with a syringe after 7 days of exponential tumour growth. Then, the cells were counted. Afterwards, the hepatoma cells were concentrated to $50 \cdot 10^6$ cells/mL in PBS. Next, 2 mL of the hepatoma cell dilution was intraperitoneally inoculated to other rats. 7 days after the tumour transplantation, the inoculated rats were weighted and anaesthetized with a ketamine/xylazine mixture (100 mg/Kg Imalgene® (Merila) and 10 mg/Kg Rompun® (Bayer)). The ascitic tumour was then harvested from the intraperitoneal cavity. Then, the gonadal WAT of these rats was extracted to obtain RNA extracts as it is described in section 8.7.3.

7.6. Molecular biology protein methods

7.6.1. Protein extraction from cell cultures

Total protein extracts were prepared from 3T3-L1 cells, primary immortalised preadipocytes and RAW 264.7 macrophages. Cells were placed on ice and were washed twice with ice-cold PBS. Then, the cells were scraped into cell lysis buffer (Table 6) supplemented with phosphatase inhibitor and protease inhibitor cocktails. The lysates were homogenised with a syringe and centrifuged at 13200 rpm for 30 min at 4 ^{0}C. Then, for the lysates from differentiated adipocytes, the lipids were removed from the supernatant to avoid the overestimation of the protein content. Afterwards, the supernatants were collected and stored at -80 ºC.

7.6.2. Membrane total extracts from cell cultures

Total membranes from differentiated 3T3-L1 adipocytes were extracted. Briefly, cells were cultured in 10 cm Ø culture dishes. At day 7 of differentiation, the culture dishes were placed on ice and washed twice with HES lysis buffer at 4 ºC. Afterwards, 1 mL of HES supplemented with proteases inhibitor was added into the cells. The cells were then scraped and homogenised with a syringe. Afterwards, the cells lysates were spun at 5000 rpm at 4ºC for 5 minutes. The lipids were removed from the supernatant and the rest was added to ultracentrifuge tubes of 1.5 mL capacity. The lysates were spun at 200000g in a TLA55 rotor for 75 minutes at 4ºC. The obtained pellets were then resuspended in 300 µL of HEPES 20 mM. Finally, the pellets were homogenised by pipetting and sonification at 4 ºC (3 pulses of 10'' with intervals of 10'').

7.6.3. Protein extraction from tissues

Protein extracts from gonadal WAT, gastrocnemius and liver were obtained. For gastrocnemius extracts, 50 mg of tissue was lysate in 1 mL of cell lysis buffer (Table 6) supplemented with phosphatase inhibitor and protease inhibitor cocktails. For WAT extracts, 250 mg of tissue was lysate in 500 µL of cell lysis buffer. For liver extracts, 50 mg of tissue was lysate in 1 mL of cell lysis buffer. Tissues were homogenised using a Polytron 3 times for 15'' at 4 ºC. Then, lysates were incubated 1h at 4ºC on a tube rotator. Finally, the samples were centrifuged at 13200 rpm for 30 minutes at 4 ºC. For western blot analyses, lipids were removed from the supernatant. Then, the supernatant was transferred into new Eppendorf tubes® and stored at 80 ºC.

7.6.4. Protein quantification

Protein quantification was performed using the Pierce™ BCA™ Protein assay kit from Thermo Scientific™. To this end, a standard curve was generated between 0.5-16 mg/mL using a 2 mg/mL BSA solution from the kit. After that, protein extracts were loaded in a 96 well-plate and 200 µL of the reaction mixture (A:B solutions in a 50:1 ration) were added to each well using a multichannel pipette. Then, the loaded plate was incubated 30 minutes at 37 ºC. Absorbance was read at 560 nm using the spectrophotometer Benchmark Plus™ (Bio-Rad Inc) and the Microplate Manager® software (Bio-Rad Inc). The protein concentration of the extracts was calculated by interpolating the absorbance values with the standard curve.

7.6.5. Western blot analysis

Protein homogenates were resolved in 8%, 10% or 12.5% acrylamide gels depending on the weight of the proteins to resolve. Protein samples were solubilised in 4x Laemmly Sample Buffer (LSB) with fresh 2-mercaptoethanol as a reducing agent. Then, protein samples were heated at 95 ºC for 5 minutes to linearise proteins. For the detection of phosphorylated proteins, protein samples were heated for 95 ºC for 2 minutes instead. Electrophoresis was run in reducing and denaturalizing conditions for a total of 90 minutes. Before the stacking of the proteins, electrophoresis was set at 80 V and then it was increased to 120 V. The samples were run together with the molecular weight marker HyperPAGE Prestained Protein Marker. Afterwards, the proteins were blotted to PVDF membranes via wet blotting system. The protein was run for 120 minutes at 250 mA with the presence of ice blocks in the transfer container. The ice blocks were changed every 60 minutes. Alternatively, for heavy proteins such as mTOR, protein transfer was run overnight at 40 V. Then, the membranes were blocked with 5% (w/v) non-fat dry milk for non-phosphorylated proteins or 3% (w/v) BSA for phosphorylated proteins in 0.1% (v/v) Tween®-20 Tris-buffered saline solution (TBST) for 1 h at RT.

Afterwards, the membranes were incubated overnight at 4 °C with different antibodies (table 2). Primary antibodies were diluted in 3% (w/v) in TBST supplemented with 3% (w/v) BSA and 0.02% (w/v) sodium azide. Then, the membranes were washed 3 times with TBST for 5 minutes. Afterwards, the membranes were incubated with secondary antibodies. Horseradish peroxidase (HRP) conjugated anti-mouse or anti-rabbit secondary antibodies were diluted 1/20000 in 5% (w/v) non-fat dry milk in TBST. After the secondary antibody incubation, membranes were washed 3 times with TBST for 5 minutes. Finally, the membranes were incubated with the western blotting detection reagent Enhanced Chemiluminescence solution to detect specific protein bands. ImageJ software from the National Institute of Mental Health (NIH) was used to quantify the autoradiograms. The composition of the used solutions and gels can be consulted in tables 5, 6.

7.7. Molecular biology RNA and DNA methods

7.7.1. RNA extraction from cell cultures

RNA was extracted from 3T3-L1 adipocytes, RAW 264.7 macrophages, primary immortalised white adipocytes and BMDM macrophages. Cell plates were put on ice and rinsed twice with PBS at 4 ºC before RNA extraction. Then, total RNA was extracted with PureLink™ RNA Mini Kit columns from Invitrogen™. Briefly, for $<5 \cdot 10^6$ cell suspension, 0.3 mL lysis buffer was added supplemented with 10 µL of 2-mercaptoethanol for each 1 mL of lysis buffer. Samples were vortexed until the cell pellet was dispersed and the cells appeared lysed. Afterwards, the cells were completely lysate by passing the lysate 5-10 times though an 18 to 21-gauge syringe needle. Then, up to 700 µL of the cell lysate was transferred to the spin cartridge tubes inserted in collection tubes. The cartridges were spun at 12000g for 30'' at RT and the flow-through volume was discarded. Then, the rest of the lysate volume was loaded and the cartridge tubes were spun again. After the sample loading, the cartridge tubes were washed with 700 µL of Wash Buffer I and spun 30'' at 12000g at RT. Then, the flow-through was discarded again and the samples were incubated 15 minutes with 80 µL of a DNase solution consisting of 8 µL of Pure Link 10x DNase Buffer, 10 µL of DNase stock solution and 62 µL of RNase-free water. After the DNase incubation, the cartridge tubes were washed with Wash Buffer II supplemented with ethanol. The cartridges were spun at 12000g for 30'' at RT and the flow-through was discarded. Then, the cartridges were washed again with Sample Buffer II. The cartridges were spun at 12000g for 2 minutes at RT to dry the membrane with bound RNA. The collection tubes were discarded and the recovery tubes were inserted in the spin cartridges. Finally, 20 µL of RNase-free water at 70 ºC was added to the spin cartridge tubes and were spun at 12000g at RT for 2 minutes to elute the RNA from the membranes into the recovery tubes. The purified RNA was quantified and stored at -80 ºC.

7.7.2. RNA extraction from tissues

RNA from the liver, gonadal WAT and gastrocnemius was extracted. For RNA liver extracts, 50 mg of tissue was lysed in 500 µL of TRIzol™. For RNA gonadal WAT extracts, 100 mg of tissue was lysed in 500 µL of TRIzol™. For RNA gastrocnemius extracts, 75 mg of tissue was lysed in 500 µL of TRIzol™. Briefly, frozen tissues were put in a tube containing the correct amount of TRIzol™. Then, the tissues were homogenised using a Polytron 3 times for 15'' at 4 ºC. The homogenised content was then transferred to a new Eppendorf tube® and was spun at 12000g for 1 minute. The lipids were discarded and the supernatant was transferred to a new Eppendorf. The lysates were incubated 5 minutes at RT. Afterwards, chloroform was added to the lysates (100 µL per each 500 µL of TRIzol™). The mixtures were vortexed 15'' and incubated 10 minutes at RT. After that, the Eppendorf tubes® were covered with parafilm and spun at 12000g for 15 minutes at 4 ºC. Then, the 2 phases were separated and the supernatants were transferred to new Eppendorf tubes®. Finally, the RNA was extracted from the supernatants using the PureLink™ RNA Mini Kit columns from Invitrogen™ as described in section 8.7.1.

7.7.3. RNA extraction from adipocyte and stromal white adipose tissue fractions

WAT homogenates were centrifuged at 1600 rpm for 10 min. The pellet contained the stromal fraction, while the lipid phase over the supernatant contained the adipocyte-enriched fraction. Both fractions were homogenised in lysis buffer from the PureLink™ RNA Mini Kit columns from Invitrogen™ with a syringe. Finally, total RNA was extracted as described in section 8.7.1.

7.7.4. RNA quantification

RNA samples were quantified using the Nanodrop™ 2000/2000c spectrometer (Thermo Scientific™) and the ND1000 software (Thermo Scientific™). The RNA purity was determined by the A_{260}/A_{280} and A_{260}/A_{230} absorbance ratio. An A_{260}/A_{280} ratio of 2.0 is accepted for pure RNA. If this ratio is lower could indicate sample contamination with DNA, proteins, phenol or other contaminants that absorb near 280 nm. Besides that, pure RNA should have a higher A_{260}/A_{280} ratio than A_{260}/A_{230} ratio. Lower ratios can be caused by EDTA, TRIzol™ or carbohydrate contamination.

7.7.5. Reverse transcription

RNA samples were diluted to 200 ng/µL in RNase-free water in a final volume of 10 µL in PCR tubes. Afterwards, samples were mixed with 1 µL/sample deoxynucleotides (10 mM stock) and 1 µL/sample oligodTs (500 µg/mL stock) to a final volume of 12 µL. The samples were heated to 65 ºC for 5 min using the 2720™ Thermal Cycler (Applied Biosystems) prior to the reverse-transcription to linearise the RNA molecules. In parallel, the SuperScript™ solution was prepared by mixing 4 µL of 5X First-Strand Buffer, 2 µL of RNAseOUT™ (40 U/µL stock) and 1 µL of SuperScript™ II reverse transcriptase (200 U/µL stock) per each sample. After the heating, 8 µL of the SuperScript™ solution was added to each sample to a final volume of 20 µL. Reverse-Transcription Polymerase Chain Reaction (RT-PCR) was set for 53 min at 42 ºC to allow cDNA polymerization. Then, the samples were heated for 15 min at 70 ºC to stop the reaction. Finally, the RNA samples were cooled down at 4 ºC to ensure cDNA preservation. The obtained cDNA was diluted to 2.5 ng/µL in Milli-Q H2O and stored at -20 ºC.

7.7.6. Genomic DNA extraction and quantification

Nuclear DNA and mtDNA was extracted from 3T3-L1 adipocytes using the DNeasy® Blood & Tissue Kit columns from Qiagen following the manufacturer's instructions. Briefly, cell suspensions of maximum $5 \cdot 10^6$ cells were spun at 300g and resuspended in 200 µL of PBS. Afterwards, 20 µL of proteinase K was added to the resuspended cell suspensions to degrade all the protein content. Then, 200 µL of Buffer AL was added and the samples were incubated at 56 ºC for 10 minutes. Then, 200 µL of 100% (v/v) ethanol was added and the samples were vortexed. Then, the samples were loaded into the DNeasy Mini spin columns placed in 2 mL collection tubes. The spin columns were then spun at 6000g for 1 minute and the flow-through was discarded along with the collection tube. The spin columns were placed in new 2 mL collection tubes and 500 µL of Buffer AW1 was loaded in each tube. The spin columns were then spun at 20000g for 3 minutes, the flow-through and the collection tubes were discarded. After the washing, the spin columns were transferred to new 1.5 mL Eppendorf tubes®. Finally, to elute the DNA from the columns, 100 µL of Buffer AE was added to each column. The columns were incubated 1 minute at RT and then spun at 6000g for 1 minute. Afterwards, the DNA samples were quantified using the Nanodrop™ 2000/2000c spectrometer (Thermo Scientific™) and the ND1000 software (Thermo Scientific™). The DNA purity was determined by measuring the A_{260}/A_{280} and A_{260}/A_{230} absorbance ratio. A_{260}/A_{280} ratio of 1.85 is accepted for pure DNA. DNA samples were diluted to 0.25 ng/µL in Milli-Q water and stored at -20 ºC.

7.7.7. Quantitative polymerase chain reaction

The SYBR® Green Master Mix was prepared by mixing 5 µL of SYBR® Green PCR Master Mix, 0.6 µL of 10 µM primer mix and 0.4 µL of Milli-Q H2O per each sample. Forward and reverse primer sequences were designed using the Primer-BLAST tool (National Centre for Biotechnology Information (NCBI)) and were purchased from Sigma-Aldrich Inc. The reaction volume was set to 10 µL per sample; 4 µL of 2.5 ng/µL cDNA and 6 µL of the SYBR Green Master Mix. QPCR negative controls were loaded to identify cross-contamination and 2 technical replicates were analysed per sample. QPCR analyses were performed on the ABI Prism 7900 HT platform (Applied Biosystems™) using the SDS software (Applied Biosystems™). This service was provided by the Genomics Facility from the Scientific and Technological Centre of the UB (CCiTUB). Gene expression measurements were normalised to acidic-ribosomal protein (*Arp*) gene expression using the $2^{-\Delta\Delta Ct}$ method. Besides that, relative mtDNA copy number analyses were performed as described above using 4 µL of 0.25 ng/µL DNA and specific primers for different mtDNA regions. MtDNA copy number data were normalised to telomerase reverse transcriptase (*Tert*) DNA content using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are listed in table 3. The qPCR was set as showed in table 16.

Table 16. Thermal cycling parameters of the thermocycler to run the qPCR with the SYBR® PCR Master Mix. A dissociation stage was always performed to rest assure that any non-specific amplicons were replicating.

Step	AmpliTaq Gold® Polymerase Activation	PCR (x40 cycles)		Dissociation Stage
		Denature	Anneal/Extend	
Temperature	95 ºC	95 ºC	60 ºC	60-95 ºC
Time	10’	15’’	1’	15’’ at 95 ºC

7.8. Metabolic analyses

7.8.1. Determination of lipolysis from the release of glycerol

The glycerol released into cell culture media was analysed to determine the basal lipolytic rates in 3T3-L1 adipocytes. This method is based in the following reactions:

Glycerol kinase (GK): Glycerol + ATP ➔ Glycerol-3-P + ADP

Glycerol dehydrogenase (GDH): Glycerol-3-P + NAD^+ ➔ Dihydroxyacetone-P + NADH + H^+

Briefly, cells were incubated with 1 mL of differentiated cell media 3 between day 6 to day 7 of differentiation. The next day, these cell media were retrieved to determine the release of glycerol during 24h. The obtained cell media were then deproteinised by adding 70 µL of $HCLO_4$ per 1 mL of media. Afterwards, the cell media were vortexed and incubated 5 minutes at RT. Then, we collected the supernatants and transferred them to new Eppendorf tubes®. The deproteinised cell media were stored at -20 ºC. To analyse the release of glycerol, we first performed the GDH reaction to convert all the glycerol to dihydroxyacetone-P and NADH. Then, we performed the GK reaction to convert all the glycerol in the presence of ATP to glycerol-3-P, which is then converted to dihydroxyacetone-P and NADPH by GDH. To this end, 250 µL of the standard curve (10 mM glycerol) and samples were loaded into semi-micro cuvettes. Then, 1 mL of the buffer consisting of 36 mL buffer GHB, 2 mL of 50 mM ATP, 2 mL of 20 mM NAD^+ and 80 µL of 1700 U/mL GDH was added to each cuvette. Cuvettes were then mixed by inversion and the absorbance was read at 340 nm (OD1, the absorbance of NADH that comes from the glycerol-3-P released from glycolysis). Then, 13 µL of diluted the GK solution was added. The GK solution was prepared by diluting 88 µL of 114 U/mL GK into 512 µL of Milli-Q water. Once the GK was added, the cuvettes were mixed by inversion and the absorbance was read again at 340 nm (OD2, the absorbance of which comes from the previously generated NADH and the new NADH generated from the glycerol released during the lipolysis). Hence, to calculate the concentration of the released glycerol we first determined the increase in absorbance in each sample (ΔOD=(OD2-OD1). With these values, a standard curve was generated to interpolate the nmols of glycerol released from the ΔOD of each sample. Since the glycerol content was determined using 250 µL of media from the 1 mL of total media, the obtained values were multiplied by 4 to determine the release of glycerol per well. Finally, the protein content per well was also determined as described in section 8.6.4. Thus, final data was showed as µmols of glycerol/mg of protein.

7.8.2. *De novo* lipogenesis determination in 3T3-L1 cells

In this study, *de novo* lipogenic rates were determined in 3T3-L1 adipocytes under the presence or the absence of insulin. Specifically, we measured the amount of glucose incorporated into lipids in 3T3-L1 adipocytes for 90 minutes by the incubation of radioactive metabolizable glucose. To this end, adipocytes were differentiated in 6-well plates. These adipocytes were serum-starved. To this end, adipocytes were rinsed 3 times with PBS to eliminate the leftover FBS. Then, the cells were incubated for 2h with 2 mL of the serum starvation media. After serum starvation, the adipocytes were rinsed 3 times with Krebs-Ringer-HEPES Buffer (KRHB). Afterwards, 965 µL of KRHB supplemented with 0.2% (w/v) BSA was added to each well. Then, 10 µL of insulin was added to the desired wells to a final concentration of 100 nM. In addition, 25 µL of 20 µCi/µL C^{14}glucose was also added to all wells to a final concentration of 0.5 µCi/µL. The adipocytes were incubated in the presence of radioactive glucose for 90 minutes and after that, lipogenesis was stopped by placing the cell plates on ice and removing the incubation media. Next, 2 washes with ice-cold PBS were performed to stop cellular metabolism. Afterwards, the cell plates were dried and placed at -80 ºC for 5 minutes to lysate them. Then, lipid extraction was performed by adding 2 mL of the DOLE reactive per well. DOLE reagent consists of 40 mL of isopropanol, 10 mL of heptane and 1 mL of H_2SO_4. In this step, the plates were sealed with parafilm and were placed in a shaker for 2h. After this time, the supernatants were recovered and transferred to new 15 mL Falcon® tubes containing 800 µL of water and 1.2 mL of heptane. This was done in a gas extraction hood. This mixture allows the separation of the organic lipid-containing fraction with the aqueous fraction. The tubes were vortexed and then, incubated for 5 minutes at RT. Finally, 800 µL from the organic phase was taken from each tube and transferred to scintillation tubes. In parallel, to analyse the amount of total radioactivity, 100 µL of the radioactive solution was placed in scintillation tubes (total dpm). Then, 3 mL of the scintillation liquid Ecolite was added in each tube to determine radioactivity using the β-counter Tri-Carb 2100TR from Packard Instrument Company. Thus, to calculate the nmols of glucose incorporated into lipids in each well, the following mathematical expression was used:

$$\frac{dpm\ vial\ x\ Organic\ phase\ volume\ (1.6\ mL) x\ nmols\ of\ glucose\ (5.56\ nmols)}{Total\ dpm\ x\ Measured\ volume\ (0.8\ mL)}$$

7.8.3. Triacylglyceride determination

The TAG content of 3T3-L1 adipocytes, and muscle and liver extracts from mice were determined in this study. Specifically, the TAG content of cellular and tissue lysates was determined before the removal of the lipid phase. To this end, the TAG content was determined using the Enzymatic-spectrophotometric Glycerol Phosphate Oxidase/Peroxidase kit from BioSystems. Briefly, the kit's reagents convert the present TAGs into quinonimine, the absorbance of which can be measured at 540 nm through spectrophotometry. Specifically, TAGs are converted into quinonimine through the following reactions:

$$Lipase: TAGs + H_2O \rightarrow Glycerol + FFAs$$

$$GK: Glycerol + ATP \rightarrow Glycerol\text{-}3\text{-}P + ADP$$

$$Glycerol\text{-}3\text{-}P\ oxidase\ (GPO): Glycerol\text{-}3\text{-}P + O_2 \rightarrow Dihydroxyacetone\text{-}P + H_2O_2$$

$$Peroxidase\ (POD): H_2O_2 + 4AAP + ESPA \rightarrow Quinonimine\ (Absorbance\ at\ 540\ nm) + H_2O$$

To perform this reaction, first of all, 15 µL of each homogenate were placed into a 96-well plate in triplicates. In addition, 5 µL of the standard solution with 10 µL of the cell lysis buffer and 15 µL of the cell lysis buffer (blank) were also placed in triplicates. Then, 200 µL of the kit's reactive were added in each well. The samples were then incubated 10 minutes at 37 ºC. Afterwards, absorbance was read at 540 nm using the spectrophotometer Benchmark Plus™ (Bio-Rad Inc) and the Microplate Manager® software (Bio-Rad Inc). Thus, the absorbance of each sample and the standard were calculated by subtracting the absorbance of the blank to each other absorbance value. Protein concentration was also analysed as described in section 8.6.4. To obtain the final data in nmols of TAG/mg of protein, these measurements were normalised by the amount of protein with the following mathematical expression:

$$\frac{\Delta Abs\ sample\ x\ nmols\ TAG\ (11.3)\ x\ Total\ lysate\ volume\ x\ 1000\ (to\ have\ mg\ of\ protein)}{\Delta Abs\ standard\ x\ Measured\ volume\ (15\ \mu L)\ x\ ng\ of\ protein\ measured\ in\ 20\ \mu L\ x\ 40}$$

7.8.4. Measurement of 2-Deoxyglucose uptake

To analyse the ability to uptake glucose in 3T3-L1 adipocytes and primary immortalised white adipocytes, we analysed the uptake of the non-metabolizable analogue of glucose 2DG. To this end, cells were seeded and differentiated in 6-well plates. At day 6 of differentiation, the adipocytes were serum-starved with serum-starvation media for 16h. Afterwards, at day 7 of differentiation, the adipocytes were rinsed 3 times with KRHB. Then, 900 µL of warm KRHB supplemented with 0.2% (w/v) BSA was added to the cells. At this point, we added insulin to the desired wells and incubate the cells for 30 minutes at 37 ºC. To analyse the glucose uptake, the cells were incubated with different insulin concentrations (0.1, 1, 10 and 100 nM from a 10 µM stock of insulin). To begin the 2DG uptake, 100 µL of the radioactive solution was added to each well. For 1 6-well plate, the radioactive solution was prepared with 150 µL of non-radioactive 1 mM 2DG, 600 µL of 0.2% (w/v) BSA in KRHB and 6 µL of 10 µCi/mL 2-deoxy-D-[2,6-^{3}H]glucose. The adipocytes were incubated 5 minutes with the radioactive solution at 37 ºC. After that, the 2DG uptake was stopped by adding 5 mL per well of the STOP buffer at 4 ºC. The adipocytes were also placed on ice. The STOP buffer was constituted of 50 mM of D-glucose in PBS. After that, the cells were rinsed 3 times with STOP buffer to eliminate the non-incorporated radioactivity into the adipocytes. Then, 800 µL of lysis buffer was added to each well. The lysis buffer was constituted of 0.1 N NaOH and 0.1% (w/v) SDS. The cells were lysed for 1h at RT in agitation and in the presence of the lysis buffer. Then, to analyse the radioactivity incorporate into the adipocytes, 250 µL of the cellular homogenates were placed in scintillation tubes with 3 mL of the scintillation liquid Ecolite. In parallel, to analyse the amount of total radioactivity, 100 µL of the radioactive solution was placed in scintillation tubes (total dpm). The amount of radioactivity was determined using the β-counter Tri-Carb 2100TR from Packard Instrument Company. Afterwards, we analysed the amount of protein in 20 µL of the cellular homogenates as described in section 8.6.4. to normalise radioactive data. To represent the final data as nmols of 2DG/mg of protein in 5 minutes of transport, the following mathematical expression was used:

$$\frac{dpm\ vial\ x\ Total\ volume\ (800\ \mu L) x\ nmols\ 2DG\ (100)\ x\ 1000\ (to\ have\ mg\ of\ protein)}{Total\ dpm\ x\ 250\ \mu L\ (Measured\ volume) x\ \mu g\ of\ protein\ measured\ in\ 20\ \mu L\ x\ 40}$$

7.8.5. Determination of glucose oxidation in 3T3-L1 adipocytes

The oxidation of radioactive glucose was determined by measuring the levels of radioactive CO_2 produced by differentiated 3T3-L1 adipocytes. Briefly, adipocytes were seeded and differentiated in 6-well plates. The cells were serum-starved with the serum-starvation medium for 2h. Afterwards, the cells were rinsed twice with KRBH. Then, we incubated the adipocytes with 500 µL of KRHB supplemented with 1% (w/v) BSA for 30 minutes. At this point, we also added 10 µL of insulin to the desired wells to a final concentration of 100 nM. In parallel, the radioactive solution was also prepared. This solution consisted of 400 µL of KRHB with 2.5 mM glucose, 50 µL of 1.6 µCi/µL C^{14}glucose (final concentration of 0.2 µCi/µL C^{14}glucose) and 50 µL of 10% (w/v) BSA. After the 30 minutes incubation, 500 µL of the glucose radioactive solution was added per each well. For the blank, KRHB was added instead of the radioactive solution. In addition, one well remained empty as a control to analyse the leak of CO_2 between wells. To analyse the production of CO_2 during the radioactive incubation, the plate was mounted as depicted in Figure 83.

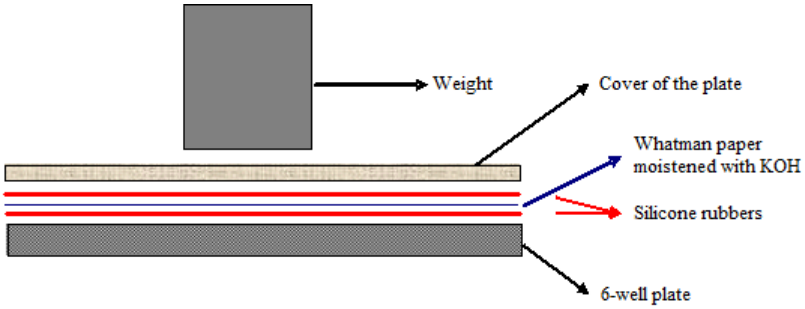


Figure 83. Schematic representation of the system to detect glucose oxidation in 6-well plates. The radioactive glucose solution was added in each well. The system was mounted in the following order: first, silicone rubber or parafilm with a small hole in the zone that covers the wells; second, pieces of Whatman paper wet with 0.1 N KOH; third, silicone rubber or parafilm without holes to seal the system, and some weight over the cover to avoid the leakage of radioactive CO_2.

Thus, the cells were incubated in these systems for 3h at 37 ºC in a stove without CO_2 supply. Afterwards, the reaction was stopped by injecting $HCLO_4$ with a syringe through the parafilm. The systems were covered again with a parafilm sheet. We allow the capture of CO_2 for 1h. Afterwards, the Whatman® filter papers were removed and placed in vials with 5 mL of the scintillation liquid EcoLite. The radioactivity was analysed in the β-counter Tri-Carb 2100TR from Packard Instrument Company. The cells were collected in 250 µL KRHB to determine the protein content as described in section 8.6.4. To express data in nmols of glucose/mg of protein x test hour we used the following mathematical expression:

$$\frac{(dpm\ sample - dpm\ blank)x\ 1250\ nmol\ of\ glucose}{Total\ dpm\ x\ Test\ hours\ (1)\ x\ mg\ of\ protein}$$

7.8.6. Determination of lactate in cellular media

The release of lactate by 3T3-L1 adipocytes was determined between day 6 to day 7 of differentiation. Briefly, cells were seeded and differentiated in 6-well plates. At day 6 of differentiation, the cell media was replaced with fresh differentiation media 3. After 24h, the cell media was retrieved to measure the amount of lactate. To this end, the amount of lactate was analysed using the commercial kit L-Lactate Assay Kit EnzyChrom™ from BioAssay Systems following the manufacturer's instructions. This kit is based on the lactate dehydrogenase (LDH) catalysed oxidation of lactate, in which the formed NADH reduces a formazan agent. The product can be measured at 565 nm, which is proportional to the amount of lactate in the sample.

Lactate dehydrogenase (LDH): Acid lactic + NAD^+ ➔ Acid pyruvic + NADH

MTT reductase: MTT + NADH ➔ MTT-H (565 nm) + NAD^+

Briefly, a standard curve from 0 to 2 mM was prepared in a 96-well plate using a 2 mM L-lactate solution. Besides that, 20 µL of the cell media of each sample was loaded in duplicates. The kit's reagent was prepared by adding 60 µL of Assay Buffer, 1 µL of Enzyme A, 1 µL of Enzyme B, 10 µL of NAD^+ and 14 µL of MTT per each sample. Then, 80 µL of the reagent solution was added per each well. The 96-well plate was covered and mixed. Afterwards, the optical density was measured for time zero (OD0) at 565 nm and after 20 minutes of incubation (OD20) at RT. Then, the ΔOD were calculated following the following mathematical expression: ΔOD=OD20-OD0. Finally, the lactate concentration was interpolated from the standard curve with the ΔOD values of each sample. Absorbance was read at 565 nm as described in 8.6.4.

7.8.7. Determination of H_2O_2 in cellular homogenates from 3T3-L1 adipocytes

We analysed the oxidative stress of 3T3-L1 adipocytes via determination of H_2O_2 levels in total lysates of these cells. To this end, the H_2O_2 levels were determined by using the Amplex® Red Hydrogen Peroxidase Assay Kit from Invitrogen™ following the manufacturer's instructions. Briefly, we first prepared the stock solutions 10 mM Amplex® Red reagent, 1x Reaction Buffer, 10 U/mL HRP solution and the 20 mM H_2O_2 standard solution. Afterwards, the standard curve of H_2O_2 was prepared from 0 to 10 µM. Then, the H_2O_2-containing samples were diluted in 1x Reaction Buffer. Afterwards, 50 µL of samples and standard curve samples were loaded in 96-well (black plates with a transparent bottom). Then, the working solution of 100 µM Amplex® Red reagent and 0.2 U/mL HRP were prepared. 50 µL of this solution was added to each sample, controls and standards. The reaction was incubated for 30 minutes at RT. Finally, fluorescence was read at 590 nm with excitation of 560 nm using the FLUOstar Optima Fluorimeter equip from BMG LABTECH.

7.8.8. Analysis of polar metabolites in 3T3-L1 adipocytes

Here, we analysed the relative amount of the polar metabolites citrate, glutamate, cysteine, asparagine, malate, phenylalanine, serine, glutamine, fumarate, proline, succinate, isoleucine, leucine, valine, glycine, alanine and acid lactic from adipocyte homogenates and conditioned cell media from day 6 to day 7 of differentiation. To this end, cells were seeded and differentiated in 10 cm Ø culture dish. At day 6 of differentiation, the differentiation cell media 3 was retrieved and replaced with fresh differentiation cell media 3. At day 7 of differentiation, the cells were lysed and the metabolites were extracted via methanol-water/chloroform extraction. Thus, with the cellular lysates, we determined the intracellular relative concentration of the metabolites, whereas with the conditioned media we analysed the production and the consumption of some metabolites comparing the number of metabolites in that conditioned media with fresh differentiation media 3. The extraction of metabolites and the GC and MS analysis was done in collaboration with Dr Cascante M. and Dr Balcells C. from the Biochemistry and Molecular Biomedicine department, UB.

Briefly, the analysed metabolites were extracted from frozen cultured plates by adding a 100% (v/v) methanol:water (1:1) solution and scrapping the cells on ice. The lysates were then sonicated using a titanium probe (VibraCell, Sonic & Materials Inc., Tune: 50, Output: 30). Then, the lysates were spun to separate the aqueous phase. After that, the aqueous phase was evaporated under airflow at RT. Once the isolation of solid residue of polar metabolites was completed, they were derivatised. This process chemically transforms the metabolites to ensure that they stay in the gas phase in the GC columns at 250-300 ºC. To this end, the solid residue was derivatised by the addition of 2% (v/v) methoxyamine (Sigma-Aldrich Inc) in pyridine and held at 37 ºC for 90 minutes. Next, MBTSTFA + 1% TBDMCS (Sigma-Aldrich Inc) was added to the samples. They were incubated for 1h at 55 ºC and transferred to GC-MS vials. Upon GC, compounds are separated based on their volatility and polarity through the interaction with the stationary phase of the chromatography. All the metabolites are in the gas phase at the working temperature of the columns (250-300 ºC). Then, the compounds coming from the GC are ionised. The path of the produced ions is modulated by charged electrodes (quadrupoles), as a function of the ion mass/charge (m/z) and each ion is detected. Each peak of the obtained chromatogram corresponds to a derivatised metabolite with a specific retention time. For each retention time, the corresponding MS spectrum is also obtained to identify each metabolite by the molecular weight and fragmentation pattern of the derivatised form. Thus, using specific standard samples in the GC-MS analysis, we can interpolate the sample results and obtain an absolute value of the amount of that metabolite in the original sample.

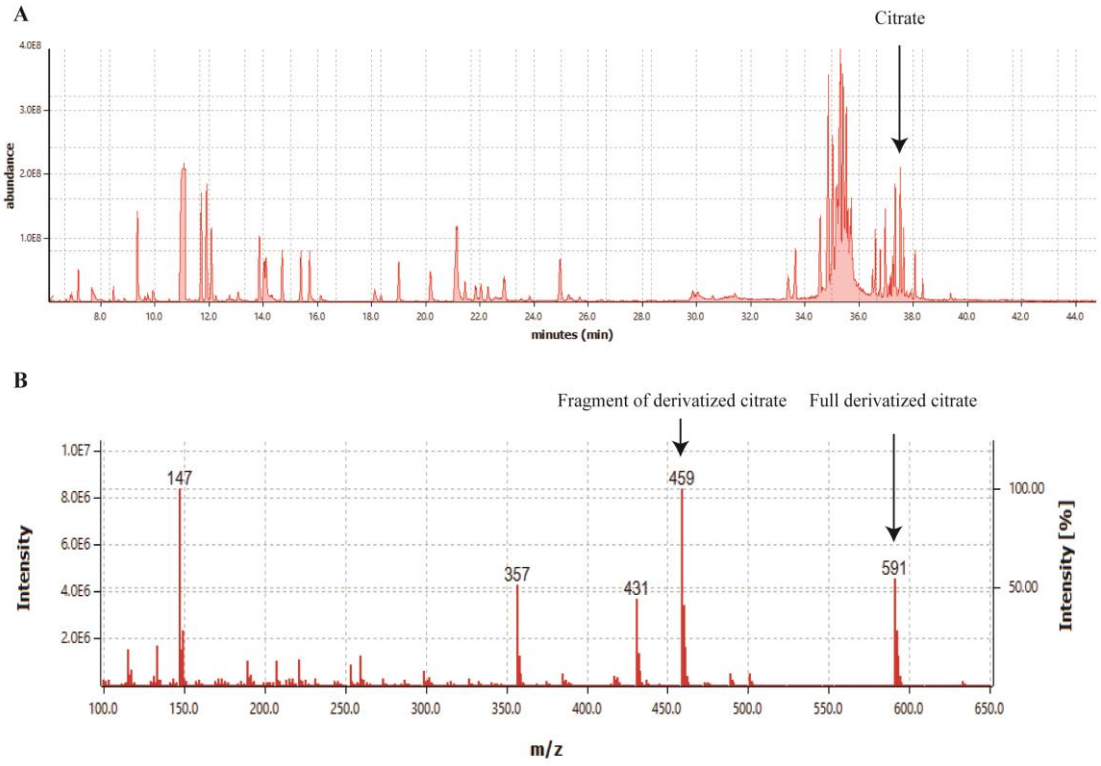


Figure 84. GC analysis of medium from *Nrg4*-silenced adipocytes and MS analysis of citrate. (**A**) GC chromatogram of the derivatised metabolites from conditioned cell media from Nrg4 KD adipocytes between day (D) 6 and D7 of differentiation. The peak around 37 minutes of retention time corresponds to the metabolite citrate. (**B**) Mass spectrum for citrate from the previous sample. The ion of 591 m/z corresponds to the full derivatised form of citrate, while the ion of 459 m/z corresponds to a fragment of the derivatised citrate.

7.9. Microscopy and flow cytometry

7.9.1. Immunolocalization of proteins in 3T3-L1 adipocytes and primary immortalised adipocytes

Here, we immunolocalised proteins to further characterise the generated primary immortalised white adipocytes. In addition, a co-localization of GLUT4 and LAMP1 was also performed. To this end, adipocytes were seeded in 6-well plates over 10 mm length coverslips. These coverslips were individually placed in 24-well plates. Then, they were washed twice with PBS avoiding to directly impact the cells with the buffer. Afterwards, the adipocytes were fixed with 3% (w/v) paraformaldehyde in PBS for 30 minutes at RT. Then, the coverslips were washed 3 times with PBS. At this point, coverslips could be stored at 4 ºC to continue the experiment another day. After the PBS washes, to reduce the autofluorescence, adipocytes were washed with 50 mM NH_4Cl in PBS for 10 minutes. After that, the H_4Cl solution was removed and the coverslips were washed with 20 mM glycine in PBS to eliminate the leftover H_4Cl.

Then, cells were permeabilised with the addition of 0.1% (v/v) Triton-X-100 in PBS for 10 minutes. Next, the coverslips were washed 3 times with PBS. For the blocking of the coverslips, they incubated with 10% (v/v) FBS in PBS for 30 minutes at RT. During this incubation, the dilutions of primary antibodies were prepared in 10% (v/v) FBS in PBS. The list of primary antibodies used in immunolocalization and their concentrations can be consulted in Table 2.3. 25 μL of primary antibody solution was prepared per each sample. This volume was placed in the cover of the 24-well plate covered with parafilm. Then, the coverslips were mounted over a drop of the primary antibody solution, with the adipocytes turned to the face of the solution. The coverslips were incubated for 1h at RT. Afterwards, the coverslips were placed again in the 24-well plate and washed 3 times with PBS for 5 minutes. Then, the coverslips were incubated with the secondary antibody solution, which was prepared in the same way as the primary antibody solution. In this study, goat anti-mouse Alexa Fluor® 488 (488 nm emission, green) and donkey anti-rabbit Alexa Fluor® 647 (647 nm emission, far-red) were used. Secondary antibodies were diluted 1/800 in 10% (v/v) FBS in PBS. Coverslips were mounted over a drop of secondary antibody solution and incubated for 1h at RT. At this point, the manipulation of the coverslips was made in the dark. After the incubation with secondary antibodies, the coverslips were placed again in the 24-well plates and washed 3 times with PBS for 5 minutes. During the last wash step, the coverslips were incubated with 10 μg/μL DAPI solution for 5 minutes. Finally, the coverslips were mounted with the Fluoromount™ mounting medium. Once mounted, the coverslips were dried overnight in a horizontal position at 4 ºC. Immunolocalization images were taken in the confocal microscope LSM 880 from Zeiss using the Zen Black and Blue software from Zeiss. The confocal microscopy service was provided by the Advanced Optical Microscopy Facility, CCiTUB. Final images were assembled and analysed using the ImageJ software from NIH.

7.9.2. MitoTracker™ staining in 3T3-L1 adipocytes

We used the MitoTracker™ red and green staining probe. The red probe is excitable at 579 nm and emits at 599 nm. Importantly, it is retained in the mitochondria thanks to the mitochondrial potential. The green probe is excited at 490 nm and it emits at 516 nm. Contrarily to the red probe, it localizes in the mitochondria independently of the mitochondrial membrane potential. However, the green probe is not well preserved upon cell fixation. Thus, we used MitoTracker™ green probe to analyse mitochondrial mass via flow cytometry in alive adipocytes, while the red probe was used to analyse the mitochondrial morphology by confocal microscopy in fixed adipocytes.

Regarding MitoTracker™ red staining, adipocytes were cultured and differentiated in 10 mm Ø coverslips. Afterwards, cell media was discarded and the adipocytes were incubated for 30 minutes at 37 ºC with 500 µL of 100 nM MitoTracker™ red probe in 1.5 mL of 10% (v/v) FBS DMEM without antibiotic supplementation. Then, the adipocytes were washed 3 times with PBS for 5 minutes. Afterwards, the adipocytes were fixed with 3% (w/v) paraformaldehyde in PBS for 30 minutes at RT. Next, to reduce the autofluorescence, the adipocytes were washed with 50 mM NH_4Cl in PBS for 10 minutes. After that, the H_4Cl solution was removed and the coverslips were washed with 20 mM glycine in PBS to eliminate the H_4Cl leftover. Then, the cells were washed 3 times with PBS for 10 minutes. During the last wash step, the cells were incubated with 10 µg/µL DAPI solution for 5 minutes. Finally, the coverslips were mounted with the Fluoromount™ mounting medium. Finally, images were taken in the confocal microscope LSM 880 from Zeiss and the Zen Black and Blue software from Zeiss as described in section 8.9.1. Regarding MitoTracker™ green staining, adipocytes were seeded and differentiated in 6-well plates. Then, cell media was discarded and changed for 1.5 mL per well of 10% (v/v) FBS DMED media without antibiotic supplementation with 500 µL of 100 nM MitoTracker™ green probe. After the MitoTracker™ incubation, the adipocytes were detached upon the addition of 500 µL of trypsin per well for 20 minutes. The obtained cell suspensions were spun at 300 rpm for 5 minutes at RT and the pellet was kept. The obtained pellets were then resuspended in 500 µL of pre-warmed KRHB buffer in flow cytometry tubes. Finally, samples were analysed in the Gallios Flow Cytometer from Beckman Coulter Inc. Flow cytometry service was provided by the Cytometry Unit, CCiTUB.

7.9.3. Determination of the mitochondrial membrane potential in 3T3-L1 adipocytes

To analyse the mitochondrial membrane potential of differentiated adipocytes, the MitoProbe™ TMRM Assay Kit from Thermo Scientific™ was used following the manufacturer's instructions. In healthy mitochondria, the tetramethylrhodamine methyl ester (TMRM) probe is sequestered, thereby emitting a red fluorescent signal (561 nm excitation, 585 nm emission). Upon mitochondrial depolarization, TMRM is released from the mitochondria, thus decreasing the red fluorescent signal. Briefly, adipocytes were grown and differentiated in 6-well plates. Then, at day 7 of differentiation, the cells were incubated with differentiation media 3 supplemented with 50 nM TMRM for 30 minutes in the dark at 37 ºC. Next, the adipocytes were detached upon the addition of 500 µL of trypsin per well for 20 minutes. The cells were spun and the pellets were resuspended in 500 µL of pre-warmed KRP-HEPES buffer in flow cytometry tubes. Finally, samples were analysed in the Gallios Flow Cytometer from Beckman Coulter as described above.

7.9.4. Oil Red O staining in adipocytes

Differentiated primary immortalised white adipocytes and 3T3-L1 adipocytes were rinsed with PBS and then fixed with 3% (w/v) paraformaldehyde for 20 minutes at RT. Then, the paraformaldehyde was removed and the cells were washed with 60% isopropanol. Afterwards, the cells were incubated with 60% Oil Red O stock (8.5 mM Oil Red O diluted in isopropanol and filtered with a 0.2 μm filter) diluted in Milli-Q water for 15 minutes at RT. Afterwards, the adipocytes were washed 3 times with PBS. Micrographs were taken in a phase-contrast microscope.

7.10. Statistical analysis

Data are presented as mean ± SEM. Comparisons between 2 experimental groups were analysed using Student's t-test. Comparisons between more than 2 experimental groups were analysed with one-way analysis of variance with Tukey's honest significant difference *post-hoc* test. P-values of significance are indicated in the figure's captions. Statistical analysis was only applied to data from n ≥3 independent experiments. Data were analysed using the Prism 6 and 8 software from GraphPad Software. Figures were assembled using Adobe illustrator® software from Adobe Systems.

8. References

Abel, E. D., Peroni, O., Kim, J. K., Kim, Y. B., Boss, O., Hadro, E., … Kahn, B. B. (2001). Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature*. https://doi.org/10.1038/35055575

Ahmad, T., Aggarwal, K., Pattnaik, B., Mukherjee, S., Sethi, T., Tiwari, B. K., … Agrawal, A. (2013). Computational classification of mitochondrial shapes reflects stress and redox state. *Cell Death and Disease*. https://doi.org/10.1038/cddis.2012.213

Ahmed, Z., Smith, B. J., & Pillay, T. S. (2000). The APS adapter protein couples the insulin receptor to the phosphorylation of c-Cbl and facilitates ligand-stimulated ubiquitination of the insulin receptor. *FEBS Letters*. https://doi.org/10.1016/S0014-5793(00)01621-5

Altiok, N., Bessereau, J. L., & Changeux, J. P. (1995). ErbB3 and ErbB2/neu mediate the effect of heregulin on acetylcholine receptor gene expression in muscle: Differential expression at the endplate. *EMBO Journal*. https://doi.org/10.1002/j.1460-2075.1995.tb00100.x

Amit, I., Citri, A., Shay, T., Lu, Y., Katz, M., Zhang, F., … Yarden, Y. (2007). A module of negative feedback regulators defines growth factor signaling. *Nature Genetics*. https://doi.org/10.1038/ng1987

Andrejeva, G., Gowan, S., Lin, G., Wong Te Fong, A. C. L., Shamsaei, E., Parkes, H. G., … Chung, Y. L. (2020). De novo phosphatidylcholine synthesis is required for autophagosome membrane formation and maintenance during autophagy. *Autophagy*. https://doi.org/10.1080/15548627.2019.1659608

Appari, M., Channon, K. M., & McNeill, E. (2018). Metabolic Regulation of Adipose Tissue Macrophage Function in Obesity and Diabetes. *Antioxidants and Redox Signaling*. https://doi.org/10.1089/ars.2017.7060

Axe, E. L., Walker, S. A., Manifava, M., Chandra, P., Roderick, H. L., Habermann, A., … Ktistakis, N. T. (2008). Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.200803137

Bacus, S. S., Gudkov, A. V., Zelnick, C. R., Chin, D., Stern, R., Stancovski, I., … Yarden, Y. (1993). Neu Differentiation Factor (Heregulin) Induces Expression of Intercellular Adhesion Molecule 1: Implications for Mammary Thmors. *Cancer Research*.

Bacus, S. S., Huberman, E., Chin, D., Kiguchi, K., Simpson, S., Lippman, M., & Lupu, R. (1992). A ligand for the erbB-2 oncogene product (gp30) induces differentiation of human breast cancer cells. *Cell Growth & Differentiation : The Molecular Biology Journal of the American Association for Cancer Research*.

Bandyopadhyay, G., Sajan, M. P., Kanoh, Y., Standaert, M. L., Quon, M. J., Lea-Currie, R., … Farese, R. V. (2002). PKC-ζ mediates insulin effects on glucose transport in cultured preadipocyte-derived human adipocytes. *Journal of Clinical Endocrinology and Metabolism*. https://doi.org/10.1210/jc.87.2.716

Bapat, S. P., Myoung Suh, J., Fang, S., Liu, S., Zhang, Y., Cheng, A., … Zheng, Y. (2015). Depletion of fat-resident T reg cells prevents age-associated insulin resistance. *Nature*. https://doi.org/10.1038/nature16151

Barton, B. E. (2001). IL-6-like cytokines and cancer cachexia: Consequences of chronic inflammation. *Immunologic Research*. https://doi.org/10.1385/IR:23:1:41

Basu, M., Frick, K., Sen-Majumdar, A., Scher, C. D., & Das, M. (1985). EGF receptor-associated DNA-nicking activity is due to a M r-100,000 dissociable protein. *Nature*. https://doi.org/10.1038/316640a0

Beguinot, F., Smith, R. J., Kahn, C. R., Maron, R., Moses, A. C., & White, M. F. (1988). Phosphorylation of Insulin-like Growth Factor I Receptor by Insulin Receptor Tyrosine Kinase in Intact Cultured Skeletal Muscle Cells. *Biochemistry*. https://doi.org/10.1021/bi00409a015

Belfiore, A., Malaguarnera, R., Vella, V., Lawrence, M. C., Sciacca, L., Frasca, F., … Vigneri, R. (2017). Insulin receptor isoforms in physiology and disease: An updated view. *Endocrine Reviews*. https://doi.org/10.1210/er.2017-00073

Bellot, G., Garcia-Medina, R., Gounon, P., Chiche, J., Roux, D., Pouysségur, J., & Mazure, N. M. (2009). Hypoxia-Induced Autophagy Is Mediated through Hypoxia-Inducible Factor Induction of BNIP3 and BNIP3L via Their BH3 Domains. *Molecular and Cellular Biology*. https://doi.org/10.1128/mcb.00166-09

Belman, J. P., Habtemichael, E. N., & Bogan, J. S. (2014). A proteolytic pathway that controls glucose uptake in fat and muscle. *Reviews in Endocrine and Metabolic Disorders*. https://doi.org/10.1007/s11154-013-9276-2

Belmonte, F., Das, S., Sysa-Shah, P., Sivakumaran, V., Stanley, B., Guo, X., … Gabrielson, K. (2015). ErbB2 overexpression upregulates antioxidant enzymes, reduces basal levels of reactive oxygen species, and protects against doxorubicin cardiotoxicity. *American Journal of Physiology - Heart and Circulatory Physiology*. https://doi.org/10.1152/ajpheart.00517.2014

Benischke, A. S., Vasanth, S., Miyai, T., Katikireddy, K. R., White, T., Chen, Y., … Jurkunas, U. V. (2017). Activation of mitophagy leads to decline in Mfn2 and loss of mitochondrial mass in Fuchs endothelial corneal dystrophy. *Scientific Reports*. https://doi.org/10.1038/s41598-017-06523-2

Bernard, J. K., McCann, S. P., Bhardwaj, V., Washington, M. K., & Frey, M. R. (2012). Neuregulin-4 is a survival factor for colon epithelial cells both in culture and in Vivo. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M112.400846

Bertolio, R., Napoletano, F., Mano, M., Maurer-Stroh, S., Fantuz, M., Zannini, A., … Del Sal, G. (2019). Sterol regulatory element binding protein 1 couples mechanical cues and lipid metabolism. *Nature Communications*. https://doi.org/10.1038/s41467-019-09152-7

Bian, Y., Sun, M., Silver, M., Ho, K. K. L., Marchionni, M. A., Caggiano, A. O., … Yan, X. (2009). Neuregulin-1 attenuated doxorubicin-induced decrease in cardiac troponins. *American Journal of Physiology - Heart and Circulatory Physiology*. https://doi.org/10.1152/ajpheart.01010.2008

Blot, V., & McGraw, T. E. (2006). GLUT4 is internalized by a cholesterol-dependent nystatin-sensitive mechanism inhibited by insulin. *EMBO Journal*. https://doi.org/10.1038/sj.emboj.7601462

Bogan, J. S., & Kandror, K. V. (2010). Biogenesis and regulation of insulin-responsive vesicles containing GLUT4. *Current Opinion in Cell Biology*. https://doi.org/10.1016/j.ceb.2010.03.012

Bogan, J. S., Hendon, N., McKee, A. E., Tsao, T. S., & Lodish, H. F. (2003). Functional cloning of TUG as a regulator of GLUT4 glucose transporter trafficking. *Nature*. https://doi.org/10.1038/nature01989

Bolinder, J., Kerckhoffs, D. A. J. M., Moberg, E., Hagström-Toft, E., & Arner, P. (2000). Rates of skeletal muscle and adipose tissue glycerol release in nonobese and obese subjects. *Diabetes*. https://doi.org/10.2337/diabetes.49.5.797

Bonnefont-Rousselot, D. (2002). Glucose and reactive oxygen species. *Current Opinion in Clinical Nutrition and Metabolic Care*. https://doi.org/10.1097/00075197-200209000-00016

Bournat, J. C., & Brown, C. W. (2010). Mitochondrial dysfunction in obesity. *Current Opinion in Endocrinology, Diabetes and Obesity*. https://doi.org/10.1097/MED.0b013e32833c3026

Brasaemle, D. L., Dolios, G., Shapiro, L., & Wang, R. (2004). Proteomic analysis of proteins associated with lipid droplets of basal and lipolytically stimulated 3T3-L1 adipocytes. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M409340200

Britsch, S., Li, L., Kirchhoff, S., Theuring, F., Brinkmann, V., Birchmeier, C., & Riethmacher, D. (1998). The ErbB2 and ErbB3 receptors and their ligand, neuregulin-1, are essential for development of the sympathetic nervous system. *Genes and Development*. https://doi.org/10.1101/gad.12.12.1825

Bryant, N. J., Govers, R., & James, D. E. (2002). Regulated transport of the glucose transporter GLUT4. *Nature Reviews Molecular Cell Biology*. https://doi.org/10.1038/nrm782

Cadwell, K. (2016). Crosstalk between autophagy and inflammatory signalling pathways: Balancing defence and homeostasis. *Nature Reviews Immunology*. https://doi.org/10.1038/nri.2016.100

Cai, H., Dong, L. Q., & Liu, F. (2016). Recent Advances in Adipose mTOR Signaling and Function: Therapeutic Prospects. *Trends in Pharmacological Sciences*. https://doi.org/10.1016/j.tips.2015.11.011

Candas, D., & Li, J. J. (2014). MnSOD in oxidative stress response-potential regulation via mitochondrial protein influx. *Antioxidants and Redox Signaling*. https://doi.org/10.1089/ars.2013.5305

Cantó, C., Chibalin, A. V., Barnes, B. R., Glund, S., Suárez, E., Ryder, J. W., … Gumà, A. (2006). Neuregulins mediate calcium-induced glucose transport during muscle contraction. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M600475200

Cantó, C., Pich, S., Paz, J. C., Sanches, R., Martínez, V., Orpinell, M., … Gumà, A. (2007). Neuregulins increase mitochondrial oxidative capacity and insulin sensitivity in skeletal muscle cells. *Diabetes*. https://doi.org/10.2337/db06-1726

Cantó, C., Suárez, E., Lizcano, J. M., Griñó, E., Shepherd, P. R., Fryer, L. G. D., … Gumà, A. (2004). Neuregulin Signaling on Glucose Transport in Muscle Cells. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M308554200

Cao, Z., Umek, R. M., & McKnight, S. L. (1991). Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells. *Genes and Development*. https://doi.org/10.1101/gad.5.9.1538

Carpenter, G. (2003). Nuclear localization and possible functions of receptor tyrosine kinases. *Current Opinion in Cell Biology*. https://doi.org/10.1016/S0955-0674(03)00015-2

Carvalho, E., Schellhorn, S. E., Zabolotny, J. M., Martin, S., Tozzo, E., Peroni, O. D., … Kahn, B. B. (2004). GLUT4 overexpression or deficiency in adipocytes of transgenic mice alters the composition of GLUT4 vesicles and the subcellular localization of GLUT4 and insulin-responsive aminopeptidase. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M312269200

Carver, R. S., Stevenson, M. C., Scheving, L. A., & Russell, W. E. (2002). Diverse expression of ErbB receptor proteins during rat liver development and regeneration. *Gastroenterology*. https://doi.org/10.1053/gast.2002.37060

Cawthorn, W. P., & Sethi, J. K. (2008). TNF-α and adipocyte biology. *FEBS Letters*. https://doi.org/10.1016/j.febslet.2007.11.051

Chan, D. C. (2006). Mitochondrial fusion and fission in mammals. *Annual Review of Cell and Developmental Biology*. https://doi.org/10.1146/annurev.cellbio.22.010305.104638

Che, M., Wang, R., Li, X., Wang, H. Y., & Zheng, X. F. S. (2016). Expanding roles of superoxide dismutases in cell regulation and cancer. *Drug Discovery Today*. https://doi.org/10.1016/j.drudis.2015.10.001

Chen, H., Detmer, S. A., Ewald, A. J., Griffin, E. E., Fraser, S. E., & Chan, D. C. (2003). Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.200211046

Chen, R., Zou, Y., Mao, D., Sun, D., Gao, G., Shi, J., … Yu, L. (2014). The general amino acid control pathway regulates mTOR and autophagy during serum/glutamine starvation. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.201403009

Chen, Z., Wang, G. X., Ma, S. L., Jung, D. Y., Ha, H., Altamimi, T., … Lin, J. D. (2017). Nrg4 promotes fuel oxidation and a healthy adipokine profile to ameliorate diet-induced metabolic disorders. *Molecular Metabolism*. https://doi.org/10.1016/j.molmet.2017.03.016

Cherrington, A. D. (1999). Control of glucose uptake and release by the liver in vivo. In *Diabetes*. https://doi.org/10.2337/diabetes.48.5.1198

Chiang, G. G., & Abraham, R. T. (2005). Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M501707200

Church, C., Brown, M., & Rodeheffer, M. S. (2015). Conditional immortalization of primary adipocyte precursor cells. *Adipocyte*. https://doi.org/10.1080/21623945.2014.995510

Clark, I. E., Dodson, M. W., Jiang, C., Cao, J. H., Huh, J. R., Seol, J. H., … Guo, M. (2006). Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature*. https://doi.org/10.1038/nature04779

Clemente-Postigo, M., Tinahones, A., Bekay, R. El, Malagón, M. M., & Tinahones, F. J. (2020). The role of Autophagy in white adipose tissue function: Implications for metabolic health. *Metabolites*. https://doi.org/10.3390/metabo10050179

Codony-Servat, J., Albanell, J., Lopez-Talavera, J. C., Arribas, J., & Baselga, J. (1999). Cleavage of the HER2 ectodomain is a pervanadate-activable process that is inhibited by the tissue inhibitor of metalloproteases-1 in breast cancer cells. *Cancer Research*.

Cole, C. L., Kleckner, I. R., Jatoi, A., Schwarz, E., & Dunne, R. F. (2018). The Role of Systemic Inflammation in Cancer-Associated Muscle Wasting and Rationale for Exercise as a Therapeutic Intervention. *JCSM Clinical Reports*. https://doi.org/10.17987/jcsm-cr.v3i2.65

Cong, L.-N. (1997). Physiological Role of Akt in Insulin-Stimulated Translocation of GLUT4 in Transfected Rat Adipose Cells. *Molecular Endocrinology*. https://doi.org/10.1210/me.11.13.1881

Corfas, G., Roy, K., & Buxbaum, J. D. (2004). Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. *Nature Neuroscience*. https://doi.org/10.1038/nn1258

Costello, L. C., & Franklin, R. B. (2013). A review of the important central role of altered citrate metabolism during the process of stem cell differentiation. *Journal of Regenerative Medicine and Tissue Engineering*. https://doi.org/10.7243/2050-1218-2-1

Cranmer-Byng, M. M., Liddle, D. M., De Boer, A. A., Monk, J. M., & Robinson, L. E. (2014). Proinflammatory effects of arachidonic acid in a lipopolysaccharide-induced inflammatory microenvironment in 3T3-L1 adipocytes in vitro. *Applied Physiology, Nutrition and Metabolism*. https://doi.org/10.1139/apnm-2014-0022

Cummins, E. P., & Taylor, C. T. (2017). Hypoxia and inflammation. *Biochemist*. https://doi.org/10.1042/bio03904034

Czech, M. P., Tencerova, M., Pedersen, D. J., & Aouadi, M. (2013). Insulin signalling mechanisms for triacylglycerol storage. *Diabetologia*. https://doi.org/10.1007/s00125-013-2869-1

D'Ignazio, L., Bandarra, D., & Rocha, S. (2016). NF-κB and HIF crosstalk in immune responses. *FEBS Journal*. https://doi.org/10.1111/febs.13578

Darlington, G. J., Ross, S. E., & MacDougald, O. A. (1998). The role of C/EBP genes in adipocyte differentiation. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.273.46.30057

Dato, V. A., & Chiabrando, G. A. (2018). The role of low-density lipoprotein receptor-related protein 1 in lipid metabolism, glucose homeostasis and inflammation. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms19061780

Degboé, Y., Rauwel, B., Baron, M., Boyer, J. F., Ruyssen-Witrand, A., Constantin, A., & Davignon, J. L. (2019). Polarization of rheumatoid macrophages by TNF targeting through an IL-10/STAT3 mechanism. *Frontiers in Immunology*. https://doi.org/10.3389/fimmu.2019.00003

Dela Cruz, C. S., & Kang, M. J. (2018). Mitochondrial dysfunction and damage associated molecular patterns (DAMPs) in chronic inflammatory diseases. *Mitochondrion*. https://doi.org/10.1016/j.mito.2017.12.001

Dibble, C. C., & Cantley, L. C. (2015). Regulation of mTORC1 by PI3K signaling. *Trends in Cell Biology*. https://doi.org/10.1016/j.tcb.2015.06.002

Ding, Y., Liu, Z., Desai, S., Zhao, Y., Liu, H., Pannell, L. K., … Tan, M. (2012). Receptor tyrosine kinase ErbB2 translocates into mitochondria and regulates cellular metabolism. *Nature Communications*. https://doi.org/10.1038/ncomms2236

Distelmaier, F., Valsecchi, F., Forkink, M., Van Emst-De Vries, S., Swarts, H. G., Rodenburg, R. J. T., … Koopman, W. J. H. (2012). Trolox-sensitive reactive oxygen species regulate mitochondrial morphology, oxidative phosphorylation and cytosolic calcium handling in healthy cells. *Antioxidants and Redox Signaling*. https://doi.org/10.1089/ars.2011.4294

Dolinsky, V. W., Sipione, S., Lehner, R., & Vance, D. E. (2001). The cloning and expression of a murine triacylglycerol hydrolase cDNA and the structure of its corresponding gene. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*. https://doi.org/10.1016/S1388-1981(01)00133-0

Duffaut, C., Galitzky, J., Lafontan, M., & Bouloumié, A. (2009). Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2009.05.002

Elachouri, G., Vidoni, S., Zanna, C., Pattyn, A., Boukhaddaoui, H., Gaget, K., … Lenaers, G. (2011). OPA1 links human mitochondrial genome maintenance to mtDNA replication and distribution. *Genome Research*. https://doi.org/10.1101/gr.108696.110

Elenius, K., Choi, C. J., Paul, S., Santiestevan, E., Nishi, E., & Klagsbrun, M. (1999). Characterization of a naturally occurring ErbB4 isoform that does not bind or activate phosphatidyl inositol 3-kinase. *Oncogene*. https://doi.org/10.1038/sj.onc.1202612

El-Hafidi, M., Franco, M., Ramírez, A. R., Sosa, J. S., Flores, J. A. P., Acosta, O. L., … Cardoso-Saldaña, G. (2018). Glycine increases insulin sensitivity and glutathione biosynthesis and protects against oxidative stress in a model of sucrose-induced insulin resistance. *Oxidative Medicine and Cellular Longevity*. https://doi.org/10.1155/2018/2101562

Endemann, G., Stanton, L. W., Madden, K. S., Bryant, C. M., White, R. T., & Protter, A. A. (1993). CD36 is a receptor for oxidized low density lipoprotein. *Journal of Biological Chemistry*.

Eskelinen, E. L., & Saftig, P. (2009). Autophagy: A lysosomal degradation pathway with a central role in health and disease. *Biochimica et Biophysica Acta - Molecular Cell Research*. https://doi.org/10.1016/j.bbamcr.2008.07.014

Eskelinen, E. L., Illert, A. L., Tanaka, Y., Schwarzmann, G., Blanz, J., Von Figura, K., & Saftig, P. (2002). Role of LAMP-2 in lysosome biogenesis and autophagy. *Molecular Biology of the Cell*. https://doi.org/10.1091/mbc.E02-02-0114

Fajas, L. (2003). Adipogenesis: A cross-talk between cell proliferation and cell differentiation. *Annals of Medicine*. https://doi.org/10.1080/07853890310009999

Farmer, S. R. (2005). Regulation of PPARγ activity during adipogenesis. *International Journal of Obesity*. https://doi.org/10.1038/sj.ijo.0802907

Farmer, S. R. (2006). Transcriptional control of adipocyte formation. *Cell Metabolism*. https://doi.org/10.1016/j.cmet.2006.07.001

Febbraio, M. A., Hiscock, N., Sacchetti, M., Fischer, C. P., & Pedersen, B. K. (2004). Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. *Diabetes*. https://doi.org/10.2337/diabetes.53.7.1643

Ferrannini, E., Barrett, E. J., Bevilacqua, S., & DeFronzo, R. A. (1983). Effect of fatty acids on glucose production and utilization in man. *Journal of Clinical Investigation*. https://doi.org/10.1172/JCI111133

Filadi, R., Pendin, Di., & Pizzo, P. (2018). Mitofusin 2: From functions to disease. *Cell Death and Disease*. https://doi.org/10.1038/s41419-017-0023-6

Fischer-Posovszky, P., Wabitsch, M., & Hochberg, Z. (2007). Endocrinology of adipose tissue - An update. *Hormone and Metabolic Research*. https://doi.org/10.1055/s-2007-976539

Flores-Guerrero, J., Osté, M., Kieneker, L., Gruppen, E., Wolak-Dinsmore, J., Otvos, J., … Dullaart, R. (2018). Plasma Branched-Chain Amino Acids and Risk of Incident Type 2 Diabetes: Results from the PREVEND Prospective Cohort Study. *Journal of Clinical Medicine*. https://doi.org/10.3390/jcm7120513

Florini, J. R., Samuel, D. S., Ewton, D. Z., Kirk, C., & Sklar, R. M. (1996). Stimulation of myogenic differentiation by a neuregulin, glial growth factor 2: Are neuregulins the long-sought muscle trophic factors secreted by nerves? *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.271.22.12699

Foley, K., Boguslavsky, S., & Klip, A. (2011). Endocytosis, recycling, and regulated exocytosis of glucose transporter 4. *Biochemistry*. https://doi.org/10.1021/bi2000356

Frey, M. R., Edelblum, K. L., Mullane, M. T., Liang, D., & Polk, D. B. (2009). The ErbB4 Growth Factor Receptor Is Required for Colon Epithelial Cell Survival in the Presence of TNF. *Gastroenterology*. https://doi.org/10.1053/j.gastro.2008.09.023

Fujisaka, S., Usui, I., Bukhari, A., Ikutani, M., Oya, T., Kanatani, Y., … Tobe, K. (2009). Regulatory mechanisms for adipose tissue M1 and M2 macrophages in diet-induced obese mice. *Diabetes*. https://doi.org/10.2337/db08-1475

Fukazawa, R., Miller, T. A., Kuramochi, Y., Frantz, S., Kim, Y. D., Marchionni, M. A., … Sawyer, D. B. (2003). Neuregulin-1 protects ventricular myocytes from anthracycline-induced apoptosis via erbB4-dependent activation of PI3-kinase/Akt. *Journal of Molecular and Cellular Cardiology*. https://doi.org/10.1016/j.yjmcc.2003.09.012

Fukumoto, H., Kayano, T., Buse, J. B., Edwards, Y., Pilch, P. F., Bell, G. I., & Seino, S. (1989). Cloning and characterization of the major insulin-responsive glucose transporter expressed in human skeletal muscle and other insulin-responsive tissues. *Journal of Biological Chemistry*.

Galluzzi, L., Baehrecke, E. H., Ballabio, A., Boya, P., Bravo-San Pedro, J. M., Cecconi, F., … Kroemer, G. (2017). Molecular definitions of autophagy and related processes. *The EMBO Journal*. https://doi.org/10.15252/embj.201796697

Ganapathy, V., Thangaraju, M., & Prasad, P. D. (2009). Nutrient transporters in cancer: Relevance to Warburg hypothesis and beyond. *Pharmacology and Therapeutics*. https://doi.org/10.1016/j.pharmthera.2008.09.005

Gao, J. G., & Simon, M. (2006). Molecular screening for GS2 lipase regulators: Inhibition of keratinocyte retinylester hydrolysis by TIP47. *Journal of Investigative Dermatology*. https://doi.org/10.1038/sj.jid.5700327

Gassmann, M., Casagranda, F., Orloli, D., Simon, H., Lai, C., Kleint, R., & Lemke, G. (1995). Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature*. https://doi.org/10.1038/378390a0

Gatica, D., Lahiri, V., & Klionsky, D. J. (2018). Cargo recognition and degradation by selective autophagy. *Nature Cell Biology*. https://doi.org/10.1038/s41556-018-0037-z

Geng, L., Liao, B., Jin, L., Huang, Z., Triggle, C. R., Ding, H., … Xu, A. (2019). Exercise Alleviates Obesity-Induced Metabolic Dysfunction via Enhancing FGF21 Sensitivity in Adipose Tissues. *Cell Reports*. https://doi.org/10.1016/j.celrep.2019.02.014

Giraud, M. N., Flück, M., Zuppinger, C., & Suter, T. M. (2005). Expressional reprogramming of survival pathways in rat cardiocytes by neuregulin-1β. *Journal of Applied Physiology*. https://doi.org/10.1152/japplphysiol.00609.2004

Goldman, S. J., Zhang, Y., & Jin, S. (2011). Autophagic degradation of mitochondria in white adipose tissue differentiation. *Antioxidants and Redox Signaling*. https://doi.org/10.1089/ars.2010.3777

González-Muñoz, E., López-Iglesias, C., Calvo, M., Palacín, M., Zorzano, A., & Camps, M. (2009). Caveolin-1 loss of function accelerates glucose transporter 4 and insulin receptor degradation in 3T3-L1 adipocytes. *Endocrinology*. https://doi.org/10.1210/en.2008-1520

Graus-Porta, D., Beerli, R. R., & Hynes, N. E. (1995). Single-chain antibody-mediated intracellular retention of ErbB-2 impairs Neu differentiation factor and epidermal growth factor signaling. *Molecular and Cellular Biology*. https://doi.org/10.1128/mcb.15.3.1182

Graus-Porta, D., Beerli, R. R., Daly, J. M., & Hynes, N. E. (1997). ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. *EMBO Journal*. https://doi.org/10.1093/emboj/16.7.1647

Greenberg, A. S., Egan, J. J., Wek, S. A., Garty, N. B., Blanchette-Mackie, E. J., & Londos, C. (1991). Perilipin, a major hormonally regulated adipocyte-specific phosphoprotein associated with the periphery of lipid storage droplets. *Journal of Biological Chemistry*.

Gregoire, F. M., Smas, C. M., & Sul, H. S. (1998). Understanding adipocyte differentiation. *Physiological Reviews*. https://doi.org/10.1152/physrev.1998.78.3.783

Gumà, A., Díaz-Sáez, F., Camps, M., & Zorzano, A. (2020). Neuregulin, an Effector on Mitochondria Metabolism That Preserves Insulin Sensitivity. *Frontiers in Physiology*. https://doi.org/10.3389/fphys.2020.00696

Gumà, A., Martínez-Redondo, V., López-Soldado, I., Cantó, C., & Zorzano, A. (2010). Emerging role of neuregulin as a modulator of muscle metabolism. *American Journal of Physiology - Endocrinology and Metabolism*. https://doi.org/10.1152/ajpendo.00541.2009

Guo, L., Zhang, P., Chen, Z., Xia, H., Li, S., Zhang, Y., … Lin, J. D. (2017). Hepatic neuregulin 4 signaling defines an endocrine checkpoint for steatosis-to-NASH progression. *Journal of Clinical Investigation*. https://doi.org/10.1172/JCI96324

Guy, P. M., Platko, J. V., Cantley, L. C., Cerione, R. A., & Carraway, K. L. (1994). Insect cell-expressed p180(erbB3) possesses an impaired tyrosine kinase activity. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.91.17.8132

Haase, J., Weyer, U., Immig, K., Klöting, N., Blüher, M., Eilers, J., … Gericke, M. (2014). Local proliferation of macrophages in adipose tissue during obesity-induced inflammation. *Diabetologia*. https://doi.org/10.1007/s00125-013-3139-y

Hamacher-Brady, A., Brady, N. R., Logue, S. E., Sayen, M. R., Jinno, M., Kirshenbaum, L. A., … Gustafsson, Å. B. (2007). Response to myocardial ischemia/reperfusion injury involves Bnip3 and autophagy. *Cell Death and Differentiation*. https://doi.org/10.1038/sj.cdd.4401936

Hamm, J. K., Park, B. H., & Farmer, S. R. (2001). A Role for C/EBPβ in Regulating Peroxisome Proliferator-activated Receptor γ Activity during Adipogenesis in 3T3-L1 Preadipocytes. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M100797200

Han, J., Meng, Q., Shen, L., & Wu, G. (2018). Interleukin-6 induces fat loss in cancer cachexia by promoting white adipose tissue lipolysis and browning. *Lipids in Health and Disease*. https://doi.org/10.1186/s12944-018-0657-0

Harari, D., Tzahar, E., Romano, J., Shelly, M., Pierce, J. H., Andrews, G. C., & Yarden, Y. (1999). Neuregulin-4: A novel growth factor that acts through the ErbB-4 receptor tyrosine kinase. *Oncogene*. https://doi.org/10.1038/sj.onc.1202631

Hardie, D. G. (2011). AMPK and autophagy get connected. *EMBO Journal*. https://doi.org/10.1038/emboj.2011.12

He, Y., Hara, H., & Núñez, G. (2016). Mechanism and Regulation of NLRP3 Inflammasome Activation. *Trends in Biochemical Sciences*. https://doi.org/10.1016/j.tibs.2016.09.002

Heid, M. E., Keyel, P. A., Kamga, C., Shiva, S., Watkins, S. C., & Salter, R. D. (2013). Mitochondrial Reactive Oxygen Species Induces NLRP3-Dependent Lysosomal Damage and Inflammasome Activation. *The Journal of Immunology*. https://doi.org/10.4049/jimmunol.1301490

Henson, E., Chen, Y., & Gibson, S. (2017). EGFR family members' regulation of autophagy is at a crossroads of cell survival and death in cancer. *Cancers*. https://doi.org/10.3390/cancers9040027

Herscovitch, M., Comb, W., Ennis, T., Coleman, K., Yong, S., Armstead, B., … Gilmore, T. D. (2008). Intermolecular disulfide bond formation in the NEMO dimer requires Cys54 and Cys347. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2007.12.123

Hill, M. M., Clark, S. F., Tucker, D. F., Birnbaum, M. J., James, D. E., & Macaulay, S. L. (1999). A Role for Protein Kinase Bβ/Akt2 in Insulin-Stimulated GLUT4 Translocation in Adipocytes. *Molecular and Cellular Biology*. https://doi.org/10.1128/mcb.19.11.7771

Holbro, T., Beerli, R. R., Maurer, F., Koziczak, M., Barbas, C. F., & Hynes, N. E. (2003). The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.1537685100

Holm, C. (2003). Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. In *Biochemical Society Transactions*. https://doi.org/10.1042/bst0311120

Holm, C., Osterlund, T., Laurell, H., & Contreras, J. A. (2000). Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Annual Review of Nutrition*. https://doi.org/10.1146/annurev.nutr.20.1.365

Holmes, W. E., Sliwkowski, M. X., Akita, R. W., Henzel, W. J., Lee, J., Park, J. W., … Vandlen, R. L. (1992). Identification of heregulin, a specific activator of p185erbB2. *Science*. https://doi.org/10.1126/science.256.5060.1205

Hou, J. C., & Pessin, J. E. (2007). Ins (endocytosis) and outs (exocytosis) of GLUT4 trafficking. *Current Opinion in Cell Biology*. https://doi.org/10.1016/j.ceb.2007.04.018

Hsieh, A. C., Costa, M., Zollo, O., Davis, C., Feldman, M. E., Testa, J. R., … Ruggero, D. (2010). Genetic Dissection of the Oncogenic mTOR Pathway Reveals Druggable Addiction to Translational Control via 4EBP-eIF4E. *Cancer Cell*. https://doi.org/10.1016/j.ccr.2010.01.021

Huh, J. Y., Park, Y. J., Ham, M., & Kim, J. B. (2014). Crosstalk between adipocytes and immune cells in adipose tissue inflammation and metabolic dysregulation in obesity. *Molecules and Cells*. https://doi.org/10.14348/molcells.2014.0074

Huynh, K. K., Eskelinen, E. L., Scott, C. C., Malevanets, A., Saftig, P., & Grinstein, S. (2007). LAMP proteins are required for fusion of lysosomes with phagosomes. *EMBO Journal*. https://doi.org/10.1038/sj.emboj.7601511

Ingerman, E., Perkins, E. M., Marino, M., Mears, J. A., McCaffery, J. M., Hinshaw, J. E., & Nunnari, J. (2005). Dnm1 forms spirals that are structurally tailored to fit mitochondria. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.200506078

Inoki, K., Li, Y., Zhu, T., Wu, J., & Guan, K. L. (2002). TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nature Cell Biology*. https://doi.org/10.1038/ncb839

Inoki, K., Zhu, T., & Guan, K. L. (2003). TSC2 Mediates Cellular Energy Response to Control Cell Growth and Survival. *Cell*. https://doi.org/10.1016/S0092-8674(03)00929-2

Jacob, S., Hauer, B., Becker, R., Artzner, S., Grauer, P., Löblein, K., … Häring, H. U. (1999). Lipolysis in skeletal muscle is rapidly regulated by low physiological doses of insulin. *Diabetologia*. https://doi.org/10.1007/s001250051288

Jiang, S., Nandy, P., Wang, W., Ma, X., Hsia, J., Wang, C., … Zhu, X. (2018). Mfn2 ablation causes an oxidative stress response and eventual neuronal death in the hippocampus and cortex. *Molecular Neurodegeneration*. https://doi.org/10.1186/s13024-018-0238-8

Jones, F. E., Golding, J. P., & Gassmann, M. (2003). ErbB4 signaling during breast and neural development: novel genetic models reveal unique ErbB4 activities. *Cell Cycle (Georgetown, Tex.)*. https://doi.org/10.4161/cc.2.6.598

Jones, J. T., Akita, R. W., & Sliwkowski, M. X. (1999a). Binding specificities and affinities of egf domains for ErbB receptors. *FEBS Letters*. https://doi.org/10.1016/S0014-5793(99)00283-5

Jones, S. M., Klinghoffer, R., Prestwich, G. D., Toker, A., & Kazlauskas, A. (1999b). PDGF induces an early and a late wave of PI 3-kinase activity, and only the late wave is required for progression through G1. *Current Biology*. https://doi.org/10.1016/S0960-9822(99)80235-8

Juge-Aubry, C. E., Gorla-Bajszczak, A., Pernin, A., Lemberger, T., Wahli, W., Burger, A. G., & Meier, C. A. (1995). Peroxisome proliferator-activated receptor mediates cross-talk with thyroid hormone receptor by competition for retinoid X receptor. Possible role of a leucine zipper-like heptad repeat. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.270.30.18117

Jung, C. H., Jun, C. B., Ro, S. H., Kim, Y. M., Otto, N. M., Cao, J., … Kim, D. H. (2009). ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Molecular Biology of the Cell*. https://doi.org/10.1091/mbc.E08-12-1249

Kahn, S. E., Hull, R. L., & Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. https://doi.org/10.1038/nature05482

Katz, M., Amit, I., & Yarden, Y. (2007). Regulation of MAPKs by growth factors and receptor tyrosine kinases. *Biochimica et Biophysica Acta - Molecular Cell Research*. https://doi.org/10.1016/j.bbamcr.2007.01.002

Kawaguchi, T., Osatomi, K., Yamashita, H., Kabashima, T., & Uyeda, K. (2002). Mechanism for fatty acid "sparing" effect on glucose-induced transcription: Regulation of carbohydrate-responsive element-binding protein by AMP-activated protein kinase. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M107895200

Kawamoto, T., Sato, J. D., Le, A., Polikoff, J., Sato, G. H., & Mendelsohn, J. (1983). Growth stimulation of A431 cells by epidermal growth factor: Identification of high-affinity receptors for epidermal

growth factor by an anti-receptor monoclonal antibody. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.80.5.1337

Kawanishi, M., Tamori, Y., Okazawa, H., Araki, S., Shinoda, H., & Kasuga, M. (2000). Role of SNAP23 in insulin-induced translocation of GLUT4 in 3T3-L1 adipocytes: Mediation of complex formation between syntaxin4 and VAMP2. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.275.11.8240

Kayano, T., Fukumoto, H., Eddy, R. L., Fan, Y. S., Byers, M. G., Shows, T. B., & Bell, G. I. (1988). Evidence for a family of human glucose transporter-like proteins. Sequence and gene localization of a protein expressed in fetal skeletal muscle and other tissues. *Journal of Biological Chemistry*.

Kim, J. B., Wright, H. M., Wright, M., & Spiegelman, B. M. (1998). ADD1/SREBP1 activates PPARγ through the production of endogenous ligand. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.95.8.4333

Kim, M., Park, H. L., Park, H. W., Ro, S. H., Nam, S. G., Reed, J. M., … Lee, J. H. (2013). Drosophila Fip200 is an essential regulator of autophagy that attenuates both growth and aging. *Autophagy*. https://doi.org/10.4161/auto.24811

Klapper, L. N., Glathe, S., Vaisman, N., Hynes, N. E., Andrews, G. C., Sela, M., & Yarden, Y. (1999). The erbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.96.9.4995

Klemm, D. J., Leitner, J. W., Watson, P., Nesterova, A., Reusch, J. E.-B., Goalstone, M. L., & Draznin, B. (2001). Insulin-induced Adipocyte Differentiation. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.m103382200

Klip, A., McGraw, T. E., & James, D. E. (2019). Thirty sweet years of GLUT4. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.REV119.008351

Knudsen, J. G., Murholm, M., Carey, A. L., Biensø, R. S., Basse, A. L., Allen, T. L., … Pilegaard, H. (2014). Role of IL-6 in exercise training- and cold-induced UCP1 expression in subcutaneous white adipose tissue. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0084910

Koh, H., Jee, K., Lee, B., Kim, J., Kim, D., Yun, Y. H., … Chung, J. (1999). Cloning and characterization of a nuclear S6 kinase, S6 kinase-related kinase (SRK); a novel nuclear target of Akt. *Oncogene*. https://doi.org/10.1038/sj.onc.1202895

Kopp, E., & Ghosh, S. (1994). Inhibition of NF-κB by sodium salicylate and aspirin. *Science*. https://doi.org/10.1126/science.8052854

Kounakis, K., Chaniotakis, M., Markaki, M., & Tavernarakis, N. (2019). Emerging roles of lipophagy in health and disease. *Frontiers in Cell and Developmental Biology*. https://doi.org/10.3389/fcell.2019.00185

Kralisch, S., Klein, J., Lossner, U., Bluher, M., Paschke, R., Stumvoll, M., & Fasshauer, M. (2005). Isoproterenol, TNFα, and insulin downregulate adipose triglyceride lipase in 3T3-L1 adipocytes. *Molecular and Cellular Endocrinology*. https://doi.org/10.1016/j.mce.2005.06.002

Kudo, M., Sugawara, A., Uruno, A., Takeuchi, K., & Ito, S. (2004). Transcription suppression of peroxisome proliferator-activated receptor γ2 gene expression by tumor necrosis factor α via an inhibition of CCAAT/enhancer-binding protein δ during the early stage of adipocyte differentiation. *Endocrinology*. https://doi.org/10.1210/en.2004-0180

Kuramochi, Y., Cote, G. M., Guo, X., Lebrasseur, N. K., Cui, L., Liao, R., & Sawyer, D. B. (2004). Cardiac endothelial cells regulate reactive oxygen species-induced cardiomyocyte apoptosis through neuregulin-1β/erbB4 signaling. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M408662200

Ky, B., Kimmel, S. E., Safa, R. N., Putt, M. E., Sweitzer, N. K., Fang, J. C., … Cappola, T. P. (2009). Neuregulin-1β is associated with disease severity and adverse outcomes in chronic heart failure. *Circulation*. https://doi.org/10.1161/CIRCULATIONAHA.109.856310

Lacey, C. A., Mitchell, W. J., Dadelahi, A. S., & Skyberg, J. A. (2018). Caspase-1 and caspase-11 mediate pyroptosis, inflammation, and control of Brucella joint infection. *Infection and Immunity*. https://doi.org/10.1128/IAI.00361-18

Larance, M., Ramm, G., Stöckli, J., Van Dam, E. M., Winata, S., Wasinger, V., … James, D. E. (2005). Characterization of the role of the Rab GTPase-activating protein AS160 in insulin-regulated GLUT4 trafficking. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M503897200

Lauterbach, M. A. R., & Wunderlich, F. T. (2017). Macrophage function in obesity-induced inflammation and insulin resistance. *Pflugers Archiv European Journal of Physiology*. https://doi.org/10.1007/s00424-017-1955-5

Lawrence, T. (2009). The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harbor Perspectives in Biology*. https://doi.org/10.1101/cshperspect.a001651

Lebrasseur, N. K., Coté, G. M., Miller, T. A., Fielding, R. A., & Sawyer, D. B. (2003). Regulation of neuregulin/ErbB signaling by contractile activity in skeletal muscle. *American Journal of Physiology - Cell Physiology*. https://doi.org/10.1152/ajpcell.00487.2002

Lee, H., Lee, I. S., & Choue, R. (2013). Obesity, inflammation and diet. *Pediatric Gastroenterology, Hepatology and Nutrition*. https://doi.org/10.5223/pghn.2013.16.3.143

Lee, J. H., Park, A., Oh, K. J., Lee, S. C., Kim, W. K., & Bae, K. H. (2019). The role of adipose tissue mitochondria: Regulation of mitochondrial function for the treatment of metabolic diseases. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms20194924

Lee, K. F., Simon, H., Chen, H., Bates, B., Hung, M. C., & Hauser, C. (1995). Requirement for neuregulin receptor erbB2 in neural and cardiac development. *Nature*. https://doi.org/10.1038/378394a0

Lee, Y. S., Kim, J. W., Osborne, O., Oh, D. Y., Sasik, R., Schenk, S., … Olefsky, J. M. (2014). Increased adipocyte O2 consumption triggers HIF-1α, causing inflammation and insulin resistance in obesity. *Cell*. https://doi.org/10.1016/j.cell.2014.05.012

Li, M., Paran, C., Wolins, N. E., & Horowitz, J. F. (2011). High muscle lipid content in obesity is not due to enhanced activation of key triglyceride esterification enzymes or the suppression of lipolytic proteins. *American Journal of Physiology - Endocrinology and Metabolism*. https://doi.org/10.1152/ajpendo.00316.2010

Li, M., Zhao, L., Liu, J., Liu, A., Jia, C., Ma, D., … Bai, X. (2010). Multi-mechanisms are involved in reactive oxygen species regulation of mTORC1 signaling. *Cellular Signalling*. https://doi.org/10.1016/j.cellsig.2010.05.015

Li, Q., & Engelhardt, J. F. (2006). Interleukin-1β induction of NFκB is partially regulated by H2O2-mediated activation of NFκB-inducing kinase. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M511153200

Li, Y., Wang, Y., Kim, E., Beemiller, P., Wang, C. Y., Swanson, J., … Guan, K. L. (2007). Bnip3 mediates the hypoxia-induced inhibition on mammalian target of rapamycin by interacting with Rheb. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M705231200

Li, Y., Xu, S., Mihaylova, M. M., Zheng, B., Hou, X., Jiang, B., … Zang, M. (2011). AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metabolism*. https://doi.org/10.1016/j.cmet.2011.03.009

Lien, C. C., Au, L. C., Tsai, Y. L., Ho, L. T., & Juan, C. C. (2009). Short-term regulation of tumor necrosis factor-α-induced lipolysis in 3T3-L1 adipocytes is mediated through the inducible nitric oxide synthase/nitric oxide-dependent pathway. *Endocrinology*. https://doi.org/10.1210/en.2009-0403

Linggi, B., & Carpenter, G. (2006). ErbB receptors: new insights on mechanisms and biology. *Trends in Cell Biology*. https://doi.org/10.1016/j.tcb.2006.10.008

Liu, H. Y., Cao, S. Y., Hong, T., Han, J., Liu, Z., & Cao, W. (2009). Insulin is a stronger inducer of insulin resistance than hyperglycemia in mice with type 1 diabetes mellitus (T1DM). *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M109.016675

Liu, J., Lee, P., Galbiati, F., Kitsis, R. N., & Lisanti, M. P. (2001). Caveolin-1 expression sensitizes fibroblastic and epithelial cells to apoptotic stimulation. *American Journal of Physiology - Cell Physiology*. https://doi.org/10.1152/ajpcell.2001.280.4.c823

Liu, L. Z., Zhao, H. L., Zuo, J., Ho, S. K. S., Chan, J. C. N., Meng, Y., … Tong, P. C. Y. (2006). Protein kinase Cζ mediates insulin-induced glucose transport through actin remodeling in L6 muscle cells. *Molecular Biology of the Cell*. https://doi.org/10.1091/mbc.E05-10-0969

Liu, T., Zhang, L., Joo, D., & Sun, S. C. (2017). NF-κB signaling in inflammation. *Signal Transduction and Targeted Therapy*. https://doi.org/10.1038/sigtrans.2017.23

Liu, X., Zhang, Z., Ruan, J., Pan, Y., Magupalli, V. G., Wu, H., & Lieberman, J. (2016). Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature*. https://doi.org/10.1038/nature18629

López-Soldado, I., Niisuke, K., Veiga, C., Adrover, A., Manzano, A., Martínez-Redondo, V., … Gumà, A. (2016). Neuregulin improves response to glucose tolerance test in control and diabetic rats. *American Journal of Physiology - Endocrinology and Metabolism*. https://doi.org/10.1152/ajpendo.00226.2015

Lu, X., Altshuler-Keylin, S., Wang, Q., Chen, Y., Sponton, C. H., Ikeda, K., … Kajimura, S. (2018). Mitophagy controls beige adipocyte maintenance through a Parkin-dependent and UCP1-independent mechanism. *Science Signaling*. https://doi.org/10.1126/scisignal.aap8526

Lumeng, C. N., & Saltiel, A. R. (2011). Inflammatory links between obesity and metabolic disease. *Journal of Clinical Investigation*. https://doi.org/10.1172/JCI57132

Lumeng, C. N., Bodzin, J. L., & Saltiel, A. R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *Journal of Clinical Investigation*. https://doi.org/10.1172/JCI29881

Luo, L., & Liu, M. (2016). Adipose tissue in control of metabolism. *Journal of Endocrinology*. https://doi.org/10.1530/JOE-16-0211

Luo, Y., Xu, W., Li, G., & Cui, W. (2018). Weighing in on mTOR complex 2 signaling: The expanding role in cell metabolism. *Oxidative Medicine and Cellular Longevity*. https://doi.org/10.1155/2018/7838647

Ma, L., Huang, Y. Z., Pitcher, G. M., Valtschanoff, J. G., Ma, Y. H., Feng, L. Y., … Mei, L. (2003). Ligand-dependent recruitment of the ErbB4 signaling complex into neuronal lipid rafts. *Journal of Neuroscience*. https://doi.org/10.1523/jneurosci.23-08-03164.2003

Ma, Y., Gao, M., & Liu, D. (2016). Preventing High Fat Diet-induced Obesity and Improving Insulin Sensitivity through Neuregulin 4 Gene Transfer. *Scientific Reports*. https://doi.org/10.1038/srep26242

Maekawa, H., Inoue, T., Ouchi, H., Jao, T. M., Inoue, R., Nishi, H., … Inagi, R. (2019). Mitochondrial Damage Causes Inflammation via cGAS-STING Signaling in Acute Kidney Injury. *Cell Reports*. https://doi.org/10.1016/j.celrep.2019.09.050

Mafakheri, S., Flörke, R. R., Kanngießer, S., Hartwig, S., Espelage, L., De Wendt, C., … Al-Hasani, H. (2018). AKT and AMP-activated protein kinase regulate TBC1D1 through phosphorylation and its interaction with the cytosolic tail of insulin-regulated aminopeptidase IRAP. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.RA118.005040

Maiuri, M. C., & Kroemer, G. (2019). Therapeutic modulation of autophagy: which disease comes first? *Cell Death and Differentiation*. https://doi.org/10.1038/s41418-019-0290-0

Malakar, P., Chartarifsky, L., Hija, A., Leibowitz, G., Glaser, B., Dor, Y., & Karni, R. (2016). Insulin receptor alternative splicing is regulated by insulin signaling and modulates beta cell survival. *Scientific Reports*. https://doi.org/10.1038/srep31222

Malka, F., Guillery, O., Cifuentes-Diaz, C., Guillou, E., Belenguer, P., Lombés, A., & Rojo, M. (2005). Separate fusion of outer and inner mitochondrial membranes. *EMBO Reports*. https://doi.org/10.1038/sj.embor.7400488

Man, S. M., & Kanneganti, T. D. (2015). Regulation of inflammasome activation. *Immunological Reviews*. https://doi.org/10.1111/imr.12296

Mandel, D. E., Malemud, C. J., & Askari, A. D. (2017). Idiopathic inflammatory myopathies: A review of the classification and impact of pathogenesis. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms18051084

Marcinkiewicz, A., Gauthier, D., Garcia, A., & Brasaemle, D. L. (2006). The phosphorylation of serine 492 of perilipin A directs lipid droplet fragmentation and dispersion. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M600171200

Marikovsky, M., Lavi, S., Pinkas-Kramarski, R., Karunagaran, D., Liu, N., Wen, D., & Yarden, Y. (1995). ErbB-3 mediates differential mitogenic effects of NDF/heregulin isoforms on mouse keratinocytes. *Oncogene*.

Markan, K. R. (2018). Defining "FGF21 Resistance" during obesity: Controversy, criteria and unresolved questions. *F1000Research*. https://doi.org/10.12688/f1000research.14117.1

Martin, S. K., Fitter, S., Dutta, A. K., Matthews, M. P., Walkley, C. R., Hall, M. N., … Zannettino, A. C. W. (2015). Brief report: The differential roles of mTORC1 and mTORC2 in mesenchymal stem cell differentiation. *Stem Cells*. https://doi.org/10.1002/stem.1931

Martina, J. A., Chen, Y., Gucek, M., & Puertollano, R. (2012). MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. *Autophagy*. https://doi.org/10.4161/auto.19653

Matarese, V., & Bernlohr, D. A. (1988). Purification of murine adipocyte lipid-binding protein. Characterization as a fatty acid- and retinoic acid-binding protein. *Journal of Biological Chemistry*.

McElroy, S. J., Castle, S. L., Bernard, J. K., Almohazey, D., Hunter, C. J., Bell, B. A., … Freyz, M. R. (2014). The ErbB4 Ligand Neuregulin-4 Protects against Experimental Necrotizing Enterocolitis. *American Journal of Pathology*. https://doi.org/10.1016/j.ajpath.2014.06.015

McLaughlin, S., & Murray, D. (2005). Plasma membrane phosphoinositide organization by protein electrostatics. *Nature*. https://doi.org/10.1038/nature04398

McLaughlin, T., Liu, L. F., Lamendola, C., Shen, L., Morton, J., Rivas, H., … Engleman, E. (2014). T-cell profile in adipose tissue is associated with insulin resistance and systemic inflammation in humans. *Arteriosclerosis, Thrombosis, and Vascular Biology*. https://doi.org/10.1161/ATVBAHA.114.304636

McLelland, G. L., Lee, S. A., McBride, H. M., & Fon, E. A. (2016). Syntaxin-17 delivers PINK1/parkin-dependent mitochondrial vesicles to the endolysosomal system. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.201603105

Melser, S., Chatelain, E. H., Lavie, J., Mahfouf, W., Jose, C., Obre, E., … Bénard, G. (2013). Rheb regulates mitophagy induced by mitochondrial energetic status. *Cell Metabolism*. https://doi.org/10.1016/j.cmet.2013.03.014

Miettinen, P. J., Berger, J. E., Meneses, J., Phung, Y., Pedersen, R. A., Werb, Z., & Derynck, R. (1995). Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature*. https://doi.org/10.1038/376337a0

Mîinea, C. P., Sano, H., Kane, S., Sano, E., Fukuda, M., Peränen, J., … Lienhard, G. E. (2005). AS160, the Akt substrate regulating GLUT4 translocation, has a functional Rab GTPase-activating protein domain. *Biochemical Journal*. https://doi.org/10.1042/BJ20050887

Miller, A. M., Asquith, D. L., Hueber, A. J., Anderson, L. A., Holmes, W. M., McKenzie, A. N., … Liew, F. Y. (2010). Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. *Circulation Research*. https://doi.org/10.1161/CIRCRESAHA.110.218867

Mizushima, N., Yoshimori, T., & Ohsumi, Y. (2011). The role of atg proteins in autophagosome formation. *Annual Review of Cell and Developmental Biology*. https://doi.org/10.1146/annurev-cellbio-092910-154005

Molofsky, A. B., Nussbaum, J. C., Liang, H. E., Dyken, S. J. V., Cheng, L. E., Mohapatra, A., … Locksley, R. M. (2013). Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *Journal of Experimental Medicine*. https://doi.org/10.1084/jem.20121964

Montagne, J., Radimerski, T., & Thomas, G. (2001). Insulin signaling: lessons from the Drosophila tuberous sclerosis complex, a tumor suppressor. *Science's STKE : Signal Transduction Knowledge Environment*. https://doi.org/10.1126/scisignal.1052001pe36

Moore, H. P. H., Silver, R. B., Mottillo, E. P., Bernlohr, D. A., & Granneman, J. G. (2005). Perilipin targets a novel pool of lipid droplets for lipolytic attack by hormone-sensitive lipase. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M506336200

Moorthy, A. K., Savinova, O. V., Ho, J. Q., Wang, V. Y. F., Vu, D., & Ghosh, G. (2006). The 20S proteasome processes NF-κB1 p105 into p50 in a translation-independent manner. *EMBO Journal*. https://doi.org/10.1038/sj.emboj.7601081

Mora, S., & Pessin, J. E. (2002). An adipocentric view of signaling and intracellular trafficking. *Diabetes/Metabolism Research and Reviews*. https://doi.org/10.1002/dmrr.321

Morano, M., Ronchi, G., Nicolò, V., Fornasari, B. E., Crosio, A., Perroteau, I., … Raimondo, S. (2018). Modulation of the Neuregulin 1/ErbB system after skeletal muscle denervation and reinnervation. *Scientific Reports*. https://doi.org/10.1038/s41598-018-23454-8

Morgan, M. J., & Liu, Z. G. (2011). Crosstalk of reactive oxygen species and NF-κB signaling. *Cell Research*. https://doi.org/10.1038/cr.2010.178

Morganstein, D. L., Christian, M., Turner, J. J. O., Parker, M. G., & White, R. (2008). Conditionally immortalized white preadipocytes: A novel adipocyte model. *Journal of Lipid Research*. https://doi.org/10.1194/jlr.D700029-JLR200

Mota, J. M., Collier, K. A., Costa, R. L. B., Taxter, T., Kalyan, A., Leite, C. A., … Carneiro, B. A. (2017). A comprehensive review of heregulins, HER3, and HER4 as potential therapeutic targets in cancer. *Oncotarget*. https://doi.org/10.18632/oncotarget.18467

Mrschtik, M., & Ryan, K. M. (2015). Lysosomal proteins in cell death and autophagy. *FEBS Journal*. https://doi.org/10.1111/febs.13253

Mueckler, M., & Holman, G. (1995). Homeostasis without a GLUT. *Nature*. https://doi.org/10.1038/377100a0

Muñoz, J. P., & Zorzano, A. (2015). Analysis of mitochondrial morphology and function under conditions of mitofusin 2 deficiency. In *Mitochondrial Medicine*. https://doi.org/10.1007/978-1-4939-2288-8_21

Muñoz, J. P., Ivanova, S., Sánchez-Wandelmer, J., Martínez-Cristóbal, P., Noguera, E., Sancho, A., … Zorzano, A. (2013). Mfn2 modulates the UPR and mitochondrial function via repression of PERK. *EMBO Journal*. https://doi.org/10.1038/emboj.2013.168

Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *Biochemical Journal*. https://doi.org/10.1042/BJ20081386

Nakae, J., Kitamura, T., Kitamura, Y., Biggs, W. H., Arden, K. C., & Accili, D. (2003). The forkhead transcription factor Fox01 regulates adipocyte differentiation. *Developmental Cell*. https://doi.org/10.1016/S1534-5807(02)00401-X

Narendra, D. P., Jin, S. M., Tanaka, A., Suen, D. F., Gautier, C. A., Shen, J., … Youle, R. J. (2010). PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biology*. https://doi.org/10.1371/journal.pbio.1000298

Neishabouri, S. H., Hutson, S. M., & Davoodi, J. (2015). Chronic activation of mTOR complex 1 by branched chain amino acids and organ hypertrophy. *Amino Acids*. https://doi.org/10.1007/s00726-015-1944-y

Newgard, C. B., & Denis McGarry, J. (1995). Metabolic coupling factors in pancreatic β-cell signal transduction. *Annual Review of Biochemistry*. https://doi.org/10.1146/annurev.bi.64.070195.003353

Newman, L. E., & Shadel, G. S. (2018). Pink1/Parkin link inflammation, mitochondrial stress, and neurodegeneration. *Journal of Cell Biology*. https://doi.org/10.1083/JCB.201808118

Nicod, N., Giusti, V., Besse, C., & Tappy, L. (2003). Metabolic adaptations to dexamethasone-induced insulin resistance in healthy volunteers. *Obesity Research*. https://doi.org/10.1038/oby.2003.90

Nie, Q., Wang, C., Song, G., Ma, H., Kong, D., Zhang, X., … Tang, Y. (2014). Mitofusin 2 deficiency leads to oxidative stress that contributes to insulin resistance in rat skeletal muscle cells. *Molecular Biology Reports*. https://doi.org/10.1007/s11033-014-3584-9

Nie, S. D., Li, X., Tang, C. E., Min, F. Y., Shi, X. J., Wu, L. Y., … Wang, S. (2018). High glucose forces a positive feedback loop connecting ErbB4 expression and mTOR/S6K pathway to aggravate the formation of tau hyperphosphorylation in differentiated SH-SY5Y cells. *Neurobiology of Aging*. https://doi.org/10.1016/j.neurobiolaging.2018.03.023

Nielsen, M. S., Madsen, P., Christensen, E. I., Nykjær, A., Gliemann, J., Kasper, D., … Petersen, C. M. (2001). The sortilin cytoplasmic tail conveys Golgi-endosome transport and binds the VHS domain of the GGA2 sorting protein. *EMBO Journal*. https://doi.org/10.1093/emboj/20.9.2180

Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., … Nagai, R. (2009). CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nature Medicine*. https://doi.org/10.1038/nm.1964

Nugroho, D. B., Ikeda, K., Barinda, A. J., Wardhana, D. A., Yagi, K., Miyata, K., … Emoto, N. (2018a). Neuregulin-4 is an angiogenic factor that is critically involved in the maintenance of adipose tissue vasculature. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2018.06.043

Nugroho, D. B., Ikeda, K., Kajimoto, K., Hirata, K. ichi, & Emoto, N. (2018b). Activation of neuregulin-4 in adipocytes improves metabolic health by enhancing adipose tissue angiogenesis. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2018.08.197

Olayioye, M. A., Beuvink, I., Horsch, K., Daly, J. M., & Hynes, N. E. (1999). ErbB receptor-induced activation of Stat transcription factors is mediated by Src tyrosine kinases. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.274.24.17209

Olson, A. L., Knight, J. B., & Pessin, J. E. (1997). Syntaxin 4, VAMP2, and/or VAMP3/cellubrevin are functional target membrane and vesicle SNAP receptors for insulin-stimulated GLUT4 translocation in adipocytes. *Molecular and Cellular Biology*. https://doi.org/10.1128/mcb.17.5.2425

Ost, M., Coleman, V., Voigt, A., van Schothorst, E. M., Keipert, S., van der Stelt, I., … Klaus, S. (2016). Muscle mitochondrial stress adaptation operates independently of endogenous FGF21 action. *Molecular Metabolism*. https://doi.org/10.1016/j.molmet.2015.11.002

Özcelik, C., Erdmann, B., Pilz, B., Wettschureck, N., Britsch, S., Hübner, N., … Garratt, A. N. (2002). Conditional mutation of the ErbB2 (HER2) receptor in cardiomyocytes leads to dilated cardiomyopathy. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.122249299

Pagano, E., & Calvo, J. C. (2003). ErbB2 and EGFR are downmodulated during the differentiation of 3T3-L1 preadipocytes. *Journal of Cellular Biochemistry*. https://doi.org/10.1002/jcb.10647

Pardridge, W. M., Boado, R. J., & Farrell, C. R. (1990). Brain-type glucose transporter (GLUT-1) is selectively localized to the blood-brain barrier: Studies with quantitative Western blotting and in situ hybridization. *Journal of Biological Chemistry*.

Park, J., Lee, S. B., Lee, S., Kim, Y., Song, S., Kim, S., … Chung, J. (2006). Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature*. https://doi.org/10.1038/nature04788

Pedersen, B. K., Steensberg, A., Keller, P., Keller, C., Fischer, C., Hiscock, N., … Febbraio, M. A. (2003). Muscle-derived interleukin-6: Lipolytic, anti-inflammatory and immune regulatory effects. *Pflugers Archiv European Journal of Physiology*. https://doi.org/10.1007/s00424-002-0981-z

Peles, E., Bacus, S. S., Koski, R. A., Lu, H. S., Wen, D., Ogden, S. G., … Yarden, Y. (1992). Isolation of the Neu HER-2 stimulatory ligand: A 44 kd glycoprotein that induces differentiation of mammary tumor cells. *Cell*. https://doi.org/10.1016/0092-8674(92)90131-U

Pellegrinelli, V., Peirce, V. J., Howard, L., Virtue, S., Türei, D., Senzacqua, M., … Vidal-Puig, A. (2018). Adipocyte-secreted BMP8b mediates adrenergic-induced remodeling of the neuro-vascular network in adipose tissue. *Nature Communications*. https://doi.org/10.1038/s41467-018-07453-x

Peppler, W. T., Townsend, L. K., Meers, G. M., Panasevich, M. R., Macpherson, R. E. K., Rector, R. S., & Wright, D. C. (2019). Acute administration of IL-6 improves indices of hepatic glucose and insulin homeostasis in lean and obese mice. *American Journal of Physiology - Gastrointestinal and Liver Physiology*. https://doi.org/10.1152/ajpgi.00097.2018

Perera, H. K. I., Clarke, M., Morris, N. J., Hong, W., Chamberlain, L. H., & Gould, G. W. (2003). Syntaxin 6 regulates Glut4 trafficking in 3T3-L1 adipocytes. *Molecular Biology of the Cell*. https://doi.org/10.1091/mbc.E02-11-0722

Pietras, R. J., Arboleda, J., Reese, D. M., Wongvipat, N., Pegram, M. D., Ramos, L., … Slamon, D. J. (1995). HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. *Oncogene*.

Pinkas-Kramarski, R., Soussan, L., Waterman, H., Levkowitz, G., Alroy, I., Klapper, L., … Yarden, Y. (1996). Diversification of Neu differentiation factor and epidermal growth factor signaling by combinatorial receptor interactions. *EMBO Journal*. https://doi.org/10.1002/j.1460-2075.1996.tb00603.x

Pinzani, M., Knauss, T. C., Pierce, G. F., Hsieh, P., Kenney, W., Dubyak, G. R., & Abboud, H. E. (1991). Mitogenic signals for platelet-derived growth factor isoforms in liver fat-storing cells. *American Journal of Physiology - Cell Physiology*. https://doi.org/10.1152/ajpcell.1991.260.3.c485

Plowman, G. D., Culouscou, J. M., Whitney, G. S., Green, J. M., Carlton, G. W., Foy, L., … Shoyab, M. (1993). Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.90.5.1746

Plowman, G. D., Whitney, G. S., Neubauer, M. G., Green, J. M., McDonald, V. L., Todaro, G. J., & Shoyab, M. (1990). Molecular cloning and expression of an additional epidermal growth factor receptor-related gene. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.87.13.4905

Poulain-Godefroy, O., Lecoeur, C., Pattou, F., Frühbeck, G., & Froguel, P. (2008). Inflammation is associated with a decrease of lipogenic factors in omental fat in women. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*. https://doi.org/10.1152/ajpregu.00926.2007

Puri, C., Vicinanza, M., & Rubinsztein, D. C. (2018). Phagophores evolve from recycling endosomes. *Autophagy*. https://doi.org/10.1080/15548627.2018.1482148

Qin, X., Jiang, B., & Zhang, Y. (2016). 4E-BP1, a multifactor regulated multifunctional protein. *Cell Cycle*. https://doi.org/10.1080/15384101.2016.1151581

Qiu, Y., Ravi, L., & Kung, H. J. (1998). Requirement of ErbB2 for signalling by interleukin-6 in prostate carcinoma cells. *Nature*. https://doi.org/10.1038/30012

Quinlan, C. L., Perevoshchikova, I. V., Hey-Mogensen, M., Orr, A. L., & Brand, M. D. (2013). Sites of reactive oxygen species generation by mitochondria oxidizing different substrates. *Redox Biology*. https://doi.org/10.1016/j.redox.2013.04.005

Rabanal-Ruiz, Y., Otten, E. G., & Korolchuk, V. I. (2017). MTORC1 as the main gateway to autophagy. *Essays in Biochemistry*. https://doi.org/10.1042/EBC20170027

Ramm, G., Slot, J. W., James, D. E., & Stoorvogel, W. (2000). Insulin recruits GLUT4 from specialized VAMP2-carrying vesicles as well as from the dynamic endosomal/trans-golgi network in rat adipocytes. *Molecular Biology of the Cell*. https://doi.org/10.1091/mbc.11.12.4079

Rao, R. R., Long, J. Z., White, J. P., Svensson, K. J., Lou, J., Lokurkar, I., … Spiegelman, B. M. (2014). Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. *Cell*. https://doi.org/10.1016/j.cell.2014.03.065

Rehman, K., & Akash, M. S. H. (2016). Mechanisms of inflammatory responses and development of insulin resistance: How are they interlinked? *Journal of Biomedical Science*. https://doi.org/10.1186/s12929-016-0303-y

Reilly, S. M., & Saltiel, A. R. (2017). Adapting to obesity with adipose tissue inflammation. *Nature Reviews Endocrinology*. https://doi.org/10.1038/nrendo.2017.90

Ren, M., Xu, G., Zeng, J., De Lemos-Chiarandini, C., Adesnik, M., & Sabatini, D. D. (1998). Hydrolysis of GTP on rab11 is required for the direct delivery of transferrin from the pericentriolar recycling compartment to the cell surface but not from sorting endosomes. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.95.11.6187

Ribon, V., Printen, J. A., Hoffman, N. G., Kay, B. K., & Saltiel, A. R. (1998). A Novel, Multifunctional c-Cbl Binding Protein in Insulin Receptor Signaling in 3T3-L1 Adipocytes. *Molecular and Cellular Biology*. https://doi.org/10.1128/mcb.18.2.872

Richard, E., Gallego-Villar, L., Rivera-Barahona, A., Oyarzábal, A., Pérez, B., Rodríguez-Pombo, P., & Desviat, L. R. (2018). Altered redox homeostasis in branched-chain amino acid disorders, organic acidurias, and homocystinuria. *Oxidative Medicine and Cellular Longevity*. https://doi.org/10.1155/2018/1246069

Rimessi, A., Previati, M., Nigro, F., Wieckowski, M. R., & Pinton, P. (2016). Mitochondrial reactive oxygen species and inflammation: Molecular mechanisms, diseases and promising therapies. *International Journal of Biochemistry and Cell Biology*. https://doi.org/10.1016/j.biocel.2016.06.015

Robinson, L. J., Pang, S., Harris, D. S., Heuser, J., & James, D. E. (1992). Translocation of the glucose transporter (GLUT4) to the cell surface in permeabilized 3T3-L1 adipocytes: Effects of ATP, insulin, and GTPγS and localization of GLUT4 to clathrin lattices. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.117.6.1181

Robinson, M. W., Harmon, C., & O'Farrelly, C. (2016). Liver immunology and its role in inflammation and homeostasis. *Cellular and Molecular Immunology*. https://doi.org/10.1038/cmi.2016.3

Rodríguez-Nuevo, A., & Zorzano, A. (2019). The sensing of mitochondrial DAMPs by non-immune cells. *Cell Stress*. https://doi.org/10.15698/cst2019.06.190

Rodríguez-Nuevo, A., Díaz-Ramos, A., Noguera, E., Díaz-Sáez, F., Duran, X., Muñoz, J. P., … Zorzano, A. (2018). Mitochondrial DNA and TLR9 drive muscle inflammation upon Opa1 deficiency. *The EMBO Journal*. https://doi.org/10.15252/embj.201796553

Rong, Y., Liu, M., Ma, L., Du, W., Zhang, H., Tian, Y., … Yu, L. (2012). Clathrin and phosphatidylinositol-4,5-bisphosphate regulate autophagic lysosome reformation. *Nature Cell Biology*. https://doi.org/10.1038/ncb2557

Ros-Baró, A., López-Iglesias, C., Peiró, S., Bellido, D., Palacín, M., Zorzano, A., & Camps, M. (2001). Lipid rafts are required for GLUT4 internalization in adipose cells. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.211341698

Rosell, M., Kaforou, M., Frontini, A., Okolo, A., Chan, Y. W., Nikolopoulou, E., … Christian, M. (2014). Brown and white adipose tissues: Intrinsic differences in gene expression and response to cold exposure in mice. *American Journal of Physiology - Endocrinology and Metabolism*. https://doi.org/10.1152/ajpendo.00473.2013

Ross, J. S., & Fletcher, J. A. (1998). The HER-2/neu oncogene in breast cancer: Prognostic factor, predictive factor, and target for therapy. *Stem Cells*. https://doi.org/10.1002/stem.160413

Rotondo, F., Ho-Palma, A. C., Remesar, X., Fernández-López, J. A., Romero, M. D. M., & Alemany, M. (2017). Glycerol is synthesized and secreted by adipocytes to dispose of excess glucose, via glycerogenesis and increased acyl-glycerol turnover. *Scientific Reports*. https://doi.org/10.1038/s41598-017-09450-4

Rotter, V., Nagaev, I., & Smith, U. (2003). Interleukin-6 (IL-6) Induces Insulin Resistance in 3T3-L1 Adipocytes and Is, Like IL-8 and Tumor Necrosis Factor-α, Overexpressed in Human Fat Cells from Insulin-resistant Subjects. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M301977200

Ruan, H., Hacohen, N., Golub, T. R., Van Parijs, L., & Lodish, H. F. (2002). Tumor necrosis factor-α suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes: Nuclear factor-κB activation by TNF-α is obligatory. *Diabetes*. https://doi.org/10.2337/diabetes.51.5.1319

Rydén, M., Dicker, A., Van Harmelen, V., Hauner, H., Brunnberg, M., Perbeck, L., … Arner, P. (2002). Mapping of early signaling events in tumor necrosis factor-α-mediated lipolysis in human fat cells. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M109498200

Saad, M. J. A., Santos, A., & Prada, P. O. (2016). Linking gut microbiota and inflammation to obesity and insulin resistance. *Physiology*. https://doi.org/10.1152/physiol.00041.2015

Samra, J. S., Clark, M. L., Humphreys, S. M., Macdonald, I. A., Bannister, P. A., & Frayn, K. N. (1998). Effects of physiological hypercortisolemia on the regulation of lipolysis in subcutaneous adipose tissue. *Journal of Clinical Endocrinology and Metabolism*. https://doi.org/10.1210/jc.83.2.626

Sano, H., Eguez, L., Teruel, M. N., Fukuda, M., Chuang, T. D., Chavez, J. A., … McGraw, T. E. (2007). Rab10, a Target of the AS160 Rab GAP, Is Required for Insulin-Stimulated Translocation of GLUT4 to the Adipocyte Plasma Membrane. *Cell Metabolism*. https://doi.org/10.1016/j.cmet.2007.03.001

Saxton, R. A., & Sabatini, D. M. (2017). mTOR Signaling in Growth, Metabolism, and Disease. *Cell*. https://doi.org/10.1016/j.cell.2017.02.004

Scheepers, A., Joost, H. G., & Schürmann, A. (2004). The glucose transporter families SGLT and GLUT: Molecular basis of normal and aberrant function. *Journal of Parenteral and Enteral Nutrition*. https://doi.org/10.1177/0148607104028005364

Scherer, T., & Buettner, C. (2011). Yin and Yang of hypothalamic insulin and leptin signaling in regulating white adipose tissue metabolism. *Reviews in Endocrine and Metabolic Disorders*. https://doi.org/10.1007/s11154-011-9190-4

Scherz-Shouval, R., & Elazar, Z. (2011). Regulation of autophagy by ROS: Physiology and pathology. *Trends in Biochemical Sciences*. https://doi.org/10.1016/j.tibs.2010.07.007

Schreiber, A. B., Libermann, T. A., Lax, I., Yarden, Y., & Schlessinger, J. (1983). Biological role of epidermal growth factor-receptor clustering. Investigation with monoclonal anti-receptor antibodies. *Journal of Biological Chemistry*.

Schumacher, M. A., Hedl, M., Abraham, C., Bernard, J. K., Lozano, P. R., Hsieh, J. J., … Frey, M. R. (2017). ErbB4 signaling stimulates pro-inflammatory macrophage apoptosis and limits colonic inflammation. *Cell Death and Disease*. https://doi.org/10.1038/cddis.2017.42

Schuppan, D., Surabattula, R., & Wang, X. Y. (2018). Determinants of fibrosis progression and regression in NASH. *Journal of Hepatology*. https://doi.org/10.1016/j.jhep.2017.11.012

Sebastián, D., Hernández-Alvarez, M. I., Segalés, J., Sorianello, E., Muñoz, J. P., Sala, D., … Zorzano, A. (2012). Mitofusin 2 (Mfn2) links mitochondrial and endoplasmic reticulum function with insulin signaling and is essential for normal glucose homeostasis. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.1108220109

Seki, E., Brenner, D. A., & Karin, M. (2012). A liver full of JNK: Signaling in regulation of cell function and disease pathogenesis, and clinical approaches. *Gastroenterology*. https://doi.org/10.1053/j.gastro.2012.06.004

Seldin, M. M., & Wong, G. W. (2012). Regulation of tissue crosstalk by skeletal muscle-derived myonectin and other myokines. *Adipocyte*. https://doi.org/10.4161/adip.20877

Šestan, M., Marinović, S., Kavazović, I., Cekinović, Đ., Wueest, S., Turk Wensveen, T., … Polić, B. (2018). Virus-Induced Interferon-γ Causes Insulin Resistance in Skeletal Muscle and Derails Glycemic Control in Obesity. *Immunity*. https://doi.org/10.1016/j.immuni.2018.05.005

Sesti, G., Marini, M. A., Tullio, A. N., Montemurro, A., Borboni, P., Fusco, A., … Lauro, R. (1991). Altered expression of the two naturally occurring human insulin receptor variants in isolated adipocytes of non-insulin-dependent diabetes mellitus patients. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/0006-291X(91)92097-4

Severinsen, M. C. K., & Pedersen, B. K. (2020). Muscle-Organ Crosstalk: The Emerging Roles of Myokines. *Endocrine Reviews*. https://doi.org/10.1210/endrev/bnaa016

Shelton, M., Ritso, M., Liu, J., O'Neil, D., Kocharyan, A., Rudnicki, M. A., … Blais, A. (2019). Gene expression profiling of skeletal myogenesis in human embryonic stem cells reveals a potential cascade of transcription factors regulating stages of myogenesis, including quiescent/activated satellite cell-like gene expression. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0222946

Shi, Y., Tan, S. H., Ng, S., Zhou, J., Yang, N. Di, Koo, G. B., … Shen, H. M. (2015). Critical role of CAV1/caveolin-1 in cell stress responses in human breast cancer cells via modulation of lysosomal function and autophagy. *Autophagy*. https://doi.org/10.1080/15548627.2015.1034411

Shiroto, T., Romero, N., Sugiyama, T., Sartoretto, J. L., Kalwa, H., Yan, Z., … Michel, T. (2014). Caveolin-1 is a critical determinant of autophagy, metabolic switching, and oxidative stress in vascular endothelium. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0087871

Smith, U., & Kahn, B. B. (2016). Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. *Journal of Internal Medicine*. https://doi.org/10.1111/joim.12540

Soltoff, S. P., & Cantley, L. C. (1996). p120cbl is a cytosolic adapter protein that associates with phosphoinositide 3-kinase in response to epidermal growth factor in PC 12 and other cells. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.271.1.563

Song, Z., Xiaoli, A. M., & Yang, F. (2018). Regulation and metabolic significance of De Novo lipogenesis in adipose tissues. *Nutrients*. https://doi.org/10.3390/nu10101383

Soriano, F. X., Liesa, M., Bach, D., Chan, D. C., Palacín, M., & Zorzano, A. (2006). Evidence for a mitochondrial regulatory pathway defined by peroxisome proliferator-activated receptor-γ coactivator-1α, estrogen-related receptor-α, and mitofusin 2. *Diabetes*. https://doi.org/10.2337/db05-0509

Sorkin, A., & Goh, L. K. (2008). Endocytosis and intracellular trafficking of ErbBs. *Experimental Cell Research*. https://doi.org/10.1016/S0014-4827(08)00404-7

Souza, S. C., De Vargas, L. M., Yamamoto, M. T., Lien, P., Franciosa, M. D., Moss, L. G., & Greenberg, A. S. (1998). Overexpression of Perilipin A and B blocks the ability of tumor necrosis factor α to increase lipolysis in 3T3-L1 adipocytes. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.273.38.24665

Stahl, A., Evans, J. G., Pattel, S., Hirsch, D., & Lodish, H. F. (2002). Insulin causes fatty acid transport protein translocation and enhanced fatty acid uptake in adipocytes. *Developmental Cell*. https://doi.org/10.1016/S1534-5807(02)00143-0

Steele-Perkins, G., Turner, J., Edman, J. C., Hari, J., Pierce, S. B., Stover, C., … Roth, R. A. (1988). Expression and characterization of a functional human insulin-like growth factor I receptor. *Journal of Biological Chemistry*.

Stephens, J. M., Lee, J., & Pilch, P. F. (1997). Tumor necrosis factor-α-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.272.2.971

Stier, A., Bize, P., Habold, C., Bouillaud, F., Massemin, S., & Criscuolo, F. (2014). Mitochondrial uncoupling prevents cold-induced oxidative stress: A case study using UCP1 knockout mice. *Journal of Experimental Biology*. https://doi.org/10.1242/jeb.092700

Strickland, D. K., Gonias, S. L., & Argraves, W. S. (2002). Diverse roles for the LDL receptor family. *Trends in Endocrinology and Metabolism*. https://doi.org/10.1016/S1043-2760(01)00526-4

Suárez, E., Bach, D., Cadefau, J., Palacín, M., Zorzano, A., & Gumà, A. (2001). A novel role of neuregulin in skeletal muscle: Neuregulin stimulates glucose uptake, glucose transporter translocation, and transporter expression in muscle cells. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M008100200

Sun, S. C. (2017). The non-canonical NF-κB pathway in immunity and inflammation. *Nature Reviews Immunology*. https://doi.org/10.1038/nri.2017.52

Szatmári, Z., Kis, V., Lippai, M., Hegedus, K., Faragó, T., Lorincz, P., … Sass, M. (2014). Rab11 facilitates cross-talk between autophagy and endosomal pathway through regulation of Hook localization. *Molecular Biology of the Cell*. https://doi.org/10.1091/mbc.E13-10-0574

Tacchini, L., De Ponti, C., Matteucci, E., Follis, R., & Desiderio, M. A. (2004). Hepatocyte growth factor-activated NF-κB regulates HIF-1 activity and ODC expression, implicated in survival, differently in different carcinoma cell lines. *Carcinogenesis*. https://doi.org/10.1093/carcin/bgh227

Takada, Y., Mukhopadhyay, A., Kundu, G. C., Mahabeleshwar, G. H., Singh, S., & Aggarwal, B. B. (2003). Hydrogen peroxide activates NF-κB through tyrosine phosphorylation of IκBα and serine phosphorylation of p65. Evidence for the involvement of IκBα kinase and Syk protein-tyrosine kinase. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M212389200

Takahashi, H., Alves, C. R. R., Stanford, K. I., Middelbeek, R. J. W., Nigro, P., Ryan, R. E., … Goodyear, L. J. (2019). TGF-β2 is an exercise-induced adipokine that regulates glucose and fatty acid metabolism. *Nature Metabolism*. https://doi.org/10.1038/s42255-018-0030-7

Takahashi, S., Kubo, K., Waguri, S., Yabashi, A., Shin, H. W., Katoh, Y., & Nakayama, K. (2012). Rab11 regulates exocytosis of recycling vesicles at the plasma membrane. *Journal of Cell Science*. https://doi.org/10.1242/jcs.102913

Tamori, Y., Masugi, J., Nishino, N., & Kasuga, M. (2002). Role of peroxisome proliferator-activated receptor-γ in maintenance of the characteristics of mature 3T3-L1 adipocytes. *Diabetes*. https://doi.org/10.2337/diabetes.51.7.2045

Tanida, I., Ueno, T., & Kominami, E. (2008). LC3 and autophagy. *Methods in Molecular Biology*. https://doi.org/10.1007/978-1-59745-157-4_4

Tanti, J. F., Grémeaux, T., Grillo, S., Calleja, V., Klippel, A., Williams, L. T., … Marchand-Brustel, Y. Le. (1996). Overexpression of a constitutively active form of phosphatidylinositol 3-kinase is sufficient to promote Glut 4 translocation in adipocytes. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.271.41.25227

Tappy, L., Randin, D., Vollenweider, P., Vollenweider, L., Paquot, N., Scherrer, U., … Jéquier, E. (1994). Mechanisms of dexamethasone-induced insulin resistance in healthy humans. *The Journal of Clinical Endocrinology & Metabolism*. https://doi.org/10.1210/jcem.79.4.7962275

Taylor, D., & Gottlieb, R. A. (2017). Parkin-mediated mitophagy is downregulated in browning of white adipose tissue. *Obesity*. https://doi.org/10.1002/oby.21786

Tezze, C., Romanello, V., Desbats, M. A., Fadini, G. P., Albiero, M., Favaro, G., … Sandri, M. (2017). Age-Associated Loss of OPA1 in Muscle Impacts Muscle Mass, Metabolic Homeostasis, Systemic Inflammation, and Epithelial Senescence. *Cell Metabolism*. https://doi.org/10.1016/j.cmet.2017.04.021

Thomanetz, V., Angliker, N., Cloëtta, D., Lustenberger, R. M., Schweighauser, M., Oliveri, F., … Rüegg, M. A. (2013). Ablation of the mTORC2 component rictor in brain or Purkinje cells affects size and neuron morphology. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.201205030

Thomas, D., & Apovian, C. (2017). Macrophage functions in lean and obese adipose tissue. *Metabolism: Clinical and Experimental*. https://doi.org/10.1016/j.metabol.2017.04.005

Threadgill, D. W., Dlugosz, A. A., Hansen, L. A., Tennenbaum, T., Lichti, U., Yee, D., … Magnuson, T. (1995). Targeted disruption of mouse EGF receptor: Effect of genetic background on mutant phenotype. *Science*. https://doi.org/10.1126/science.7618084

Todaro, G. J., & Green, H. (1963). Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.17.2.299

Uldry, M., Ibberson, M., Hosokawa, M., & Thorens, B. (2002). GLUT2 is a high affinity glucosamine transporter. *FEBS Letters*. https://doi.org/10.1016/S0014-5793(02)03058-2

Valladares, A., Roncero, C., Benito, M., & Porras, A. (2001). TNF-α inhibits UCP-1 expression in brown adipocytes via ERKs - Opposite effect of p38MAPK. *FEBS Letters*. https://doi.org/10.1016/S0014-5793(01)02264-5

Van Uden, P., Kenneth, N. S., & Rocha, S. (2008). Regulation of hypoxia-inducible factor-1αa by NF-κB. *Biochemical Journal*. https://doi.org/10.1042/BJ20080476

Villalobo, A., García-Andrés, C., & Molina-Ortiz, P. (2003). Translocation of ErbB receptors into the nucleus. *Clinical and Translational Oncology*. https://doi.org/10.1007/BF02711468

Villena, J. A., Roy, S., Sarkadi-Nagy, E., Kim, K. H., & Hei, S. S. (2004). Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: Ectopic expression of desnutrin increases triglyceride hydrolysis. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M403855200

Vittoria Barone, M., Crozat, A., Tabaee, A., Philipson, L., & Ron, D. (1994). CHOP (GADD153) and its oncogenic variant, TLS-CHOP, have opposing effects on the induction of G1/S arrest. *Genes and Development*. https://doi.org/10.1101/gad.8.4.453

Wallis, M. G., Lankford, M. F., & Keller, S. R. (2007). Vasopressin is a physiological substrate for the insulin-regulated aminopeptidase IRAP. *American Journal of Physiology - Endocrinology and Metabolism*. https://doi.org/10.1152/ajpendo.00440.2007

Wang, G. X., Zhao, X. Y., Meng, Z. X., Kern, M., Dietrich, A., Chen, Z., … Lin, J. D. (2014). The brown fat-enriched secreted factor Nrg4 preserves metabolic homeostasis through attenuation of hepatic lipogenesis. *Nature Medicine*. https://doi.org/10.1038/nm.3713

Wang, L., Li, L., Ran, X., Long, M., Zhang, M., Tao, Y., … Ren, J. (2013). Lipopolysaccharides reduce adipogenesis in 3T3-L1 adipocytes through activation of NF-κB pathway and downregulation of AMPK expression. *Cardiovascular Toxicology*. https://doi.org/10.1007/s12012-013-9214-2

Wang, P., Yang, F. J., Du, H., Guan, Y. F., Xu, T. Y., Xu, X. W., … Miao, C. Y. (2011). Involvement of leptin receptor long isoform (LepRb)-STAT3 signaling pathway in brain fat mass- and obesity-associated (FTO) downregulation during energy restriction. *Molecular Medicine*. https://doi.org/10.2119/molmed.2010.000134

Watson, R. T., Kanzaki, M., & Pessin, J. E. (2004). Regulated membrane trafficking of the insulin-responsive glucose transporter 4 in adipocytes. *Endocrine Reviews*. https://doi.org/10.1210/er.2003-0011

Watson, R. T., Shigematsu, S., Chiang, S. H., Mora, S., Kanzaki, M., Macara, I. G., … Pessin, J. E. (2001). Lipid raft microdomain compartmentalization of TC10 is required for insulin signaling and GLUT4 translocation. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.200102078

Weatherill, A. R., Lee, J. Y., Zhao, L., Lemay, D. G., Youn, H. S., & Hwang, D. H. (2005). Saturated and Polyunsaturated Fatty Acids Reciprocally Modulate Dendritic Cell Functions Mediated through TLR4. *The Journal of Immunology*. https://doi.org/10.4049/jimmunol.174.9.5390

Wilcke, M., Johannes, L., Galli, T., Mayau, V., Goud, B., & Salamero, J. (2000). Rab11 regulates the compartmentalization of early endosomes required for efficient transport from early endosomes to the trans-Golgi network. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.151.6.1207

Willems, P. H. G. M., Rossignol, R., Dieteren, C. E. J., Murphy, M. P., & Koopman, W. J. H. (2015). Redox Homeostasis and Mitochondrial Dynamics. *Cell Metabolism*. https://doi.org/10.1016/j.cmet.2015.06.006

Williams, N. C., & O'Neill, L. A. J. (2018). A role for the krebs cycle intermediate citrate in metabolic reprogramming in innate immunity and inflammation. *Frontiers in Immunology*. https://doi.org/10.3389/fimmu.2018.00141

Wolsk, E., Mygind, H., Grøndahl, T. S., Pedersen, B. K., & Van Hall, G. (2010). IL-6 selectively stimulates fat metabolism in human skeletal muscle. *American Journal of Physiology - Endocrinology and Metabolism*. https://doi.org/10.1152/ajpendo.00328.2010

Worthylake, R., & Wiley, H. S. (1997). Structural aspects of the epidermal growth factor receptor required for transmodulation of erbB-2/neu. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.272.13.8594

Wu, Q., Kazantzis, M., Doege, H., Ortegon, A. M., Tsang, B., Falcon, A., & Stahl, A. (2006). Fatty acid transport protein 1 is required for nonshivering thermogenesis in brown adipose tissue. *Diabetes*. https://doi.org/10.2337/db06-0749

Wu, X., Xu, W., Feng, X., He, Y., Liu, X., Gao, Y., … Ye, Z. (2015). TNF-a mediated inflammatory macrophage polarization contributes to the pathogenesis of steroid-induced osteonecrosis in mice. *International Journal of Immunopathology and Pharmacology*. https://doi.org/10.1177/0394632015593228

Wu, Z., Bucher, N. L., & Farmer, S. R. (1996). Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids. *Molecular and Cellular Biology*. https://doi.org/10.1128/mcb.16.8.4128

Xie, B., Chen, Q., Chen, L., Sheng, Y., Wang, H. Y., & Chen, S. (2016). The inactivation of RabGAP function of AS160 promotes lysosomal degradation of glut4 and causes postprandial hyperglycemia and hyperinsulinemia. *Diabetes*. https://doi.org/10.2337/db16-0416

Xiong, W., Ma, Z., An, D., Liu, Z., Cai, W., Bai, Y., … Xu, Di. (2019). Mitofusin 2 participates in mitophagy and mitochondrial fusion against angiotensin II-induced cardiomyocyte injury. *Frontiers in Physiology*. https://doi.org/10.3389/fphys.2019.00411

Xue, C., Liang, F., Mahmood, R., Vuolo, M., Wyckoff, J., Qian, H., … Segall, J. E. (2006). ErbB3-dependent motility and intravasation in breast cancer metastasis. *Cancer Research*. https://doi.org/10.1158/0008-5472.CAN-05-0550

Yamaguchi, T., Omatsu, N., Matsushita, S., & Osumi, T. (2004). CGI-58 interacts with perilipin and is localized to lipid droplets: Possible involvement of CGI-58 mislocalization in Chanarin-Dorfman syndrome. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M403920200

Yan, P. J., Xu, Y., Wan, Q., Feng, J., Li, H., Gao, C. L., … Zhang, Z. H. (2017). Decreased plasma neuregulin 4 concentration is associated with increased high-sensitivity C-reactive protein in newly diagnosed type 2 diabetes mellitus patients: a cross-sectional study. *Acta Diabetologica*. https://doi.org/10.1007/s00592-017-1044-4

Yan, P., Xu, Y., Wan, Q., Feng, J., Li, H., Yang, J., … Zhang, Z. (2018). Plasma neuregulin 4 levels are associated with metabolic syndrome in patients newly diagnosed with type 2 diabetes mellitus. *Disease Markers*. https://doi.org/10.1155/2018/6974191

Ye, J. (2008). Regulation of PPARγ function by TNF-α. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2008.07.068

Yeh, T. Y. J., Sbodio, J. I., Tsun, Z. Y., Luo, B., & Chi, N. W. (2007). Insulin-stimulated exocytosis of GLUT4 is enhanced by IRAP and its partner tankyrase. *Biochemical Journal*. https://doi.org/10.1042/BJ20060793

Yeh, W. C., Cao, Z., Classon, M., & McKnight, S. L. (1995). Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes and Development*. https://doi.org/10.1101/gad.9.2.168

Yekollu, S. K., Thomas, R., & O'Sullivan, B. (2011). Targeting curcusomes to inflammatory dendritic cells inhibits NF-κB and improves insulin resistance in obese mice. *Diabetes*. https://doi.org/10.2337/db11-0275

Yi, L., Shen, H., Zhao, M., Shao, P., Liu, C., Cui, J., … Zhang, X. (2017). Inflammation-mediated SOD-2 upregulation contributes to epithelial-mesenchymal transition and migration of tumor cells in aflatoxin G1-induced lung adenocarcinoma. *Scientific Reports*. https://doi.org/10.1038/s41598-017-08537-2

Yokozeki, T., Wakatsuki, S., Hatsuzawa, K., Black, R. A., Wada, I., & Sehara-Fujisawa, A. (2007). Meltrin β (ADAM19) mediates ectodomain shedding of Neuregulin β1 in the Golgi apparatus: Fluorescence correlation spectroscopic observation of the dynamics of ectodomain shedding in living cells. *Genes to Cells*. https://doi.org/10.1111/j.1365-2443.2007.01060.x

Yoo, S. Y., Yoo, J. Y., Kim, H. B., Baik, T. K., Lee, J. H., & Woo, R. S. (2019). Neuregulin-1 protects neuronal cells against damage due to CoCl2-induced hypoxia by suppressing hypoxia-inducible factor-1α and p53 in SH-SY5Y cells. *International Neurourology Journal*. https://doi.org/10.5213/inj.1938190.095

Yoshida, K., Ono, M., & Sawada, H. (1999). Lipopolysaccharide-induced vacuoles in macrophages: Their origin is plasma membrane-derived organelles and endoplasmic reticulum, but not lysosomes. *Journal of Endotoxin Research*. https://doi.org/10.1177/09680519990050030601

Young, A. R. J., Narita, M., Ferreira, M., Kirschner, K., Sadaie, M., Darot, J. F. J., … Narita, M. (2009). Autophagy mediates the mitotic senescence transition. *Genes and Development*. https://doi.org/10.1101/gad.519709

Yu, C., Cresswell, J., Löffler, M. G., & Bogan, J. S. (2007). The Glucose transporter 4-regulating protein TUG is essential for highly insulin-responsive glucose uptake in 3T3-L1 adipocytes. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M610824200

Yu, D. (2001). Mechanisms of ErbB2-mediated paclitaxel resistance and trastuzumab-mediated paclitaxel sensitization in ErbB2-overexpressing breast cancers. In *Seminars in Oncology*. https://doi.org/10.1053/sonc.2001.28545

Yu, L., Chen, Y., & Tooze, S. A. (2018). Autophagy pathway: Cellular and molecular mechanisms. *Autophagy*. https://doi.org/10.1080/15548627.2017.1378838

Zeng, F., Wang, Y., Kloepfer, L. A., Wang, S., & Harris, R. C. (2018). ErbB4 deletion predisposes to development of metabolic syndrome in mice. *American Journal of Physiology - Endocrinology and Metabolism*. https://doi.org/10.1152/ajpendo.00166.2018

Zerial, M., & McBride, H. (2001). Rab proteins as membrane organizers. *Nature Reviews Molecular Cell Biology*. https://doi.org/10.1038/35052055

Zhang, B., Berger, J., Hu, E., Szalkowski, D., White-Carrington, S., Spiegelman, B. M., & Moller, D. E. (1996). Negative regulation of peroxisome proliferator-activated receptor-gamma gene expression contributes to the antiadipogenic effects of tumor necrosis factor-alpha. *Molecular Endocrinology*. https://doi.org/10.1210/mend.10.11.8923470

Zhang, C., He, Y., Okutsu, M., Ong, L. C., Jin, Y., Zheng, L., … Yan, Z. (2013). Autophagy is involved in adipogenic differentiation by repressesing proteasome-dependent PPARγ2 degradation. *American Journal of Physiology - Endocrinology and Metabolism*. https://doi.org/10.1152/ajpendo.00640.2012

Zhang, H. H., Halbleib, M., Ahmad, F., Manganiello, V. C., & Greenberg, A. S. (2002). Tumor necrosis factor-α stimulates lipolysis in differentiated human adipocytes through activation of extracellular signal-related kinase and elevation of intracellular cAMP. *Diabetes*. https://doi.org/10.2337/diabetes.51.10.2929

Zhang, X., Ramírez, C. M., Aryal, B., Madrigal-Matute, J., Liu, X., Diaz, A., … Fernández-Hernando, C. (2020). Cav-1 (Caveolin-1) Deficiency Increases Autophagy in the Endothelium and Attenuates Vascular Inflammation and Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*. https://doi.org/10.1161/ATVBAHA.120.314291

Zhao, Y. Y., Feron, O., Dessy, C., Han, X., Marchionni, M. A., & Kelly, R. A. (1999). Neuregulin signaling in the heart: Dynamic targeting of erbB4 to caveolar microdomains in cardiac myocytes. *Circulation Research*. https://doi.org/10.1161/01.RES.84.12.1380

Zhenyukh, O., Civantos, E., Ruiz-Ortega, M., Sánchez, M. S., Vázquez, C., Peiró, C., … Mas, S. (2017). High concentration of branched-chain amino acids promotes oxidative stress, inflammation and migration of human peripheral blood mononuclear cells via mTORC1 activation. *Free Radical Biology and Medicine*. https://doi.org/10.1016/j.freeradbiomed.2017.01.009

Zhou, X., & You, S. (2014). Rosiglitazone inhibits hepatic insulin resistance induced by chronic pancreatitis and IKK-β/NF-κB expression in liver. *Pancreas*. https://doi.org/10.1097/MPA.0000000000000173

Zhu, Y., Massen, S., Terenzio, M., Lang, V., Chen-Lindner, S., Eils, R., … Brady, N. R. (2013). Modulation of serines 17 and 24 in the LC3-interacting region of Bnip3 determines pro-survival mitophagy versus apoptosis. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M112.399345

Zimmermann, R., Strauss, J. G., Haemmerle, G., Schoiswohl, G., Birner-Gruenberger, R., Riederer, M., … Zechner, R. (2004). Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science*. https://doi.org/10.1126/science.1100747

Zorzano, A., Liesa, M., Sebastián, D., Segalés, J., & Palacín, M. (2010). Mitochondrial fusion proteins: Dual regulators of morphology and metabolism. *Seminars in Cell and Developmental Biology*. https://doi.org/10.1016/j.semcdb.2010.01.002

9. Resumen en castellano

9.1 Introducción

Las neuregulinas son proteínas que participan en la señalización celular, siendo ligandos de los receptores tirosina quinasa ErbB. Constituyen una subfamilia de proteínas del factor de crecimiento epidérmico (EGF). Específicamente, las neuregulinas se unen directamente a los receptores ErbB3 y ErbB4, los cuales pueden heterodimerizar con ErbB2 o ErbB1 (también conocido como el receptor del factor de crecimiento epidérmico, (EGFR)) para inducir señalización celular. En el año 2014 se descubrió que una de las isoformas de neuregulina, la neuregulina-4 (NRG4), era altamente expresada en el tejido adiposo de ratón, concretamente en el tejido adiposo marrón (BAT) (*Wang et al., 2014*). Los autores determinaron mediante ensayos de unión a receptor que la NRG4 solo podía unirse directamente al receptor ErbB4, tal y como otros autores ya habían demostrado previamente (*Harari et al., 1999*). Además, los autores generaron ratones que carecían del gen *Nrg4* (KO) y encontraron que incrementaba la expresión de varios genes lipogénicos en el hígado de estos ratones. Por el contrario, los autores describieron que la lipogénesis hepática se veía atenuada en ratones transgénicos que sobre expresaban *Nrg4*. Además, los niveles de triacilglicéridos (TAG) en plasma se veían reducidos y los animales ganaban menos peso corporal cuando eran alimentados con una dieta alta en grasas (HFD). En este contexto, otros autores concluyeron que la NRG4 constituye un factor protector contra los efectos deletéreos de la obesidad, incrementando la sensibilidad a la insulina en ratones (*Chen et al., 2017*).

En otro estudio fue descrito que NRG4 es un factor molecular clave en la progresión de la esteatosis hepática hacia esteatohepatitis no alcohólica (NASH) (*Guo et al., 2017*). Por lo tanto, en todos los estudios mencionados se subraya la importancia del factor de crecimiento NRG4 en la regulación del metabolismo hepático. Además del rol de la NRG4 en el metabolismo hepático, ahora sabemos que es una adipoquina expresada en el BAT y en el tejido adiposo blanco (WAT) que ejerce multitud de funciones que contribuyen al mantenimiento del correcto funcionamiento metabólico en ratones (*Gumà et al., 2020*). Sin embargo, las acciones locales del factor NRG4 en la fisiología del adipocito continúan siendo un misterio.

La isoforma neuregulina-1 (NRG1) tiene gran importancia en el metabolismo del músculo esquelético. Específicamente, promueve el metabolismo oxidativo y la biogénesis mitocondrial en el músculo esquelético (*Cantó et al., 2016*; *Cantó et al., 2007*). Por lo tanto, se podría pensar que la NRG4 también podría contribuir a la regulación del metabolismo del músculo esquelético. Sin embargo, la función de este factor de crecimiento en la biología del músculo esquelético está aún por explorar.

Por otro lado, la NRG4 regula y es regulada por la inflamación. Así, algunos autores han mostrado como la expresión genética de *Nrg4* se veía disminuida por la activación de la vía NF-κB debida a la acción del factor de necrosis tumoral α (TNFα) (*Wang et al., 2014; Chen et al., 2017*). Por otro lado, Chen *et al* descubrieron que la expresión genética de citoquinas proinflamatorias como la interleucina (IL) 1β o el *Tnfa* aumentaban en el WAT de ratones deficientes en *Nrg4*. Por lo tanto, dichos estudios mostraron por primera vez la relación entre la inflamación del WAT y la expresión de *Nrg4* sin profundizar en la naturaleza de dicha inflamación. En otras palabras, sigue sin saberse si la ausencia de *Nrg4* es capaz de generar inflamación autónoma en adipocitos, lo que a su vez contribuiría a la inflamación total del WAT.

El síndrome metabólico y el desarrollo de la resistencia a la insulina en patologías como la diabetes de tipo II (T2D) son orquestados por la emergencia de inflamación en el WAT (*Appari et al., 2018*). A pesar de que los macrófagos proinflamatorios contribuyen en mayor medida en la inflamación del tejido, la respuesta inflamatoria inicial es causada por los propios adipocitos. Los adipocitos no forman parte directa del sistema inmunitario, pero secretan multitud de factores como citoquinas proinflamatorias que regulan el comportamiento de células inmunitarias como los macrófagos. Así, a raíz del estrés metabólico causado por la obesidad en el adipocito, éste aumenta la expresión de citoquinas proinflamatorias como el *Tnfa*. A su vez, estos factores proinflamatorios secretados por los adipocitos señalizan sobre los macrófagos residentes del WAT e inducir su polarización hacia un perfil proinflamatorio. Estos macrófagos proinflamatorios amplifican la respuesta inflamatoria inicial aumentando la expresión de factores proinflamatorios y quimiocinas que facilitan la infiltración de nuevas células inmunes al WAT. Por ello, la respuesta inflamatoria autónoma del adipocito es clave en el desarrollo de la inflamación del WAT, que contribuye a sufrir síndrome metabólico y resistencia insulínica inducida por obesidad.

Además de los estudios de inflamación del WAT, otros estudios demuestran que la NRG4 puede señalizar sobre macrófagos proinflamatorios (*Schumacher et al., 2017*). En concreto, se descubrió que los macrófagos proinflamatorios extraídos de un modelo murino de colitis inflamatoria inducida por sulfato dextrano (SDS) aumentaban la expresión del receptor de NRG4 ErbB4. En este contexto, el tratamiento con NRG4 en estos ratones redujo la expresión de citoquinas proinflamatorias en lisados de colon, sugiriendo que la NRG4 tendría un papel antiinflamatorio sobre los macrófagos. Además, los autores mostraron que el tratamiento con NRG4 inducía muerte celular en macrófagos proinflamatorios.

Podemos hipotetizar que la NRG4 tendría un papel clave en la prevención de la inflamación de macrófagos residentes del WAT, teniendo en cuenta la importancia de los macrófagos en el mantenimiento de la homeostasis del WAT y en el papel fundamental que juegan en el desarrollo del síndrome metabólico. Sin embargo, actualmente dicha afirmación es solamente una hipótesis ya que el papel de la NRG4 secretada por adipocitos en macrófagos residentes aún no ha sido analizado.

9.2. Objetivos

Como se ha descrito previamente, el factor de crecimiento NRG4 ha emergido como una nueva adipoquina clave en el mantenimiento del metabolismo hepático. Además, numerosos estudios han destacado la importancia de la NRG4 como un factor antinflamatorio en modelos de colitis. Sin embargo, se desconoce si este factor puede regular de forma autocrina la fisiología de los adipocitos o los macrófagos residentes del WAT. Por otro lado, las acciones distales de la NRG4 en el control de la fisiología del músculo esquelético aún no han sido analizadas. Por todo ello, esta tesis tiene como objetivo general la generación de diferentes modelos celulares y murinos para analizar la función de la NRG4 en diferentes tejidos y células. Los objetivos específicos de esta tesis son los siguientes:

- **Estudiar las acciones locales de la NRG4 en adipocitos 3T3-L1.** Para ello, se analizará la capacidad de diferenciación, la sensibilidad a la insulina y el estado inflamatorio de los adipocitos 3T3-L1 con la expresión genética de *Nrg4* o *Erbb4* silenciada.
- **Generar adipocitos primarios inmortalizados como un nuevo modelo celular para estudiar las acciones locales de la NRG4**. Para ello, se optimizará un protocolo de generación de adipocitos primarios inmortalizados. Después, se eliminarán los genes de los receptores *Erbb2* y *Erbb4* mediante el sistema de recombinación genética Cre-LoxP con el objetivo de estudiar los efectos locales de NRG4 en la fisiología de los adipocitos primarios.
- **Evaluar los efectos de la NRG4 secretada por adipocitos en la polarización de macrófagos.** Para ello, se generarán macrófagos derivados de médula ósea (BMDM). Se analizará la polarización de los macrófagos BMDM y RAW 264.7 después de ser tratados con medio condicionado de adipocitos control y deficientes en NRG4.
- **Analizar los efectos distales de la adipoquina NRG4 en el músculo esquelético de ratón.** Para ello, se caracterizará el fenotipo de ratones *Erbb4 knockout* (KO) músculo-específico a dos y seis meses de edad.

9.3. Resultados

9.3.1. Patrón de expresión de los receptores ErbB y de las diferentes isoformas de neuregulina en células 3T3-L1 y en tejido adiposo blanco

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.2. Generación y caracterización de las células 3T3-L1 con *Nrg4* silenciada

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.3. Los adipocitos *Nrg4* KD presentan resistencia a la acción de la insulina

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.4. Los adipocitos *Nrg4* KD presentan inflamación autónoma celular

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.5. El tratamiento con los agentes antiinflamatorios salicilato sódico y dexametasona recuperan la expresión del receptor de la insulina en los adipocitos *Nrg4* KD

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.6. El marcador de autofagia LC3-II aumenta en los adipocitos *Nrg4* KD

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.7. El inhibidor lisosomal bafilomicina A1 recupera el contenido en proteína GLUT4 en los adipocitos deficientes en NRG4

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.8. El contenido en proteína TBC1D4 se encuentra reducido en los adipocitos *Nrg4* KD

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.9. El contenido mitocondrial se encuentra reducido en los adipocitos *Nrg4* KD

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.10. La morfología de la red mitocondrial se ve afectada en los adipocitos *Nrg4* KD

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.11. Los adipocitos *Nrg4* KD presentan estrés oxidativo celular

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.12. El estrés oxidativo es la causa de la inflamación autónoma celular de los adipocitos *Nrg4* KD

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.13. El eje de señalización NRG4-ErbB4 controla la degradación de GLUT4 y la reducción de la expresión del receptor de insulina

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.14. La ausencia de señalización de ErbB4 genera inflamación en los adipocitos 3T3-L1

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.15. Los adipocitos primarios inmortalizados *Erbb4 knockout* presentan inflamación celular autónoma

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.16. El tratamiento con medio condicionado de adipocitos 3T3-L1 recupera y previene la polarización proinflamatoria de los macrófagos

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.17 Caracterización fenotípica de ratones *Erbb4 knockout* músculo-específico

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.18. Caracterización molecular del músculo esquelético de los ratones *Erbb4* KO

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.19. Caracterización molecular del tejido adiposo blanco de los ratones *Erbb4* KO músculo-específico

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.3.20. Caracterización molecular del hígado de los ratones *Erbb4* KO músculo-específico

AVÍS IMPORTANT
Els apartats 4, 7.3.6., 7.3.8. i 9.3. de la present tesi doctoral han estat protegits per l'autor en existir la participación d'empreses, conveni de confidencialitat o la possibilitat de generar patents.

AVISO IMPORTANTE

Los apartados 4, 7.3.6., 7.3.8. y 9.3. presentes en la tesis doctoral han estado protegidos por el autor al existir la participación de empresas, convenio de confidencialidad o la posibilidad de generar patentes.

IMPORTANT NOTE

The sections 4, 7.3.6, 7.3.8. and 9.3. present in this doctoral thesis have been protected by the author due to the participation of companies, confidentiality agreements or the possibility of generating patents.

9.4. Discusión

En esta tesis, hemos arrojado luz sobre la función que desempeña la NRG4 en la fisiología de los adipocitos. Hemos descrito cómo la ausencia de NRG4 en adipocitos 3T3-L1 *Nrg4* KD genera resistencia insulínica. Esto sería consecuencia de la emergencia de inflamación autónoma celular, que a su vez, reduce la expresión del *InsR*; además de la degradación de las GSVs vía autofagia. La degradación de GLUT4 y de otras proteínas asociadas a las GSVs observada en las células *Nrg4* KD no es consecuencia de la respuesta autoinflamatoria, ya que el tratamiento con agentes antiinflamatorios no fue capaz de recuperar el contenido en proteína de dichos marcadores. Sin embargo, la acción de los agentes antiinflamatorios sí que recuperó la expresión del *InsR*, confirmando que la reducción en la expresión de este marcador es consecuencia directa de la inflamación.

Las GSVs son degradadas vía autofagia en los adipocitos deficientes de NRG4. Así, el flujo autofágico se encuentra aumentado de forma basal en estas células, lo que probablemente es consecuencia del mal funcionamiento de mTORc1. En efecto, los adipocitos *Nrg4* KD presentaron niveles de fosforilación reducidos de la proteína mTOR en condiciones basales. De un modo similar, en presencia de insulina, estos adipocitos fueron incapaces de inducir la fosforilación de la proteína mTOR. Nuestros datos indican que la NRG4 podría regular vía ErbB4 la vía de mTORc1, modulando así el flujo autofágico. De hecho, estudios previos han descrito cómo la señalización de ErbB4 puede inducir la activación de mTORc1 en neuronas (*Nie et al., 2018*), lo que apoya nuestra hipótesis. Nuestros resultados muestran un incremento en los niveles de la proteína LC3-II en las células *Nrg4* KD en condiciones basales, lo cual indica un aumento de la cantidad de autofagosomas. Por ello, la NRG4 juega un papel fundamental en la represión de la vía autofágica en condiciones basales. Este resultado abre un nuevo campo de investigación en el que se deberían evaluar los mecanismos exactos por los que la vía NRG4-ErbB4 regula el flujo autofágico en adipocitos. En este contexto, la caída de la proteína GLUT4 en adipocitos *Nrg4* KD es consecuencia del elevado flujo autofágico presente en estas células, dado que el tratamiento con bafilomicina A1 recuperó el contenido en GLUT4, IRAP y sintaxina-6. Por lo tanto, nuestros datos indican que no solo GLUT4 sería degradado vía autofágica en los adipocitos *Nrg4* KD, sino que, la totalidad de las GSVs serían degradadas.

El tráfico de GLUT4 ha sido muy estudiado durante los últimos años (*Hou et al., 2007*). Hoy en día sabemos que las GSVs se translocan a la membrana plasmática por la acción de la insulina. Sin embargo, existe un cierto grado de translocación basal de las GSVs en ausencia de insulina. Después de la translocación de estas vesículas, éstas son recicladas por endocitosis. Los endosomas reinternalizados acaban en los endosomas de reciclaje y, a desde ahí, las proteínas de las GSVs parten al trans-Golgi o a la reserva de GSVs para ser funcionales de nuevo. Sin embargo, a raíz de nuestros resultados, hipotetizamos que no todas las GSVs son recicladas al completo, sino que parte de los endosomas reinternalizados acaban siendo degradados vía autofagia. Estudios previos observaron cómo la falta de función de la proteína TBC1D4 aumenta la degradación de GLUT4 por la vía autofágica (*Xie et al., 2016*). En este contexto, la caída de los niveles de la proteína TBC1D4 observada en los adipocitos *Nrg4* KD podría causar la pérdida de retención de las GSVs, aumentando la translocación de éstas en condiciones basales. Esto, sumado al mal funcionamiento de mTORc1, conduciría a los endosomas de las proteínas asociadas a las GSVs a su degradación por la vía lisosomal. Sin embargo, esto no es más que una hipótesis por el momento, debido a que no hemos esclarecido por completo la ruta seguida por las GSVs hacia su degradación lisosomal en los adipocitos *Nrg4* KD.

Además de las células 3T3-L1 *Nrg4* KD, los adipocitos 3T3-L1 *Erbb4* KD también presentaron inflamación autónoma celular. Esta inflamación se caracteriza por el aumento en la expresión génica de citoquinas proinflamatorias como *il1b*, *Il6* y *Tnfa*, además de por la reducción de los niveles de la proteína IκB. Como se ha descrito previamente, la expresión de *Nrg4* en adipocitos se reduce por la respuesta inflamatoria inducida por TNFα (*Wang et al., 2014*). Estudios previos también mostraron como la ausencia de NRG4 genera inflamación generalizada en el WAT (*Chen et al., 2017*). Sin embargo, aquí mostramos por primera vez cómo la ausencia de NRG4 en adipocitos es capaz de causar inflamación autónoma en estas células por sí misma. Además, los adipocitos *Nrg4* KD mostraron un aumento en la expresión de los receptores ErbB, especialmente de *Erbb4*. El tratamiento con agentes antiinflamatorios redujo la expresión de estos receptores en los adipocitos, mientras que la presencia de inflamación en macrófagos, músculo esquelético e hígado aumentó la expresión de este gen. Por lo tanto, todas las evidencias indican que la expresión de *Erbb4* es modulada por la inflamación, lo que subrayaría la importancia antiinflamatoria del eje de señalización NRG4-ErbB4 en diferentes tejidos. En el contexto de los adipocitos, el eje de señalización NRG4-ErbB4 desempeñaría un papel clave en la prevención de la inflamación de los macrófagos residentes del WAT, previniendo el desarrollo de patologías metabólicas como el síndrome metabólico. La ausencia de *Erbb4* también generó inflamación en adipocitos primarios *Erbb4* KO. Esto confirma nuestros hallazgos en un modelo más fisiológico que la línea secundaria celular 3T3-L1.

La respuesta inflamatoria celular presente en las células *Nrg4* KD es consecuencia de la emergencia de estrés oxidativo celular por un mal funcionamiento mitocondrial. Observamos una caída del contenido en proteína de TIM44 y MFN2 en estos adipocitos, los cuales son degradados vía lisosomal como también ocurre con GLUT4. Es por ello que la desregulación basal de mTORc1 en estos adipocitos podría estar afectando también a la mitofagia en los adipocitos *Nrg4* KD. En este contexto, estos adipocitos mostraron redes mitocondriales con morfología condensada, lo que es consistente con la presencia de estrés oxidativo. Además, el contenido mitocondrial total se mostró reducido en estos adipocitos. Todas estas alteraciones mitocondriales llevan a la aparición de estrés oxidativo celular. De hecho, estudios previos han descrito cómo las neuregulinas regulan la homeostasis mitocondrial en el músculo esquelético (Cantó et al., 2007). Además, otros estudios subrayan la importancia de las neuregulinas en la prevención de estrés oxidativo en el músculo cardíaco (*Belmonte et al., 2015*). Así, en los últimos años, la evidencia científica ha señalado la disfunción mitocondrial como una de las primeras causas de diferentes patologías autoinflamatorias (*Dela Cruz et al., 2018*). En efecto, las mitocondrias son una fuente mayoritaria de diferentes DAMPs como el mtRNA o las mtROS. El mecanismo exacto por el que las mtROS activan la vía NF-κB sigue siendo motivo de discusión (*Morgan et al., 2011*).

Sin embargo, en los adipocitos *Nrg4* KD el tratamiento con el agente antioxidante NAC revirtió el fenotipo proinflamatorio, demostrando que las ROS son los principales DAMPs que inician la respuesta inflamatoria autónoma en estas células. Así, la inflamación celular que se da como consecuencia de la presencia de estrés oxidativo sería importante en el origen de las patologías inflamatorias tales como el síndrome metabólico y la T2D. Por lo tanto, esta respuesta inflamatoria que involucra a la mitocondria y a la NRG4 constituye un nuevo eje terapéutico para el tratamiento de las patologías metabólicas. Como resumen, en la Figura 78 se representa de forma esquemática todas las alteraciones moleculares que la disrupción del eje de señalización NRG4-ErbB4 causa en el adipocito. La NRG4 no solo regula la respuesta inflamatoria en los adipocitos, sino que también en los macrófagos. Específicamente, la NRG4 secretada por los adipocitos 3T3-L1 recuperó y previno la polarización proinflamatoria M1 en BMDM y macrófagos RAW 264.7 inducida por LPS. Por lo tanto, la acción de NRG4 no solo sería importante promoviendo la eliminación de macrófagos proinflamatorios (*Schucmacher et al., 2017*), sino que también, sería clave en la prevención y reversión de la polarización M1 de los macrófagos. El eje de señalización NRG4-ErbB4 en los macrófagos tendría un papel clave en la resolución de la inflamación tisular, debido a que éstos aumentan la expresión del receptor *Erbb4*. Así, hipotetizamos que la caída de la expresión de *Nrg4* por parte de los adipocitos contribuiría al desarrollo de inflamación crónica por parte de los macrófagos. Por ello, la interrelación entre la inflamación de los macrófagos y la expresión de *Nrg4* por parte de los adipocitos podría ser relevante en el desarrollo de patologías metabólicas.

Además, la NRG4 también tiene efectos distales que regulan la fisiología del músculo esquelético en ratones. La ausencia del receptor ErbB4 en el músculo esquelético de ratones *Erbb4* KO músculo-específico de 2 meses de edad generó inflamación en el tejido. Esta inflamación también se observó en el músculo esquelético de ratones de 6 meses de edad. La emergencia de inflamación muscular se correlacionó con un aumento en la expresión de *Fgf21* en el músculo esquelético, lo que indicaría la presencia de estrés mitocondrial. En efecto, estudios recientes han descrito como el FGF21 es una mioquina cuya expresión se induce por la presencia de estrés mitocondrial en el músculo (*Tezze et al., 2017*). Por lo tanto, hipotetizamos que la NRG4 contribuye al mantenimiento de la homeostasis mitocondrial en músculo de forma sistémica, previniendo la aparición de inflamación. Consecuentemente, la masa del tejido adiposo de ratones *Erbb4* KO alimentados con HFD se redujo, al igual que la expresión hepática de *Nrg4*. Ambas observaciones podrían explicarse por la presencia de inflamación sistémica originada por la inflamación muscular de los ratones *Erbb4* KO.

Sin embargo, la presencia de inflamación sistémica no ha sido demostrada aún en estos ratones, por lo que esto es solo una hipótesis. Aun así, podemos asegurar que la ausencia de ErbB4 en el músculo esquelético impacta en la fisiología de otros tejidos como el WAT y el hígado en los ratones *Erbb4* KO músculo-específico.

9.5. Conclusiones

1. Los adipocitos 3T3-L1 *Nrg4* KD presentan resistencia a la acción de la insulina. Tanto la expresión del receptor de la insulina como el contenido en proteína del transportador de glucosa GLUT4 se redujeron en los adipocitos *Nrg4* KD. Además, el contenido en proteína de las proteínas asociadas a las vesículas de GLUT4, IRAP y sintaxina-6, también disminuyó.

2. Los adipocitos 3T3-L1 *Nrg4* KD presentan inflamación autónoma celular. Dicha inflamación se caracteriza por la activación de la vía NF-κB que aumenta la expresión de citoquinas proinflamatorias. Por otro lado, el tratamiento con TNFα reduce la expresión de *Nrg4* en los adipocitos control.

3. El tratamiento con agentes antiinflamatorios, como la dexametasona o el salicilato sódico, recuperan la expresión y el contenido en proteína del receptor de la insulina en los adipocitos *Nrg4* KD, pero no de GLUT4.

4. La NRG4 regula la activación de mTORc1 en condiciones basales y en presencia de insulina en los adipocitos 3T3-L1. Como consecuencia, los adipocitos deficientes de NRG4 presentan un flujo autofágico acelerado. Así, GLUT4, IRAP y sintaxina-6 son degradados en estos adipocitos vía lisosomal, dado que el tratamiento con bafilomicina A1 recupera el contenido en proteína de dichos marcadores.

5. Los adipocitos *Nrg4* KD presentan una disfunción mitocondrial caracterizada por la reducción en la masa mitocondrial total y por la presencia de redes mitocondriales condensadas. En este estudio, hemos detectado niveles reducidos de las proteínas MFN2 y TIM44, las cuales son degradadas vía lisosomal en estos adipocitos. Como consecuencia, la disfunción mitocondrial genera estrés oxidativo en los adipocitos *Nrg4* KD. Así, la presencia de mtROS genera inflamación autónoma celular en los adipocitos deficientes de NRG4.

6. El silenciamiento de *Erbb4* en adipocitos 3T3-L1 reduce la expresión proteica de GLUT4, IRAP y sintaxina-6. Del mismo modo, el bloqueo del dominio de unión a ligando del receptor ErbB4 en adipocitos control también reduce el contenido en proteína de GLUT4 e IRAP. El bloqueo del receptor genera inflamación autónoma celular. Además, los adipocitos primarios inmortalizados *Erbb4* KO presentan inflamación autónoma celular y reducción en la expresión

del *InsR*. Todo ello indica que la disrupción en el eje de señalización NRG4-ErbB4 causa la inflamación autónoma celular y la degradación de las GSVs en los adipocitos.

7. La eliminación del gen *Erbb2* en preadipocitos inmortalizados primarios impide la progresión de la adipogénesis. Del mismo modo, los preadipocitos *Erbb2* KD no pueden diferenciar a adipocitos maduros. Así, la expresión de *Nrg1* y *Erbb2* es mayor en los preadipocitos que en los adipocitos maduros, por lo que ambas proteínas podrían jugar un papel clave en la inducción de la adipogénesis en preadipocitos.

8. Los macrófagos proinflamatorios M1 aumentan la expresión de *Erbb4*. La suplementación de NRG4 producida por los adipocitos vía medio condicionado de células 3T3-L1 recupera y previene la polarización M1 de los BMDM y los macrófagos RAW 264.7 inducida por LPS. Por el contrario, la suplementación con medio condicionado proveniente de adipocitos *Nrg4* KD genera inflamación en los BMDM no polarizados. Por lo que la NRG4 previene y promueve la resolución de la inflamación de los macrófagos.

9. La tolerancia a la glucosa y a la insulina no se ve afectada en los ratones *Erbb4* KO músculo-específico. Por el contrario, los animales *Erbb4* KO alimentados con HFD tienden a aumentar la sensibilidad a la glucosa y a la insulina. Por lo tanto, la ausencia de *Erbb4* en músculo no causa resistencia insulínica en ratones.

10. El eje de señalización NRG4-ErbB4 previene la inflamación del músculo esquelético en ratones. Así, la supresión del gen *Erbb4* en el músculo esquelético en ratones aumenta la expresión de citoquinas proinflamatorias y de *Fgf21*. En animales alimentados con ND, la expresión hepática de *Nrg4* se muestra reducida en los ratones *Erbb4* KO, por lo que el músculo esquelético es capaz de regular la expresión hepática de este factor.

11. La inflamación inducida por la dieta HFD no empeora en los ratones *Erbb4* KO músculo-específico. Así, la inflamación presente en el WAT de los ratones de 6 meses de edad alimentados con HFD se observa invariable entre los diferentes genotipos. Sin embargo, en estas condiciones, el contenido de TAGs en el músculo esquelético y la masa del WAT gonadal se reduce en los ratones *Erbb4* KO músculo-específico. Por lo tanto, la inflamación muscular de los ratones *Erbb4* KO podría inducir el catabolismo lipídico de los TAGs en el WAT vía inflamación sistémica.