UNIVERSITAT DE BARCELONA

Mechanisms of LXR-mediated control of obesity-induced metaflammation and insulin-resistance

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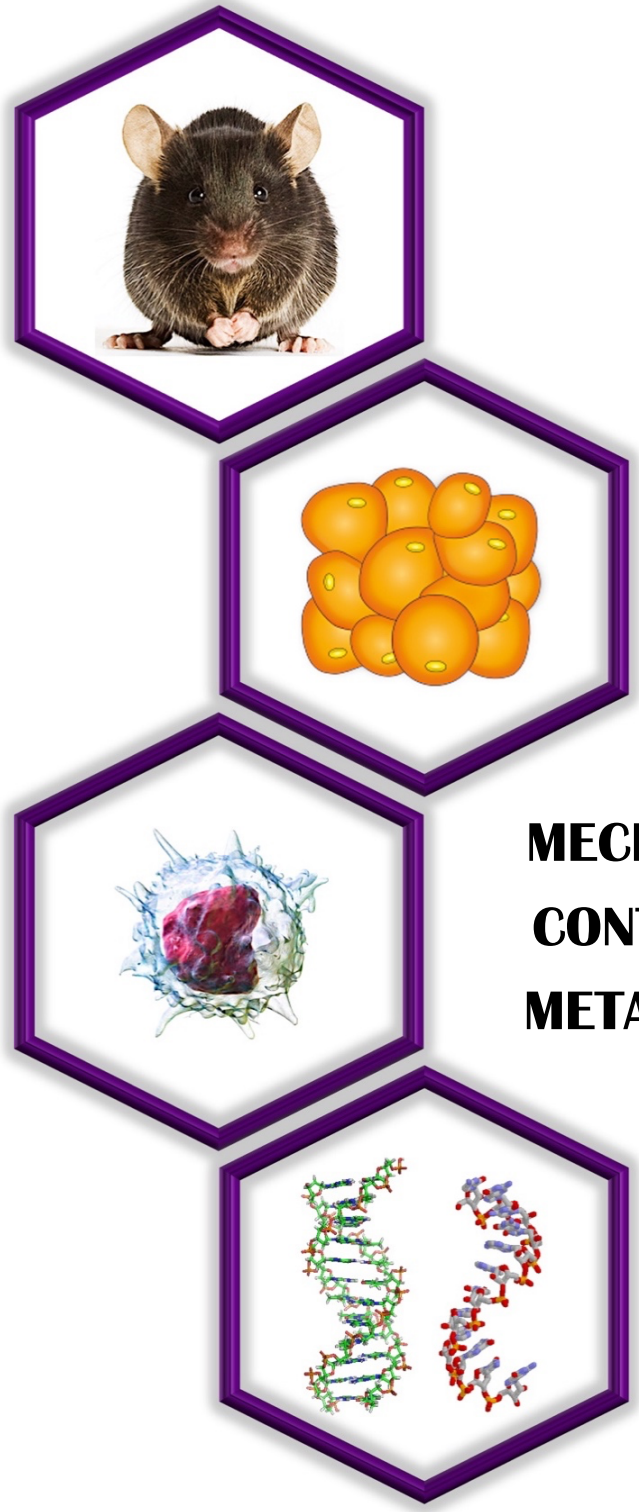

MECHANISMS OF LXR-MEDIATED CONTROL OF OBESITY-INDUCED METAFLAMMATION AND INSULIN-RESISTANCE

Nicole Alejandra Letelier Torres

2021

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Departamento de Biología Celular, Fisiología e Inmunología.

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MECANISMOS MEDIADOS POR LXR PARA EL CONTROL DE LA METAINFLAMACIÓN Y LA RESISTENCIA A LA INSULINA INDUCIDAS POR OBESIDAD

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Para optar al grado de:

Doctor por la Universidad de Barcelona

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Nicole Alejandra Letelier Torres

Barcelona, Septiembre 2021

It is never too late to be what

you might have been.

(Mary Ann Evans)

The development of this doctoral thesis has been possible thanks to the following financial resources:

Grant 201605.31 from Fundació La Marató de TV3 to A.F. Valledor.

Grant SAF2017-89510R from Ministerio Español de Ciencia e Innovación to A. Valledor and C. Caelles. Grants SAF2017-90604-REDT and SAF2015-71878-REDT from Ministerio Español de Ciencia e Innovación to the NuRCaMeIn network.

Fondo Europeo de Desarrollo Regional (FEDER): ***Una manera de hacer Europa.***

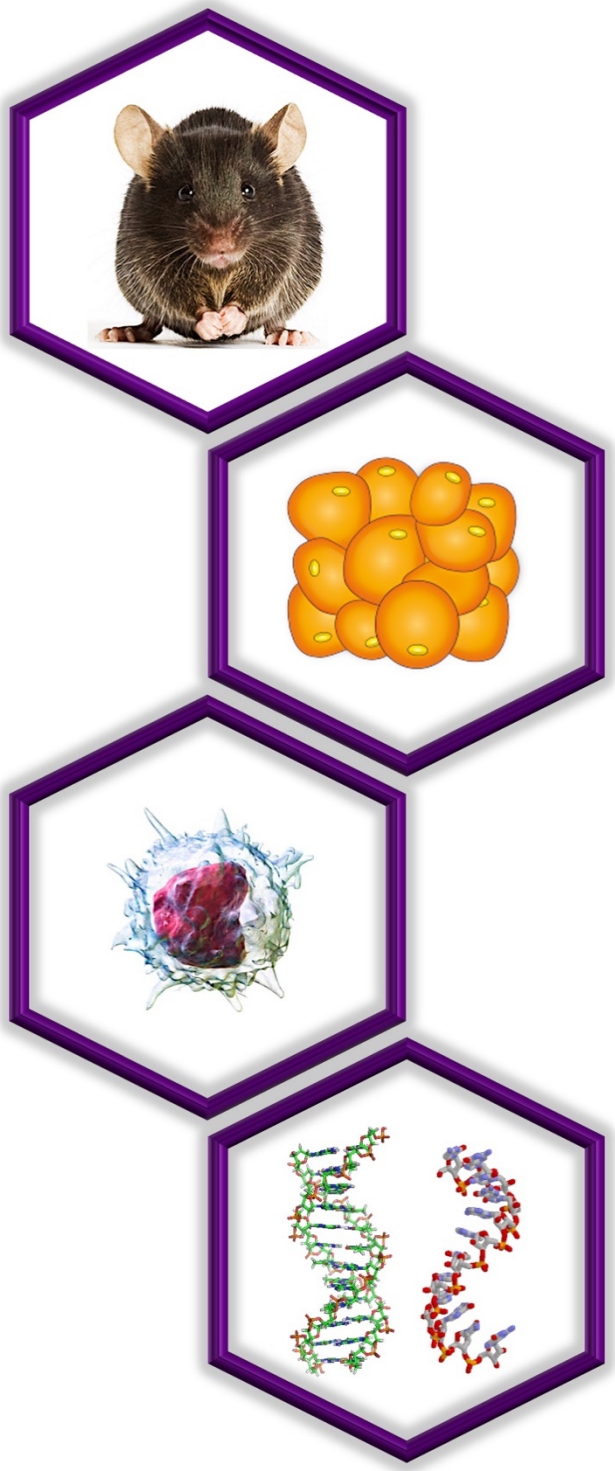
BECAS CHILE/2016-72170639 fellowship from Agencia Nacional de Investigación y Desarrollo de Chile (ANID) to N.A. Letelier.

ABSTRACT

Obesity is nowadays considered a pandemic disease, and its incidence correlates with the concomitant increase of a set of associated comorbidities, including T2D, cardiovascular diseases, fatty liver disease, among others, which are all grouped within the metabolic syndrome. Insulin resistance is frequently developed in obesity and seems to be a common link to these associated diseases. Many efforts have focused in deciphering the mechanisms underlying insulin resistance, being metaflammation, that is the chronic low grade inflammatory response in the adipose tissue associated to obesity, the most well-documented. In this scenario, it is urgent to explore the relevance of anti-inflammatory actors as insulin-sensitizing agents.

The nuclear receptors LXRs represent interesting therapeutic targets that can be pharmacologically activated by synthetic high affinity agonists. Accumulated evidence, including work from our group, indicates the importance of the LXR pathway in the negative control of inflammatory conditions. In this work we have focused in better understanding several aspects of LXR biology. On one side, using primary murine macrophages, our results suggest that negative crosstalk between LXRs and the pro-inflammatory transcription factor IRF1 occurs in a gene-specific manner during the macrophage response to IFN-γ. Notably, IRF1 plays a fine-tuning role in the control of the expression of the LXR target CD38. On the other side, we have evaluated the consequences of LXR activation in a murine model of obesity-associated insulin resistance. Pharmacological administration of an LXR agonist resulted in decreased body weight and basal glycemia and in improved systemic insulin sensitivity in diet-induced obese mice. However, these effects did not correlate with broad inhibitory actions on metaflammation. Our results suggest that the insulin-sensitizing actions mediated by LXRs require functional systemic expression of the multi-functional protein CD38, but these effects do not depend on CD38 expression specifically in bone marrow-derived cells. In addition, our studies indicate that CD38-deficient mice are partially protected from both the increase in adiposity and the development of insulin resistance in response to a high fat diet, and this phenotype is not mediated by the lack of expression of CD38 in bone marrow-derived cells.

In summary, this work unravels the importance of the multi-enzymatic protein CD38 in the insulin-sensitizing actions of the LXR pathway and opens the door to more precisely characterize the function of the LXR-CD38 axis specifically in metabolic cell compartments.



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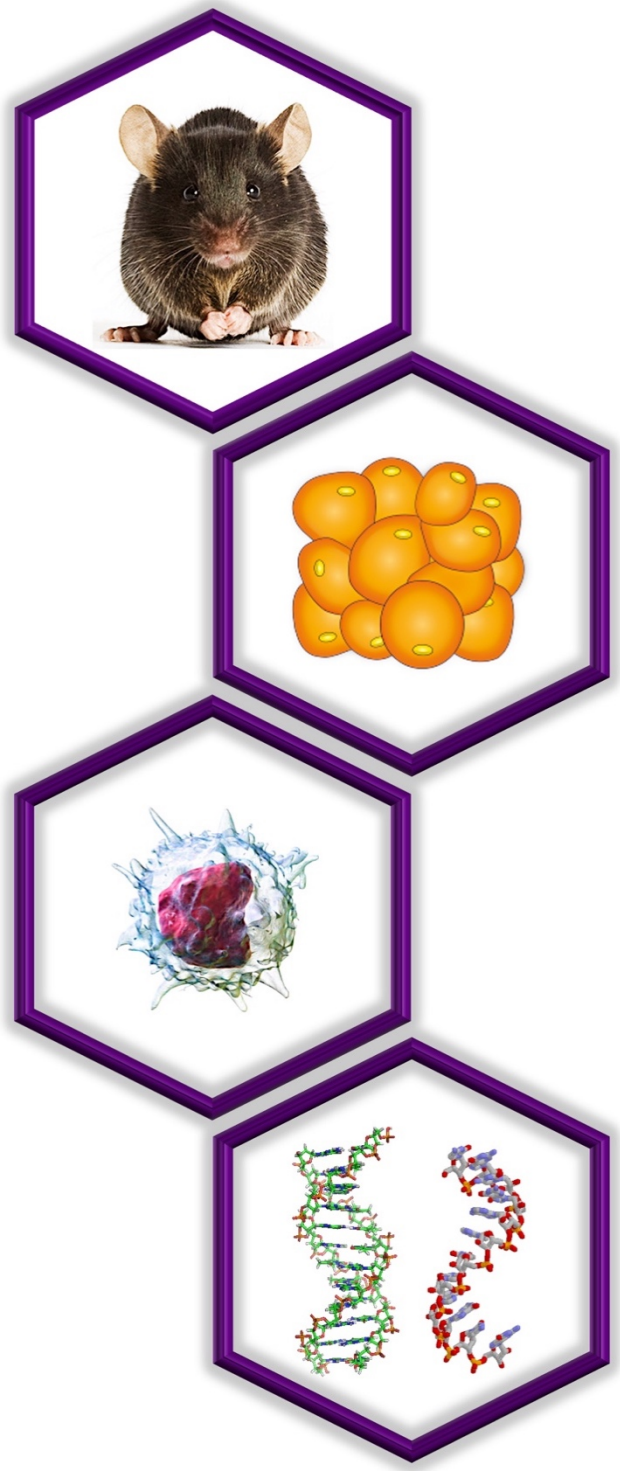
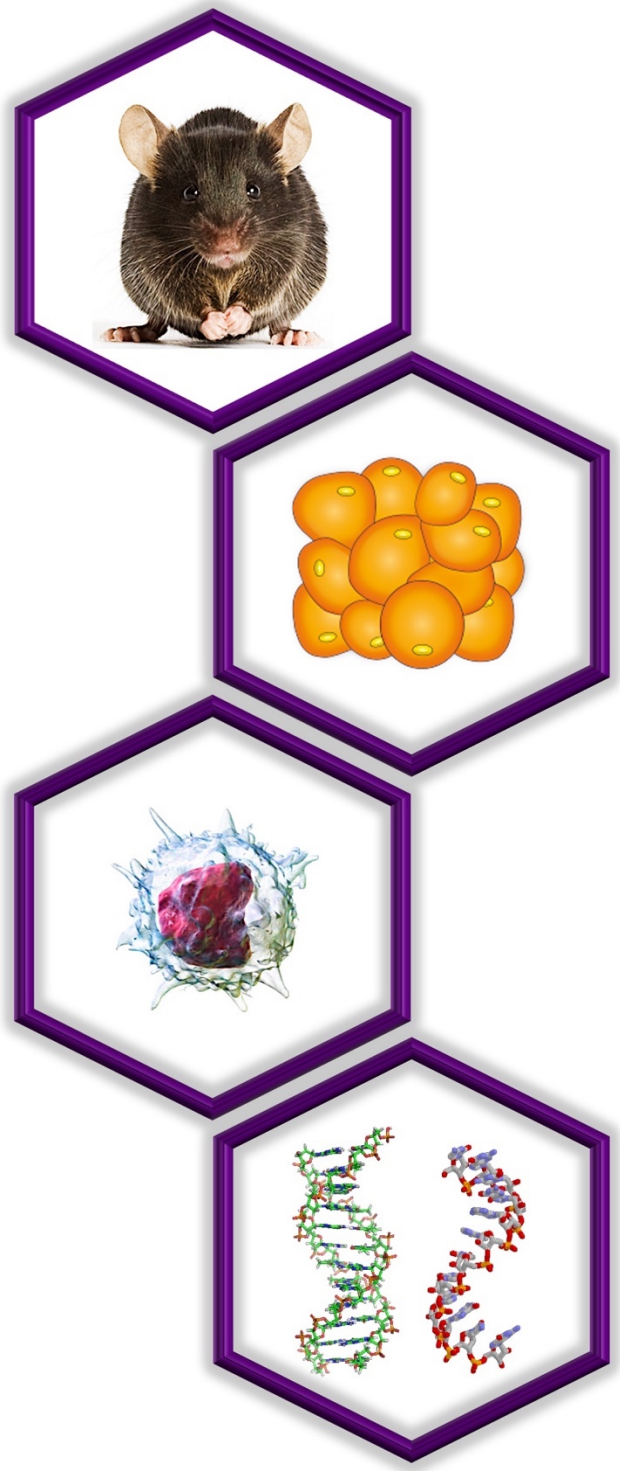


ABBREVIATIONS

ABC	ATP-binding cassette
AIM	Apoptotic inhibitory factor secreted by macrophages
APC	Antigen-presenting cell
ATDCs	Adipose tissue dendritic cells
ATMs	Adipose tissue macrophages
Bcl-2	B-cell lymphoma 2
BCR	B-cell receptor
BMI	Body mass index
Bregs	Regulatory B cells
cADPR	Cyclic adenosine diphosphate ribose
cAMP	Cyclic adenosine monophosphate
CCL2 (MCP-1)	C-C motif chemokine ligand (Monocyte chemoattractant protein 1)
CD38	Cluster of differentiation CD38
CHL	Cholesterol
ChREBP	Carbohydrate response element binding protein
CLSs	Crown-like structures
COX	Cyclooxygenase
DC	Dendritic cell
DPP-4	Dipeptidyl peptidase 4
DPP-4i	Dipeptidyl peptidase 4 inhibitors
ER	Endoplasmatic reticulum
FcγR	Fc-gamma receptor
FFAs	Free fatty acids
Flt3L	Fms-like tyrosine kinase 3 ligand
GLP-1	Glucagon-like peptide-1
GLUT4	Glucose transporter 4
GM-CSF	Granulocyte/macrophage-colony stimulating factor
HFD	High fat diet
IDOL	Inducible degrader of LDL receptor
IFN-γ	Interferon gamma
Ig	Immunoglobulin
IKK	IκB kinase
IKKi	Inducible IκB kinase
IL	Interleukin
ILC2	Lymphoid cells type 2
iNKT	Invariant natural killer T
iNOS	Inducible nitric oxide synthase
InR	Insulin receptor
IRF	Interferon regulatory factor
IRS	Insulin receptor substrate
ISRE	Interferon-stimulated regulatory elements
JAK	Janus kinase

JNK	c-Jun N-terminal kinase
LDL	Low density lipoprotein
LPS	Lipopolysaccharide
LXR	Liver X receptor
LXRE	LXR response element
MAPK	Mitogen-activated protein kinase
MetS	Metabolic syndrome
MERTK	c-Mer tyrosine Kinase
MHC	Major histocompatibility complex
NAADP	Nicotinic acid adenine dinucleotide phosphate
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NAFLD	Non-alcoholic fatty liver disease
NAMPT	Nicotinamide phosphoribosyltransferase
NCoR	Nuclear receptor co-repressor
NF-κB	Nuclear factor-kappa B
NK	Natural killer
NKT	Natural killer T cell
NO	Nitric oxide
NOS	Nitric oxide synthase
NR	Nuclear receptor
NSAIDs	Nonsteroidal anti-inflammatory drugs
oxLDL	Oxidized low density lipoprotein
PEPCK	Phosphoenolpyruvate carboxykinase
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator-1α
PI3K	Phosphoinositide 3-kinase
PKB	Protein kinase B
PPAR	Peroxisome proliferator-activated receptor
PPARγ	Peroxisome proliferator-activated receptor gamma
PPRs	Pattern recognition receptors
RIP 140	Receptor-interacting protein 140
ROR	RAR-related orphan receptor
ROS	Reactive oxygen species
RXR	Retinoic X receptor
SAT	Subcutaneous adipose tissue
Sirt	Sirtuin
SREBP-1c	Sterol regulatory element-binding protein 1c
STAT1	Signal transducer and activator of transcription 1
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TCR	T cell receptor
Th1	Helper type 1 T lymphocyte

Th2	Helper type 2 T lymphocyte
Th17	Helper type 17 T lymphocyte
TNF-α	Tumor necrosis factor alpha
Treg	Regulatory T cells
TZDs	Thiazolidinediones
VAT	Visceral adipose tissue
WAT	White adipose tissue
WHO	World Health Organization
WT	Wild-type



INTRODUCTION

1.- OBESITY AND DIABETES

According to the World Health Organization (WHO), in 2013, approximately 347 million people globally had diabetes, of which type 2 diabetes (T2D) accounted for around 90% of cases. Moreover, a large-scale study estimates that the world prevalence of diabetes could be up to 7.7% (439 million adults) by 2030 (Shaw et al., 2010). The incidence of T2D is increasing worldwide specially as a result of the rise in obesity, a major risk factor for the development of this disease (Grundy, 2012). Decreased responsiveness of tissues to insulin, known as insulin resistance, is an early trait in the development of T2D and is accompanied by glucose intolerance and hyperinsulinemia. With time, failure of pancreatic insulin-secreting cells (beta cells (β-cells)) is the major determinant of progression to hyperglycemia, the hallmark of diabetes (Kahn et al., 2006). In addition, obesity and T2D are grouped in a constellation of diseases known as the metabolic syndrome (MetS) (Figure 1) in which insulin resistance is an underlying common factor.

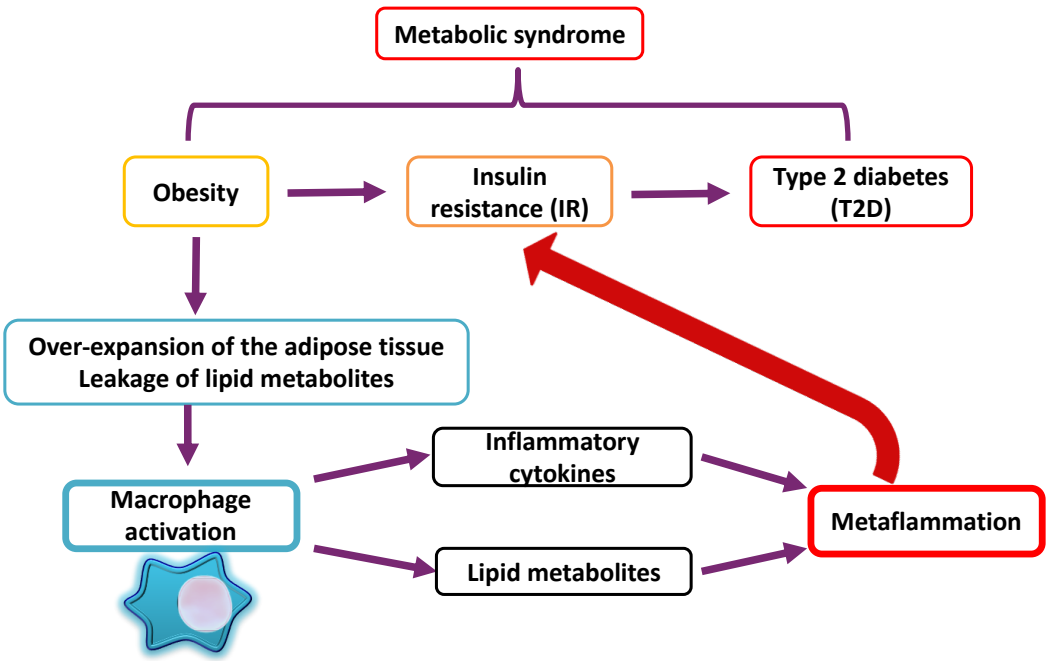


Figure 1. Obesity, T2D and metaflammation. Obesity-induced insulin resistance is an early trait in the development of T2D. In obesity, the over-expansion of the adipose tissue, the leakage of lipid metabolites and their accumulation in other tissues promote macrophage infiltration and activation, leading to metaflammation, which is considered a major etiologic factor in obesity-associated insulin resistance.

Obesity is defined as abnormal or excessive fat accumulation in the body and it is crudely calculated in terms of body mass index (BMI) (Olaogun et al., 2020). The BMI measurement does not take sex differences in the distribution of fat and the age-related decline in muscle mass into consideration, but it correlates with the degree of insulin resistance linearly. Due to the limitations of BMI measurements, it is preferable to use abdominal obesity (independent of total obesity) to determine higher risks to develop insulin resistance and, eventually, diabetes.

Some environmental factors have been linked to T2D, which can be grouped into factors in-utero, early life exposure, and adult social risk factors including socioeconomic background, smoking, and alcohol consumption (Olaogun et al., 2020). Emphasis on modifiable risk factors like smoking and alcohol abuse that may further accelerate β-cell failure in lean individuals may prevent further progression of the disease (George et al., 2015). Some studies have also associated differences in the progression of diabetes to ethnicity. In the East Asian population, for example, T2D is mostly characterized by β-cell dysfunction, and reduced insulin secretory capacity is the principal contributor to the disease (Yabe et al., 2015). Moreover, several genes are indirectly related to the risk to develop T2D through their influence on obesity and lifestyle, most of which contribute to β-cell function, and to the genetic predisposition to early destruction and apoptosis (Frayling et al., 2007). Additionally, epigenetic mechanisms have been proposed to occur in islets from patients with T2D (Kameswaran et al., 2014) and specific microRNAs have been associated to pancreatic β-cell development, function, and adaptive turnover (Poy et al., 2009).

In addition, the over-expansion of the adipose tissue in obese individuals is followed by the leakage of lipid metabolites and their accumulation in other tissues, which promote immune cell infiltration and activation leading to a state of low-grade chronic inflammation recognized as metaflammation (Figure 1) (See *Section 2.-Metaflammation*). Metaflammation is considered a major etiologic factor in obesity-induced insulin resistance, as well as a common trait to other pathologies grouped in the metabolic syndrome.

On the other hand, it has been recently suggested that obesity-induced T2D could be classified as an autoimmune disease (Prasad et al., 2020). However, the autoimmune process in T2D is completely different from that in type 1 diabetes (T1D). T1D is characterized by the failure of the pancreas because of a loss in tolerance for a number of β-cell antigens including insulin, and the adaptive autoimmune response amplifies inflammation. In contrast, in T2D, inflammation precedes the adaptive autoimmune response. Unfortunately, the self-antigens that initiate T cell infiltration into the adipose tissue are still unknown.

1.1 Pathophysiology of obesity-induced Insulin resistance

In general terms, glucose homeostasis depends on the normal functioning of pancreatic β-cells and the normal sensitivity of peripheral tissues to insulin.

1.1.1 Insulin Resistance

Insulin is secreted by pancreatic β-cells in response to hyperglycemia and has very important endocrine functions mostly related to carbohydrate and lipid homeostasis (Jia et al., 2016). For instance, insulin reduces glycemia, by inducing glucose uptake in the adipose tissue and the skeletal muscle and suppressing hepatic gluconeogenesis. Insulin also promotes glycogen, lipid and protein synthesis in specific target tissues. Insulin signaling is triggered by its binding to the insulin receptor (InR), which activates the intrinsic tyrosine kinase activity of the InR and its subsequent autophosphorylation. These phosphotyrosine residues are docking sites for the recruitment of proteins from the InR substrate (IRS) family, such as IRS-1 and IRS-2, which are also phosphorylated on tyrosine residues by the InR, and thereby, constitute docking platforms for the engagement of downstream proteins (Bedinger and Adams, 2015). Among the different signaling networks downstream of the InR, phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt/PKB) and extracellular signal-regulated kinase (ERK) are important mediators of the biological effects of insulin (Bedinger and Adams, 2015).

Insulin resistance is defined by an augmented insulin requirement of the organism in order to keep normal levels of glucose in the blood and is a consequence of defective InR signalling in the three-major insulin-sensitive tissues: skeletal muscle, liver, and adipose tissue. Several metabolic processes are impaired due to insulin resistance, including glucose uptake, glycogen synthesis and glucose oxidation (Ormazabal et al., 2018).

Several non-mutually exclusive hypotheses try to explain the link between obesity and insulin resistance. One prevailing hypothesis is based on the overflow of fatty acids out of the adipose tissue and their accumulation in other tissues. Excess fatty acid storage in peripheral tissues such as the liver and skeletal muscle may result in incomplete fatty acid oxidation in the mitochondria, thus producing an excess of lipid intermediates that impair cellular function, a process known as lipotoxicity. Several lipotoxic intermediates such as diacylglycerols and ceramides have been shown to inhibit InR signalling (revieved by Lair et al., 2020).

Obesity-associated chronic tissue inflammation also impacts insulin sensitivity (Figure 2) (Olefsky and Glass, 2009). Adipose tissue remodelling during obesity generates intrinsic and extrinsic signals

capable of triggering an inflammatory response. Activation of intracellular pro-inflammatory signalling pathways in myeloid and insulin-targeted cells translates into increased production of pro-inflammatory cytokines, endothelial adhesion molecules, and chemotactic mediators that promote the infiltration of monocytes into the adipose tissue and their differentiation toward pro-inflammatory macrophages (See *Section 2.-Metaflammation*). Thus, recruitment of immune cells further supports the inflammatory process (Mclaughlin et al., 2017; Reilly and Saltiel, 2017; Shoelson et al., 2006). As an example, adipose tissues from obese rodents and humans show enhanced secretion of the pro-inflammatory cytokine tumour necrosis factor-alpha (TNF-α), which can inhibit InR signalling. This observation was supported by assays in which TNF-α neutralizing antibodies improved insulin sensitivity and glucose metabolism in obese mice (Hotamisligil et al., 1993, 1995; Spiegelman and Hotamisligil, 1993).

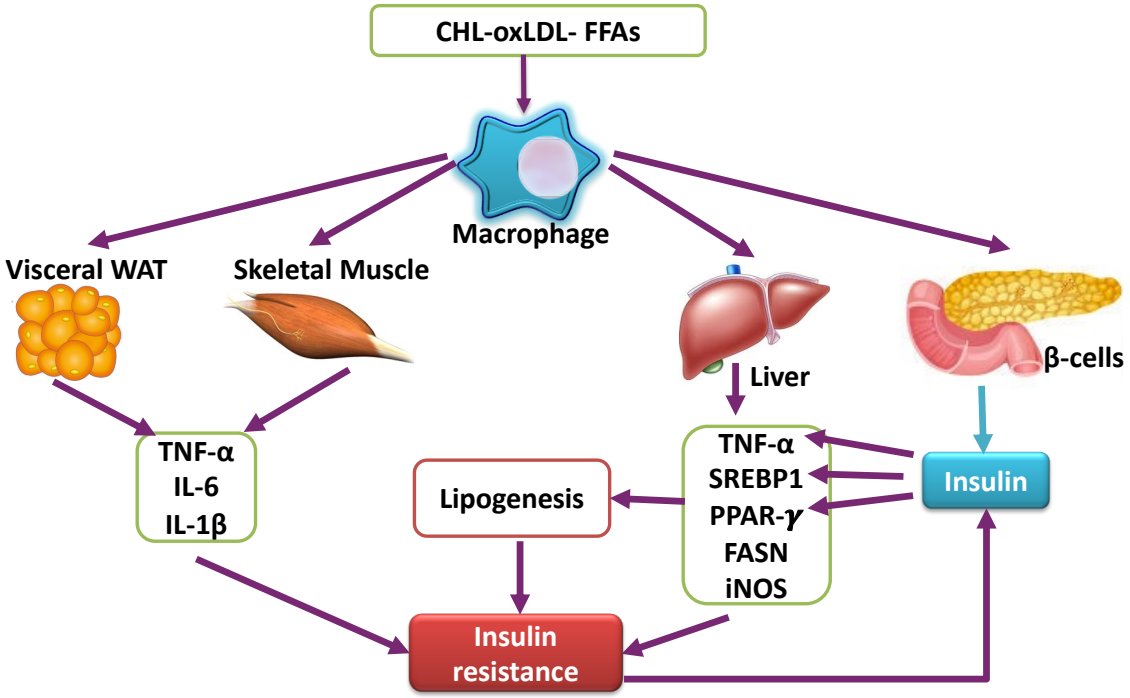


Figure 2. The vicious cycle of insulin resistance and lipid-induced metaflammation. Macrophages activated by oxidized low density lipoproteins (oxLDL), cholesterol (CHL) or free fatty acids (FFAs) infiltrate white adipose tissues (WAT), skeletal muscle, and liver, releasing pro-inflammatory mediators, such as TNF-α, interleukin (IL)-1β, and IL-6, that promote insulin resistance. This is compensated through the hypersecretion of insulin by pancreatic β-cells. The excess of insulin further upregulates the expression of inflammatory mediators (e.g. TNF-α and inducible nitric oxide synthase (iNOS)), as well as of pro-lipogenic genes (e.g. peroxisome proliferator activated receptor gamma (PPARγ) and sterol regulatory element-binding protein 1 (SREBP1)), resulting in an excessive generation of lipids and their derivatives, which in turn restart the cycle, activate macrophages within insulin target tissues and exacerbate insulin resistance.

At the molecular level, several cytokines, such as TNF-α, lipotoxic intermediates, and cellular stress signals, activate different inflammatory pathways, including the c-Jun N-terminal kinase (JNK) pathway (Zatterale et al., 2020). JNK activity is increased in the adipose tissue in two different murine models of obesity, the *ob*/*ob* mice and the diet-induced obesity model, as compared to control mice. Selective deletion of JNK in macrophages have a protective role against insulin resistance in mice (Han et al., 2013). Once activated, JNK exerts a dual effect. On one side, it promotes a pro-inflammatory transcriptional program and, on the other side, it downregulates the InR signalling pathway through inhibitory serine/threonine phosphorylation of IRS-1 and -2, thereby negatively impacting PI3K/AKT activation (Figure 3) (Gual et al., 2005). Apart from the effects mediated by JNK activation, several other signalling modules indirectly contribute to insulin resistance (See *Section 2.-Metaflammation*).

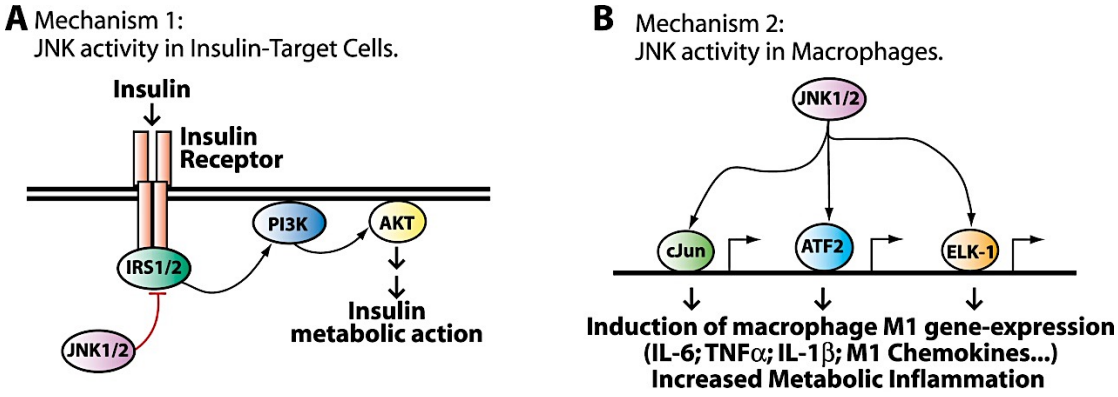


Figure 3. Molecular mechanisms of JNK1 and JNK2 in obesity-driven insulin resistance. (A) In insulin-target cells, JNK1/2 directly phosphorylate IRS1 and IRS2 at serine and threonine residues leading to reduced tyrosine-phosphorylation of IRS1/2 molecules and decreased recruitment of the PI3K-AKT signalling pathway in response to insulin. (B) JNK1/2 play a major role in obesity-driven macrophage activation, leading to increased levels of inflammatory "M1" cytokines driving insulin resistance (adapted from Solinas and Becattini 2017).

1.1.2 β-cell Dysfunction

Pancreatic islet β-cells are characterized by their ability to produce, store, and secrete insulin, which is tightly controlled by plasma glucose, incretin hormones and other factors. In fact, β-cells can respond to extremely small changes in glycemia (between 4.5 and 8 mM), triggering significant changes in insulin secretion within just minutes through a complex process of stimulus-secretion coupling (Heimberg et al., 1993). However, the chronic exposure to elevated levels of glucose, free fatty acids and inflammatory mediators, characteristic of obesity, results in a progressive β-cell adaptive response and failure (Figure 4) (Donath and Shoelson, 2011; Gupta et al., 2017; Hudish et

al., 2019; Poitout et al., 2010). Under these circumstances, β-cells initially mount a compensatory response through hypersecretion of insulin to meet the high metabolic demand. This response triggers a vicious cycle in which excessive insulin production generates insulin resistance in insulin target cells, thus preventing the regulation of hyperglycemia, which is a signal for pancreatic β-cells to drop even more insulin. To satisfy the overproduction of insulin, there is an increase in β-cell mass, accompanied by the induction of an adaptive unfolded protein response and an improvement of mitochondrial function, but these modifications cannot be sustained over time and, eventually, β-cells show endoplasmic reticulum stress, mitochondrial dysfunction, and/or oxidative stress, and are able to secrete inflammatory mediators (Gupta et al., 2017; Halban et al., 2014). Stressed β-cells may undergo cell death, dedifferentiation, transdifferentiation, or phenotypic alterations that compromise their function (Hudish et al., 2019; Sun et al., 2020)(Figure 3). Disrupted β-cell function can feedback to exacerbate insulin resistance and subsequent diabetes.

Although further studies are still needed to establish the factors that lead to the destruction of β-cells, the transition from an adaptive β-cell response to a pathological β-cell response represents a critical step in the progression to diabetes. Interestingly, β-cell defects tend to be more important in the Asian population in contrast to the predominance of insulin resistance in the Caucasian population (Yabe et al., 2015).

Moreover, the existence of different β-cell subpopulations with distinguished function, location, and expression profiles has been described (Benninger et al., 2018; Gutierrez et al., 2017). Four antigenically diverse β-cell subpopulations have been identified in human islets, each with a distinct gene expression profile and insulin secretion capability (Dorrell et al., 2016). The most abundant population is β1, characterized by low basal insulin secretion; conversely, the β4 population displays high insulin secretion capacity. Furthermore, a significant increase in the β4 population has been described in T2D patients, without significant changes in the other subpopulations. A recent study identified a new population of β-cells, termed “extreme” β-cells, located in the center of the islet and characterized by a distinct polarization pattern and a higher proinsulin and ribosomal RNA content (Farack et al., 2019). Additional subpopulations of β-cells include “hub” β-cells that display pacemaker activities to coordinate the insulin response to glucose and “virgin” β-cells, which are postulated to represent a population of immature β-cells that form a neogenic niche at the periphery of the islet (Johnston et al., 2016; van der Meulen et al., 2017) However, the extent to which these populations represent distinct β-cell subtypes *versus* a phenotypic continuum of a homogeneous cell population remains to be determined.

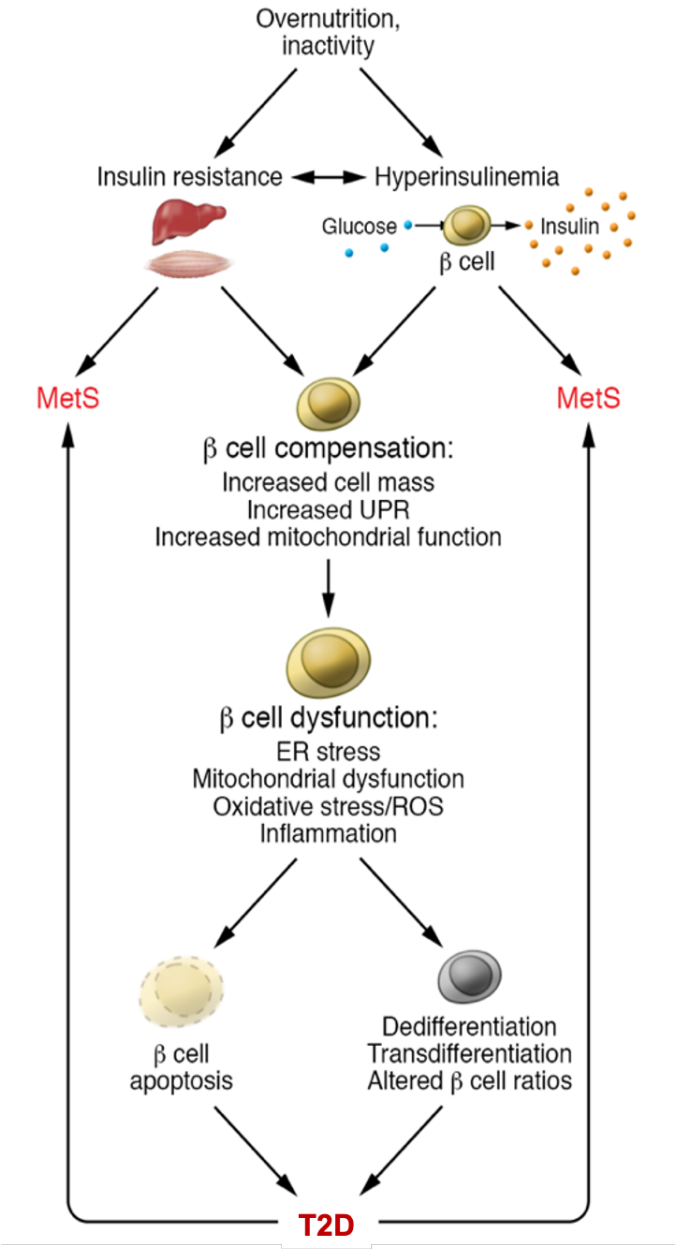


Figure 4. Chronicle of an announced death: obesity and β-cell dysfunction. Abdominal fat accumulation induces insulin hypersecretion from pancreatic β-cells in response to insulin resistance. Initially, β-cells can functionally compensate for the increased metabolic demand by increasing β-cell mass, inducing an unfolded protein response (UPR) and improving mitochondrial function. However, β-cell compensation cannot be sustained, and β-cells become dysfunctional, presenting with endoplasmic reticulum (ER) stress, mitochondrial dysfunction, oxidative stress, and markers of inflammation. Ultimately, stressed β-cells undergo cell death, dedifferentiation, transdifferentiation, or phenotypic alterations that compromise their function (Hudish et al., 2019).

2.- METAFLAMMATION

As introduced in *Section 1.- Obesity and Diabetes*, obesity is usually associated with metaflammation, a low-grade, chronic inflammatory state initially orchestrated by metabolic cells within the adipose tissue in response to excess nutrients and energy and further supported by infiltrated immune cells. The concept of metaflammation was based on the initial observation that obese individuals are characterized by the permanently elevated serum levels of inflammatory markers such as C-reactive protein, TNF-α or IL-6 (reviewed by Kuryłowicz and Kózniewski, 2020; Saltiel and Olefsky, 2017).

Lipid accumulation beyond the oxidative and storage capacity of certain tissues (e.g. adipose tissue, muscle and liver) results in lipotoxicity, with the presence of toxic lipid intermediates that inhibit insulin action (See also *Section 1.1 Insulin Resistance*) and promote immune cell infiltration (Prieur et al., 2010). In contrast to acute inflammation, metaflammation does not resolve over time; tissues remain in an anabolic state, leading to tissue remodelling and systemic metabolic deterioration over time, although the mechanisms that maintain chronicity and prevent inflammatory resolution are poorly understood (Gregor and Hotamisligil, 2011).

Obesity-induced metaflammation implies important changes in the frequency and activity of immune cell populations at different physiologic sites (Figure 4). In general, macrophages that are resident in different organs can exist in polarized forms. Classically activated macrophages exert pro-inflammatory activities (also recognized as M1 macrophages), whereas alternatively activated macrophages (M2 macrophages) have an anti-inflammatory role. In obesity, there is increased accumulation of macrophages with a pro-inflammatory phenotype in different tissues, which helps to reduce insulin sensitivity. Recent evidence suggests that innate immune cells other than macrophages, such as natural killer (NK) cells and innate lymphoid cells type 2 (ILC2), may be also involved in obesity-induced insulin resistance (reviewed by Prasad et al., 2020).

The numbers of cellular components of the adaptive immune system also differ between obese and lean organisms. For example, helper type 1 T lymphocytes (Th1 cells), which secrete inflammatory cytokines, and $CD8^+$ cytotoxic T cells, exist in higher frequencies in several tissues of obese organisms, as compared to lean organisms (Cipolletta et al., 2012; Feuerer et al., 2009; Nishimura et al., 2009; Odegaard et al., 2007; Winer et al., 2009). Conversely, regulatory T cells (Treg), which are important to maintain the tolerance toward self-antigens and exert anti-inflammatory activities,

are reduced during adipose tissue inflammation (Prasad et al., 2020). In humans, a systemic increase in Th1 responses over helper type 2 T lymphocyte (Th2) responses has been linked with disturbances in glucose homeostasis leading to the development of insulin resistance and T2D (Zhou et al., 2018). T2D patients are characterized by elevated concentrations of interferon (IFN)-γ and TNF-α (Th1 profile) and decreased concentrations of IL-4, IL-10, and IL-35 (Th2 and Treg profiles). The specific contribution of immune subsets in different tissues has been further described in the following sections.

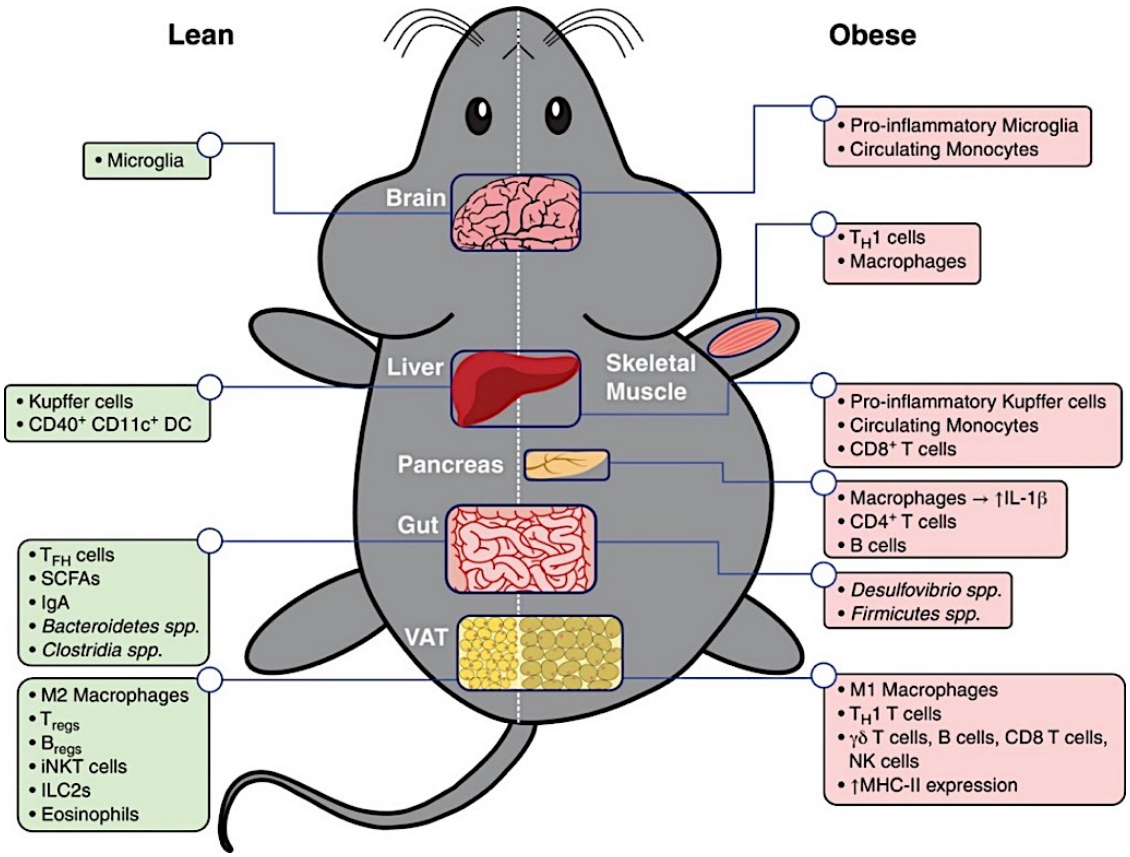


Figure 5: Differences in immune cell subsets between lean and obese mice. Obesity leads to a marked decrease in anti-inflammatory immune subsets and an increase in pro-inflammatory immune subsets at various physiologic sites. Microglia acquire a pro-inflammatory phenotype. Skeletal muscles accumulate pro-inflammatory Th1 lymphocytes and macrophages. There is a reduction in anti-inflammatory dendritic cells in the liver, whereas Kupffer cells become pro-inflammatory. In the pancreas, there is an accumulation of macrophages, $CD4^+$ T lymphocytes and B lymphocytes. In the adipose tissue, there is a reduction in the numbers of anti-inflammatory immune cells (including regulatory T cells (Treg), and alternatively activated (M2) macrophages) along with an increase in pro-inflammatory immune subsets (e.g. Th1 cells, $CD8^+$ T cells, and classically activated (M1) macrophages) (Prasad et al., 2020).

At the molecular level, several pro-inflammatory signals result in the activation of the NF-κB pathway in different cell types. The effects on this pathway are initiated by the activation of an IκB kinase (IKK) complex (containing IKKα and IKKβ subunits), which induces proteasomal degradation of IκBα, leading to the nuclear translocation of NF-κB. In the nucleus, NF-κB activates the

transcription of pro-inflammatory genes, such as IL-6, TNF-α, IFN-γ, monocyte chemotactic protein-1 (MCP-1), and IL-1β, which exacerbate the progression of insulin resistance (Shoelson et al., 2006).

Enhanced production of IL-1β by pro-inflammatory macrophages in insulin-sensitive tissues is largely associated to development of T2D (Donath and Shoelson, 2011; Sims and Smith, 2010). IL-1β impairs insulin-stimulated glucose uptake and lipogenesis by downregulation of IRS-1 and the subsequent translocation of glucose transporter type 4 (GLUT4) to the membrane in murine and human adipocytes (Ballak et al., 2015; Lagathu et al., 2006). IL-1β also induces the production of IL-8, which serves as a chemoattractant for the adhesion of human monocytes to the endothelium and their extravasation to the underlying tissue (Jaganathan et al., 2018).

IL-6, which is largely produced by activated macrophages within the adipose tissue, inhibits the insulin-stimulated tyrosine phosphorylation of IRSs both in the liver and in the adipose tissue (Fève and Bastard, 2009; Zatterale et al., 2020). However, the effects of IL-6 in skeletal muscle are controversial. In this sense, IL-6 has been shown to increase GLUT4 translocation to the plasma membrane and promote insulin-stimulated glucose uptake in myotubes. However, IL-6 is also capable of reducing insulin-stimulated glucose uptake through JNK activation (Carey et al., 2006; Nieto-Vazquez et al., 2008).

A more detailed review of the aspects governing metaflammation in insulin-target tissues is presented in the following sections.

2.1 Metaflammation in white adipose tissues

White adipose tissue inflammation is considered a major contributing factor in the development of obesity-associated insulin resistance. Adipocyte hypertrophy triggers the activation of pro-inflammatory pathways (Burhans et al., 2019; Unamuno et al., 2018). As discussed earlier in this introduction, recent studies suggest that mitochondria are essential for the maintenance of metabolic homeostasis in adipocytes. These observations have raised the hypothesis that mitochondrial dysfunction in adipocytes, which results in impaired fatty acid oxidation and in the accumulation of toxic lipid intermediates, is a primary cause of adipose tissue inflammation and adipocyte cell death (Gonzalez-Franquesa and Patti, 2017; Heinonen et al., 2015; Qatanani et al., 2013; Woo et al., 2019)

Excess of free fatty acids (as well as other stimuli) leads to NF-κB activation and subsequent production of pro-inflammatory cytokines and chemokines by adipocytes. In this sense, genetic ablation of IKKβ in adipocytes blocks the induction of IL-6 and TNF-α in response to free fatty acids (Jiao et al., 2011). In addition, the adipocyte stress response drives mast cell, T lymphocyte and NK cell activation, resulting in local production of the cytokine IFN-γ. These pro-inflammatory cytokines license adipose tissue macrophages toward a pro-inflammatory state and make them more sensitive to a range of other pro-inflammatory stimuli, including leukotrienes, free fatty acid-fetuin A complexes, and danger-associated molecular patterns from necrotic adipocytes (Wensveen et al., 2015a).

Intracellular signalling to IFN-γ involves the activation of Janus kinase (JAK)-1 and -2, subsequent phosphorylation of the transcription factor signal-transducer and activator of transcription (STAT)-1 at tyrosine-701 and dimerization and nuclear translocation of STAT-1. As a result, STAT-1 binds to IFN-γ activated sequences, inducing the expression of primary response genes, such as the transcription factor IFN regulatory factor-1 (IRF-1). Next, STAT-1 forms complexes with IRF1, binds to regulatory regions containing IFN-γ-stimulated regulatory elements (ISRE) and promotes the transcription of a secondary wave of IFN-γ-regulated genes, thus amplifying the inflammatory response to this cytokine (Levy and Darnell, 2002).

Macrophages are indeed a predominant immune cell type that accumulates in the adipose tissue during the course of obesity, constituting 30–50% of the non-adipocyte cell fraction compared with a 10% in lean mice (Russo and Lumeng, 2018; Weisberg et al., 2003). A comparison between different fat deposits suggests that in obese and normal-weight individuals, visceral adipose tissue (VAT) contains more macrophages than subcutaneous adipose tissue (SAT) (Harman-Boehm et al., 2007; Huber et al., 2008; Okamoto et al., 2007; Xu et al., 2003; Zhu et al., 2015). Despite the fact that the subcutaneous adipose tissue has been shown to be more active than the visceral adipose tissue in cytokine synthesis (Kuryłowicz and Kózniewski, 2020), obesity is associated with the increased pro-inflammatory activity of the visceral adipose tissue, especially in metabolic syndrome subjects (Jonas et al., 2015).

Obesity and insulin resistance correlate with an increased ratio of pro-inflammatory (M1-like, polarized or recruited) macrophages versus anti-inflammatory (M2-like or resident) macrophages in the adipose tissue (Reilly and Saltiel, 2017). These M1-like macrophages are characterized by an F4/80, CD11b and CD11c phenotype and secrete TNF-α, IL-6 and IL-1 β (Figure 6). Conversely, M2

macrophages express the cell-surface markers CD11b, F4/80, CD301 and CD206, and secrete IL-4, IL-10 and IL-1 receptor antagonist (IL-1Ra).

Experiments with animals on a high fat diet suggest that despite the majority of adipose tissue macrophages are recruited from the circulation, the expansion of the resident macrophage population also contributes to the development of an inflammatory microenvironment in the adipose tissue from obese individuals (Ryysy et al., 2000). In humans, the origin of macrophages accumulated in the adipose tissue remains unclear (Weisberg et al., 2003). A major stimulus for this recruitment is adipocyte stress as adipose tissue macrophages accumulate around dead adipocytes forming clusters called crown-like structures.

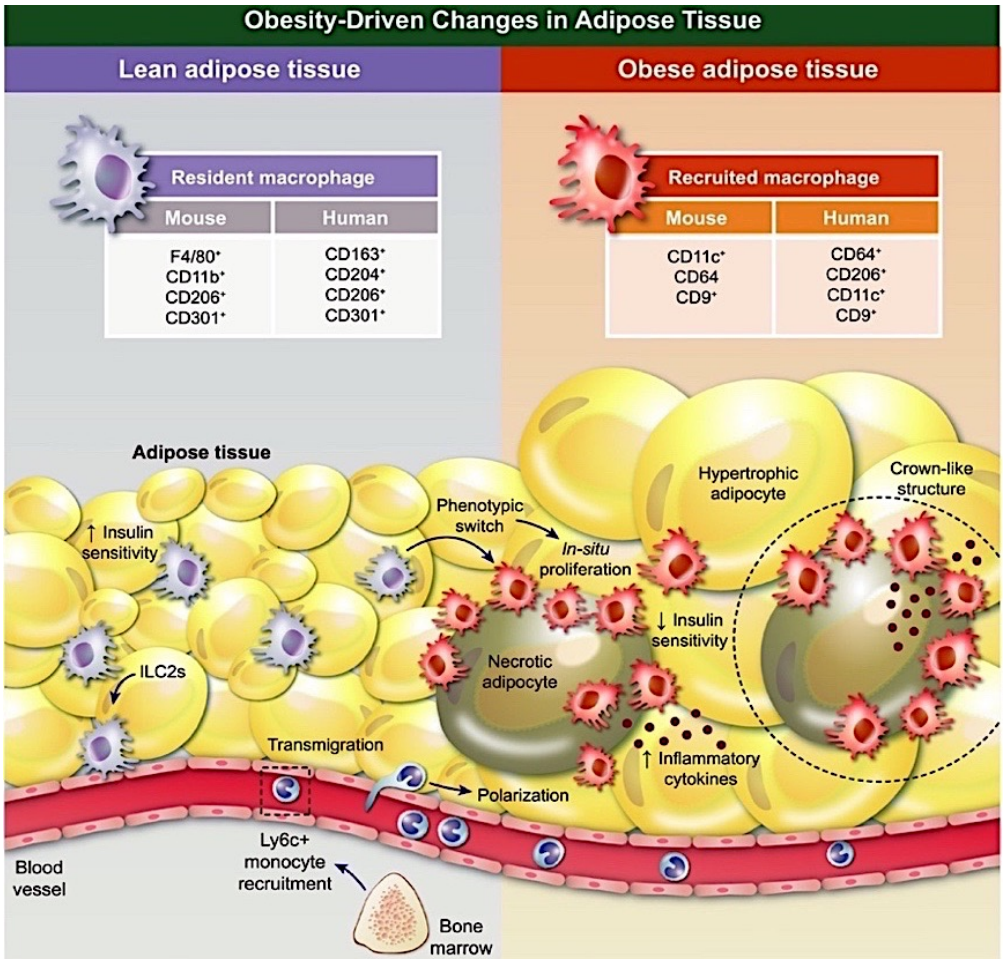


Figure 6. Obesity-driven changes in adipose tissue-associated macrophages. In lean adipose tissue, macrophages polarize toward an anti-inflammatory phenotype. In obesity, toxic lipid intermediates, excessive leptin production and inflammatory chemokines induce Ly6C$^+$ monocyte recruitment giving rise to tissue pro-inflammatory macrophages. Recruited macrophages, undergo a drastic change in distribution, forming crown-like structures around dead adipocytes and increasing their production of pro-inflammatory mediators (adapted from Russo and Lumeng, 2018)).

While white adipose tissue macrophages are considered important contributors to insulin resistance, the dysregulation of other innate immune cells also participate in the initiation or development of adipose tissue inflammation (Figure 7) (Lee, Wollam, and Olefsky 2018).

NK cells are thought to be the main producers of IFN-γ in the white adipose tissue mostly because their numbers within this site increase as early as four weeks after the start of a high fat diet (Fernø et al., 2020). *Wensveen et. al (2015)* demonstrated that NK cell depletion in obese mice blunted the accumulation of macrophages in visceral adipose tissue (Wensveen et al., 2015b). This observation suggests that NK cells are implicated in the onset of the inflammatory response in the adipose tissue preceding the infiltration of macrophages (Figure 7) (Prasad et al., 2020).

Neutrophils comprise up to 90% of all granulocytes in the blood but are relatively rare in the adipose tissue of non-obese mice. However, they are the firsts recruiters to the adipose tissue already three days after the initiation of a high fat diet in mice, and this recruitment is prolonged for over 90 days on a high fat diet (Talukdar et al., 2012). Neutrophils that infiltrate the adipose tissue promote inflammation through the production of TNF-α, MCP-1, and the protease elastase. In this regard, the administration of a pharmacologic elastase inhibitor or the specific genetic ablation of elastase in neutrophils attenuated macrophage influx into the adipose tissue of obese mice and improved insulin sensitivity. These observations suggests that elastase secreted from neutrophils plays a role in recruiting macrophages to the adipose tissue and, perhaps, influencing their polarization state (Talukdar et al., 2012).

Dendritic cells are specialized antigen-presenting cells that link the innate and adaptive immune systems by presenting antigens to T cells (Steinman, 2008; Zatterale et al., 2020). Dendritic cells infiltrate the adipose tissue of mice fed a high fat diet and the subcutaneous adipose tissue of obese humans. Blocking their accumulation improves insulin sensitivity and glucose homeostasis in obese mice (Cho et al., 2016; Hotamisligil, 2017). They likely contribute to the pro-inflammatory microenvironment through macrophage recruitment and IL-6 production (Stefanovic-Racic et al., 2012). Mice with a deficiency in dendritic cells caused by deletion of Fms-like tyrosine kinase 3 ligand (Flt3L) have reduced macrophage numbers in the adipose tissue and liver as well as improved insulin sensitivity in diet-induced obesity, aspects that were reversed through administration of recombinant Flt3L (Chung et al., 2018). Due to shared expression of markers such as CD11b, F4/80 and CD11c, in most studies, it was not possible to clearly distinguish and differentiate adipose tissue macrophages from dendritic cells.

The white adipose tissue is also a reservoir for mast cells during obesity and/or T2D in both mice and humans (Lackey and Olefsky, 2016; Liu et al., 2009). Mast cells infiltrating the white adipose tissue have an immature phenotype in lean mice, whereas obesity progressively triggers their maturation (Hirai et al., 2014). Mast cells are characterized by their high capacity of degranulation, being able to secrete large amounts of pro-inflammatory mediators, such as histamine, cytokines, and chemokines (Żelechowska et al., 2018). Mast cells promote adipose tissue metaflammation by enhancing macrophage infiltration, and their deficiency is associated with improved insulin sensitivity (Liu et al., 2009).

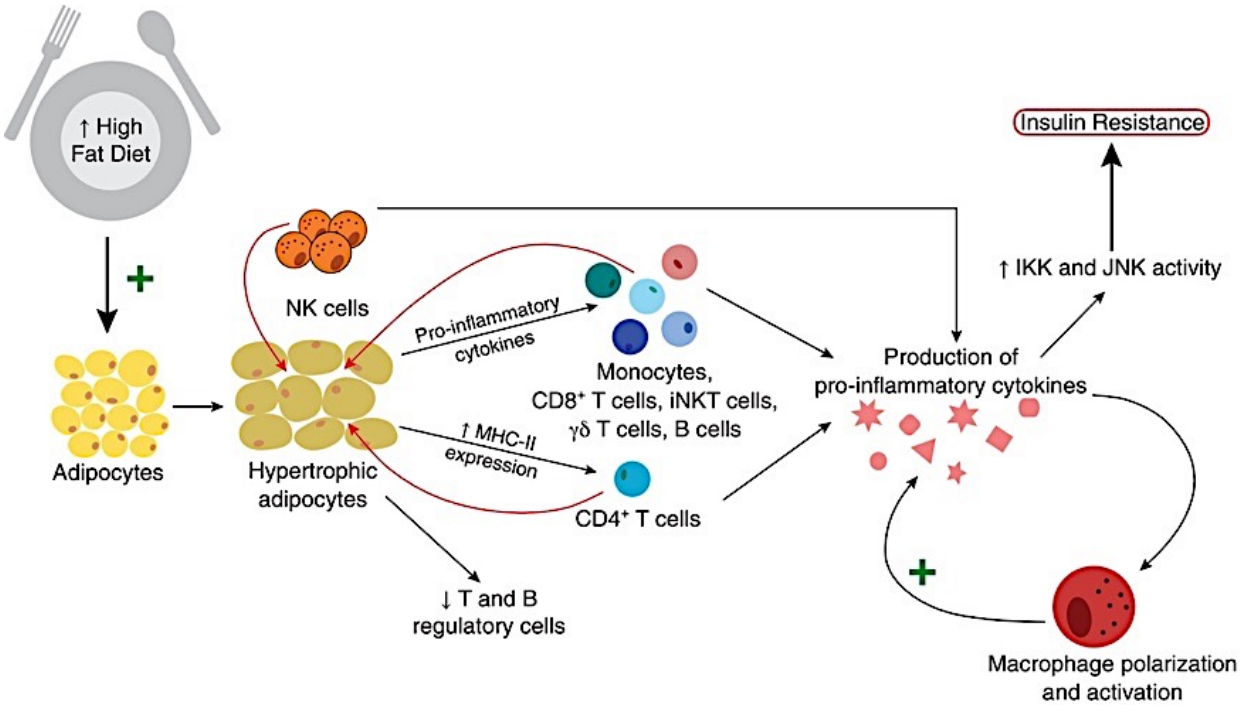


Figure 7. Events depicting obesity-induced insulin resistance in the adipose tissue. High fat diet intake leads to adipocyte hypertrophy and the accumulation of NK cells producing pro-inflammatory cytokines, which provide the first round of inflammatory signals for the recruitment of other immune cells. Recruitment of pro-inflammatory immune cells is accompanied by a decrease in Treg and Breg populations. The production of large amounts of pro-inflammatory mediators by infiltrated immune cells leads to the polarization of monocyte-derived macrophages to a classically activated phenotype (M1), which further exacerbates inflammation. Pro-inflammatory molecules activate JNK and IKK in different target cells, which inhibits insulin signaling and results in insulin resistance (Prasad et al., 2020).

Obesity-induced metaflammation triggers a shift in the frequency of T cells populations within the adipose tissue. In mice, obesity promotes Th1 (IFN-γ-producing subset) and cytotoxic T cell accumulation, with a relative loss of Treg, which may be a causative mechanism of subacute, chronic inflammation maintenance in the adipose tissue (Cipolletta et al., 2012; Feuerer et al., 2009; Nishimura et al., 2009; Odegaard et al., 2007; Winer et al., 2009). The presence of Treg in the adipose

tissue is positively associated with insulin sensitivity and with the improvement of glucose homeostasis in obese mice (Winer et al., 2009). In general, the infiltration of T lymphocytes in the adipose tissue precedes that of monocytes and their differentiation to macrophages. Thus, cytokines secreted by T cells are important determinants of monocyte recruitment.

Helper T cell activation depends on antigen presentation via major histocompatibility complex (MHC)II, costimulatory signals and secretion of polarizing cytokines by antigen-presenting cells. Adipose tissue macrophages can act as antigen-presenting cells for helper T cells (Morris et al., 2013). However, recent studies indicate that the expression of MHCII by adipocytes is essential for IFN-γ production by Th1 cells in the adipose tissue during obesity, which suggests that adipocytes may also participate in helper T cell activation (Deng et al., 2017). In addition, increased IFN-γ leads to decreased expression of the receptor for IL-33 in adipose tissue Treg. In fact, the Treg population in the adipose tissue from lean mice is characterized by a unique genetic signature and a specific TCR repertoire. Maintenance of these cells depends on IL-33 production by adipocytes and mesenchymal stromal cells (Cipolletta et al., 2012; Feuerer et al., 2009; Kolodin et al., 2015; Li et al., 2018). Therefore the actions of increased IFN-γ on IL33 receptor expression in adipose tissue Treg help explain the selective depletion of these cells during obesity (Deng et al., 2017).

Apart from conventional T cells, other minority populations, namely $\gamma\delta$ T cells and invariant natural killer T (iNKT) cells, have been related to obesity-induced insulin resistance. Mice deficient for $\gamma\delta$ T cells showed increased insulin sensitivity and lower macrophage accumulation compared to their wild-type (WT) counterparts (Mehta et al., 2015; Menn et al., 2019), whereas in human studies, the number of $\gamma\delta$ T cells decreased with obesity and showed inverse correlation with BMI (Costanzo et al., 2015). $\gamma\delta$ T cells in the adipose tissue are able to produce the pro-inflammatory cytokine IL-17 (Kohlgruber et al., 2018), which inhibited the expression of the transcription factor PPARγ in the adipose tissue in a model of obesity-induced insulin resistance (Lee et al., 2017). Importantly, PPARγ has been well documented as a key regulator of adipogenesis (Rosen et al., 1999).

iNKT cells recognize a non-classical MHC (CD1d) presenting glycolipids rather than peptides. Interestingly, the principal MHC expressed by adipocytes is CD1d, which participates in the activation of iNKT cells to produce IL-2 and IL-10. These cytokines help maintain the Treg population in the adipose tissue under normal conditions. A decrease in the frequency of iNKT cells has been shown in obese organisms, in parallel to the accumulation of pro-inflammatory macrophages and the decline in the adipose tissue Treg population (Huh et al., 2017), which suggests that an

imbalance in iNKT cell numbers may contribute to the establishment of the pro-inflammatory microenvironment in the adipose tissue during obesity (Lynch et al., 2012).

Pro-inflammatory B cells, which can produce pathogenic autoantibodies, have been also identified in the adipose tissue. However, B cells alone are not sufficient to cause insulin resistance. Instead, they exert pro-inflammatory effects through their interaction with T cells (Winer et al. 2011). Obese mice with a B cell deficiency displayed reduced insulin resistance, and the transfer of B lymphocytes from obese donor mice caused impaired insulin sensitivity and glucose homeostasis in recipient mice (DeFuria et al., 2013; Hotamisligil, 2017; Winer et al., 2011). Moreover, a decrease in regulatory B cells (Breg) that produce IL-10 in the adipose tissue is also associated to obesity, thus contributing to the pro-inflammatory environment (Nishimura et al., 2013).

Cytokines secreted by the adipose tissue are collectively known as adipokines. These include pro-inflammatory cytokines described above, which are synthesized by the different cell populations within the adipose tissue and have autocrine and paracrine effects (e.g., TNF-α, IL-6 IL-1β). In addition, several adipokines are almost exclusively produced by adipocytes. Adiponectin, for example, is a hormone characterized by anti-inflammatory, anti-atherogenic, and anti-oxidative properties (Woo et al., 2019), which also exhibits favourable insulin-sensitizing actions and may increase insulin secretion (Kadowaki et al., 2006). Mitochondrial function in white adipocytes is essential for the synthesis of adiponectin. In several studies, adiponectin levels measured in serum and adipose tissue of obese individuals were significantly lower compared to normal weight subjects (Jonas et al., 2017).

Leptin, another adipokine, has an important role in the control of food intake by the central nervous system and in energy expenditure in peripheral tissues. Obesity is accompanied by hyperleptinemia and resistance to leptin in both animals and humans. Leptin also has important effects in insulin target tissues, leading to reduced insulin secretion by pancreatic β-cells, increased lipid accumulation in the liver and enhanced glucose uptake and oxidation in the skeletal muscle. In addition, leptin provides a link between the nutritional status and immunity. In this regard, leptin has been shown to upregulate the synthesis of multiple inflammatory cytokines, including TNF-α and IL-6 (Katsiki et al., 2018), as well as to inhibit immune cell apoptosis while promoting their proliferation (Francisco et al., 2018). Moreover, leptin also increases the cytotoxic activity of NK cells, the activation of granulocytes, and the activation and polarization of macrophages and Th1 cells.

2.2 Metaflammation in the pancreas

Helper T cells within pancreatic lymph nodes, activated by antigen-presenting cells, are the principal promoters of pancreatic inflammation during obesity-induced insulin resistance (Kuryłowicz and Kózniewski, 2020). There are abundant numbers of macrophages in pancreatic islets of T2D patients (Böni-Schnetzler and Meier, 2019), which are polarized toward a pro-inflammatory phenotype. However, their origin, either recruited or resident, is still unclear (Böni-Schnetzler and Meier, 2019; Dror et al., 2017)

Metaflammation in the pancreas is accompanied by the deposition of an amyloid polypeptide, also named amylin, in pancreatic islets. Amylin is a hormone co-secreted with insulin by pancreatic β-cells, and, just like insulin, its secretion proportionally increases as insulin resistance progresses (Jurgens et al., 2011). Amylin is a potent inducer of IL-1β expression.

As introduced in *Section 1.2 β-cell Dysfunction*, several studies suggest that IL-1β is a key factor in human β-cell dysfunction. IL-1β activates the expression of additional cytokines (IL-6, TNF-α) and chemokines (IL-8, chemokine (C-X-C motif) ligand 1 (CXCL1) and C-C motif chemokine ligand 2 (CCL2)) in pancreatic β-cells. Combined actions of IL-1β and IL-6 induces pancreatic β-cell apoptosis, compromising insulin secretion (Böni-Schnetzler et al., 2009; Bonnet and Scheen, 2018; Donath et al., 2019; Ellingsgaard et al., 2008; Kuryłowicz and Kózniewski, 2020). Moreover, abundant levels of IFN-γ also cooperate with IL-1β to induce pancreatic β-cell death through a non-canonical NF-κB pathway (Meyerovich et al., 2016).

Interestingly, the blood levels of IL-1 receptor antagonist (IL-1Ra) are elevated during obesity and T2D, but are decreased in islets from patients with T2D (Kuryłowicz and Kózniewski, 2020). IL-1Ra is able to bind to the receptor for IL-1 preventing its intracellular signalling. Mice with a β-cell-specific deficiency in IL-1Ra displayed impaired insulin secretion, and reduced β-cell proliferation (Böni-Schnetzler et al., 2018). The authors identified a link between IL-1β-mediated inhibition of islet proliferation genes, β-cell function, and the transcription factor E2F1. The administration of recombinant human IL-1Ra (anakinra) reduced pancreatic metaflammation in T2D animal models and patients, which was accompanied by an increased insulin to proinsulin plasma ratio, and an improvement of glycemia control and of peripheral insulin sensitivity. These observations pointed to the therapeutic potential of IL-1 blockade in T2D (Larsen et al., 2007).

3. PHARMACOLOGIC TREATMENT OF INSULIN RESISTANCE AND TYPE 2 DIABETES AND THEIR EFFECTS IN METAFLAMMATION

Several antidiabetic drugs that control glycemia and have hypolipidemic properties can also exert direct or indirect immunomodularory effects at least in animal models of diabetes, with a reduction in inflammatory cell infiltration to peripheral tissues and pancreatic islets and the subsequent decrease in the local synthesis of pro-inflammatory mediators.

3.1 Anti-inflammatory effects of antidiabetic drugs

Metformin is the first-line medication for the treatment of T2D, particularly in obese individuals. At the molecular level, metformin increases the oxidation of free fatty acids in the liver and in the skeletal muscle via phosphorylation and activation of adenosine monophosphate-activated protein kinase (AMPK), a cellular energy sensor activated under metabolic stress. Activated AMPK inhibits NF-κB activation in several cell types. The combined effects of metformin translated in reduced lipotoxicity and improved insulin sensitivity (Rena et al., 2017). Studies with animal models of high fat diet-induced obesity suggest that metformin exerts anti-inflammatory effects, such as downregulation of the levels of TNF-α and an increase in the numbers of Treg (Kim et al., 2016; Rena et al., 2017). However, the data from clinical studies regarding the anti-inflammatory properties of metformin are inconsistent (Kuryłowicz and Kózniewski, 2020).

Sulphonylureas inhibit the ATP-sensitive potassium channel (K_{ATP}), causing cell membrane depolarization and the intracellular influx of calcium through voltage-dependent calcium channels. In pancreatic β-cells, these effects result in the stimulation of insulin release. Some of these compounds exert anti-inflammatory actions apart from their potent hypoglycemic effects. In macrophages, K_{ATP} channels stimulate pro-inflammatory actions mediated by the MAPK and NF-κB pathways, while the generic sulfonylurea glibenclamide rescues these effects (Kothari et al., 2016). In addition, glibenclamide decreases IL-1β secretion in murine and human macrophages through the inhibition of the inflammasome (Lamkanfi et al., 2009).

Rosiglitazone and pioglitazone are compounds from the thiazolidinedione group that act as selective agonists of the nuclear receptor PPAR-γ. Through positive and negative regulation of transcription, PPAR-γ exerts key actions in promoting adipogenesis, but also in the silencing of the inflammatory response in the adipose tissue, in the steatotic liver, and in many other scenarios (Kuryłowicz and Kózniewski, 2020). The administration of pioglitazone to obese mice reduced the levels of MCP-1

and TNF-α, as well as the density of crown-like structures in the adipose tissue formed by dead or dying adipocytes surrounded by macrophages (Miyazawa et al., 2018). Moreover, pioglitazone induced adiponectin secretion in murine preadipocytes and led to a decrease in the ratio of hepatic pro-inflammatory to anti-inflammatory macrophages, which correlated with a reduction in inflammatory parameters in circulation (Boettcher et al., 2012; Chen et al., 2015).

Sodium-glucose cotransporter 2 inhibitors (SGLT-2i) are used to decrease the plasma levels of glucose by inhibiting renal glucose reabsorption. These drugs also improve insulin and lipids levels (Bonnet and Scheen, 2018). In animal models of T2D, SGLT-2i (such as empagliflozin, dapagliflozin, ipragliflozin, and luseogliflozin) reduced the serum concentrations of several inflammatory mediators (e.g. IL-1β, IL-6, TNF-α, MCP-1, among others) (Bonora et al., 2020). Moreover, the treatment of diabetic mice with empagliflozin reduced pro-inflammatory macrophage infiltration to the adipose tissue and the liver, with concomitant expansion of the anti-inflammatory macrophage population. However, the evidence for anti-inflammatory effects of SGLT-2i from clinical trials in human subjects is contradictory (Bonora et al., 2020; Garvey et al., 2018).

Recently, the field has extended the focus toward the modulation of incretins as anti-diabetic treatments. Incretins are a group of hormones released after eating, which stimulate the secretion of insulin from pancreatic β-cells. Glucagon-like peptide-1 (GLP-1) is an incretin primarily secreted by intestinal L cells in response to food intake (Huang et al., 2015a). GLP-1 binds to a specific receptor (GLP-1R), increasing in the intracellular concentration of cyclic adenosine monophosphate (cAMP) and therefore stimulating insulin secretion. GLP-1 also enhances β-cell proliferation and survival, protecting them from lipotoxicity-induced apoptosis. A GLP-1R agonist, exendin-4, reduced human islet inflammation through inhibition of the NF-κB pathway (Huang et al., 2015a; Pugazhenthi et al., 2010). In several animal models of T2D and obesity, GLP-1R agonists decreased macrophage infiltration to the adipose tissue, liver and skeletal muscle, correlating with decreased synthesis of inflammatory mediators and increased insulin sensitivity (Lee and Jun, 2016). In clinical trials, the administration of GLP-1R agonists to diabetic patients ameliorated the pro-inflammatory activation of macrophages and decreased the serum levels of TNF-α, IL-1β, and IL-6 while increasing the levels of adiponectin (Hogan et al., 2014).

Dipeptidyl peptidase 4 (DPP-4) is a protease that degrades GLP-1, and their inhibitors (DPP-4i) prevent the inactivation of endogenous GLP-1, thus potentiating its influence on β-cells. DPP-4i are members of a family of hypoglycaemic incretin-based drugs routinely used for T2D treatment. Due to the ubiquitous tissue expression of DPP-4, these compounds also have immunomodulatory

effects. For example, treatment with DPP-4i results in increased levels of cAMP in the cytosol, which prevents NF-κB nuclear translocation, and thereby suppresses the production of pro-inflammatory mediators in response to the bacterial component lipopolysaccharide (LPS) (Yang, Yuan, and Zhou 2014). In a model of obese insulin-resistant mice, the DPP-4i sitagliptin reduced inflammatory cell infiltration in the adipose tissue and in pancreatic islets. In the same line, its administration to diabetic patients reduced the expression of inflammatory cytokines and improved the unfavourable ratio of pro-inflammatory/anti-inflammatory phenotypes of peripheral blood monocytes (Dobrian et al., 2011; Xourgia et al., 2019).

In vitro and animal studies suggest that insulin itself can also exert anti-inflammatory actions through different molecular mechanisms. Administration of insulin in obese non-diabetic subjects decreased the generation of ROS by peripheral blood mononuclear cells via inhibition of the NF-κB pathway. The serum levels of several pro-inflammatory markers were also downregulated (Dandona et al., 2001). Upregulation of InR expression has been reported during T cell activation. Stimulation of T cells with insulin induced a shift in T cell differentiation toward a Th2 phenotype, decreasing the Th1/Th2 and the IFN-γ/IL-4 ratios. This effect of insulin in changing T cell polarization may contribute to its anti-inflammatory role in metaflammation (Viardot et al., 2007). On the other hand, insulin activates the PI3K pathway in macrophages, with significant anti-apoptotic effects, thus improving their phagocytosis and oxidative burst capacity (Sun et al., 2014).

3.2 Anti-Inflammatory strategies for the treatment of metaflammation

Nonsteroidal anti-inflammatory drugs (NSAIDs) represent an heterogeneous group of compounds with anti-inflammatory, antipyretic, and analgesic properties that result from the inhibition of cyclooxygenases (COX) (Kuryłowicz and Kózniewski, 2020). Salicylates induce the acetylation of COX-1 and COX2 and irreversibly inhibit the synthesis of prostaglandins. Other NSAIDs interact with COX enzymes by competitive antagonism with different selectivity. In a meta-analysis with a large number of T2D patients, high doses of salicylates (≥3000 mg/day) could effectively reduce fasting plasma glucose and triglyceride levels with a simultaneous increase in plasma fasting insulin levels and a relatively low risk of adverse effects (Fang et al., 2013). A clinical trial demonstrated that salsalate decreased hepatic insulin resistance through interference with the NF-κB pathway, thus reducing liver inflammation (Chai et al., 2011).

Since metaflammation is a consequence of the imbalance between pro- and anti-inflammatory cytokines, therapies aimed at neutralizing pro-inflammatory cytokines are being tested as an

alternative in experimental models of metabolic disease. In this regard, however, the use of anti-TNF-α antibodies has rendered contradictory results. On one side, increased insulin sensitivity through the restoration of the InR signalling cascade in the adipose tissue and the liver was observed in initial studies (Hotamisligil et al., 1993). However, more recent studies suggest that this line of treatment improves inflammatory markers and total adiponectin in patients with metabolic syndrome, without improving insulin sensitivity. In addition, increased muscle adiposity was suggested as part of the effects of TNF-α neutralization (Lo et al., 2007). Anti-IL-1β antibodies have been tested in mice with promising results. Neutralization of IL-1β diminished islet infiltration, β-cell apoptosis, and led to improved insulin secretion and glycemia control in mice on a high fat diet (Sauter et al., 2015).

In addition, the administration of anti-CD3 antibodies, which promotes tolerance by selectively depleting pathogenic T cells while preserving Treg differentiation, resulted in long-term restoration of insulin sensitivity and glucose tolerance in mice with diet-induced obesity (Winer et al., 2009). Similarly, treatment with an IL-2/anti-IL-2 complex, a mediator of Treg expansion, also led to the improvement of glucose tolerance and restoration of insulin sensitivity in high fat diet-fed mice (Feuerer et al., 2009). These results suggest that specific lines of immunotherapy also represent promising strategies in the treatment of metaflammation-related metabolic diseases.

4. LIVER X RECEPTORS (LXRs)

Liver X receptors (LXRs) are transcription factors that belong to the nuclear receptor family. In general, members of the nuclear receptor family are ligand-activated transcription factors that act as hormone or metabolite sensors. Through a combination of mechanisms, they exert both positive and negative control of gene expression in a spatial and temporal manner. The expression of many metabolic genes is indeed controlled by members of this family, which translates in the regulation of diverse aspects of metabolism, including mitochondrial biogenesis, fatty acid synthesis and oxidation, lipid transport, adipogenesis, insulin responsiveness, and cholesterol catabolism. In addition, several nuclear receptors exert important actions in the negative control of inflammation and cell proliferation. For these reasons, nuclear receptors are considered key players in obesity and diabetes and are major targets for drug discovery (Evans and Mangelsdorf, 2014).

LXRs, in particular, are sterol sensors that regulate several aspects of lipid and glucose metabolism (Calkin and Tontonoz, 2012)(see *Section 4.1 LXRs, Metabolism and Obesity*). Several endogenous cholesterol metabolites, including specific oxidized forms of cholesterol (oxysterols) and desmosterol, as well as the plant sterol β-sitosterol, act as natural ligands of LXRs (Guillemot-Legris et al., 2016). High affinity synthetic agonists have also been developed, namely T0901317 and GW3965 (Valledor & Ricote, 2004).

Two isoforms of LXR have been identified so far, LXRα (NR1H3) and LXRβ (NR1H2), encoded by two different genes with more than 75% of aminoacid identity in their DNA-binding domains and ligand-binding domains. The expression of LXRβ is rather ubiquitous, whereas LXRα is distributed in a tissue-restricted fashion, with abundant expression in highly metabolic tissues (such as liver, adipose tissue, intestine, lung, kidney and spleen) and in macrophages (Chawla et al., 2001). In human cells, LXR agonists are able to induce LXRα expression in a positive feedback loop (Laffitte et al., 2001).

LXRs form obligate heterodimers with retinoid X receptors (RXRs), which are also members of the nuclear receptor family. LXR-RXR heterodimers recognize specific response elements on their target genes, called LXR response elements (LXREs) (Figure 8). In the absence of ligand, the heterodimer binds to the LXRE and actively represses the transcription of target genes through the recruitment of complexes formed by corepressor molecules such as nuclear receptor corepressor (NCoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) (Chen and Evans, 1995;

Hörlein et al., 1995). Upon binding either natural or synthetic agonists, corepressors are released in exchange for coactivators, resulting in transcriptional activation (Wagner et al., 2003). Like most nuclear receptors, LXRs undergo diverse post-transcriptional modifications including acetylation, ubiquitination, phosphorylation and SUMOylation (Becares et al., 2017).

In addition to the positive effects on gene transcription, activated LXRs can negatively affect the expression of many genes, including genes encoding important inflammatory mediators (See *Section 4.2 LXR and Immune system*). Several mechanisms contribute to the antagonizing actions of the LXR pathway on pro-inflammatory signalling (Glaría et al., 2020).

4.1 LXR, metabolism and obesity

LXRs are key players in the control of metabolism. They positively control the expression of a number of genes involved in lipid metabolism, including cholesterol efflux and elimination (Calkin and Tontonoz, 2012; Evans and Mangelsdorf, 2014). In more detail, LXRs modulate cholesterol homeostasis through promoting reverse cholesterol transport via induction of several genes encoding cholesterol transporters, apolipoproteins, and lipid metabolizing enzymes. These actions are known to prevent atherosclerosis. In this sense, the activation of LXRs induces the expression of sterol transporters from the ATP-binding cassette (ABC) family, namely ABC transporter A1 (ABCA1) and ABCG1, whose primary roles are to maintain lipid homeostasis by promoting the efflux of cellular cholesterol and phospholipids (Venkateswaran et al., 2000; Wang et al., 2004) (Figure 8). In addition, the induction of two other ABC transporters, ABCG5 and ABCG8, is responsible for the decrease of dietary sterol absorption and the increase of sterol excretion in response to LXR activation (van der Veen et al., 2009). Moreover, LXRs can reduce the cellular uptake of cholesterol by activating the expression of inducible degrader of low-density lipoprotein (LDL) receptor (Idol), an E3 ubiquitin ligase that targets several members of the LDL receptor family for degradation (Hong et al., 2010; Zelcer et al., 2009).

LXRs also promote lipogenesis through the induction of sterol regulatory element-binding protein 1c (SREBP-1c), carbohydrate response element-binding protein (ChREBP), fatty acid synthase (FAS), and stearoyl CoA desaturases 1 (SCD1) and SCD2 (Cha and Repa, 2007; Repa et al., 2000; Schultz et al., 2000). Both SREBP-1c and ChREBP further control the expression of these, and other enzymes involved in the synthesis and esterification of fatty acids, causing an increase in the circulating triglyceride levels in mice treated with LXR agonists, which results in liver steatosis.

LXRs also participate in the regulation of glucose metabolism. In the liver, LXR ligands downregulate the expression of genes implicated in gluconeogenesis, including peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), through the interaction of agonist-bound LXR with the corepressor receptor-interacting protein 140 (RIP140) (Herzog et al., 2007). In addition, LXR activation induces the expression of hepatic glucokinase (GK), promoting glucose catabolism in the liver (Herzog et al., 2007; Stulnig et al., 2002a). These effects translated in reduced glucogenesis in WT but not in LXRα/β-deficient mice upon administration of an LXR agonist (Stulnig et al., 2002a). Such actions of LXR agonists were subsequently extended to a diabetic model in mice (*db*/*db* mice) and to a model of obesity induced by high fat diet in rats (Cao et al., 2003).

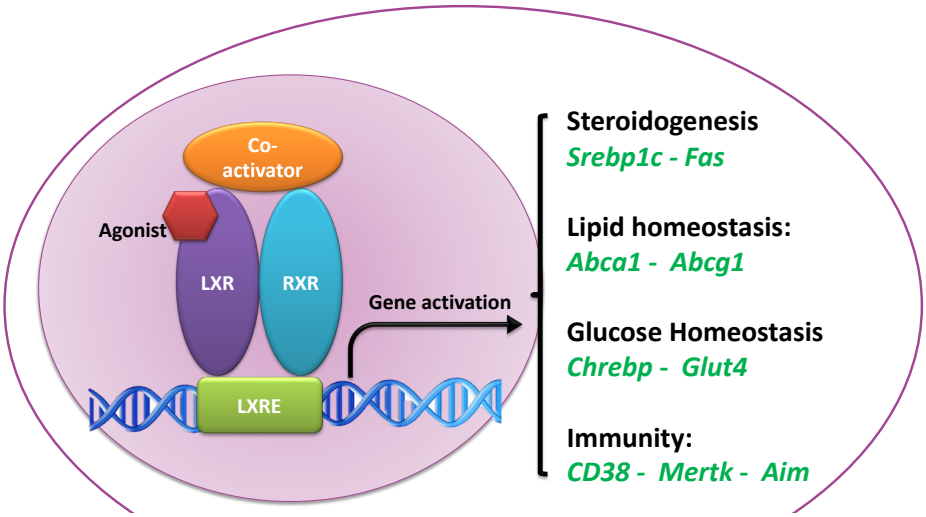


Figure 8. LXR-RXR heterodimers regulate positively the expression of genes involved in lipid and glucose homeostasis and in immune responses. Activated LXR-RXR heterodimers induce the expression of important regulators of lipid (e.g. SREBP1c, FAS, ABCA1, ABCG1) or carbohydrate (e.g. ChREBP, GLUT4) metabolism. In addition, LXR-RXR heterodimers control the expression of genes with important functions in the immune system (e.g. CD38, MERTK, AIM). Most of the genes that are regulated positively by LXRs (indicated in green) contain an LXRE in their regulatory region. AIM, apoptosis inhibitory factor secreted by macrophages; MERTK, MER proto-oncogene tyrosine kinase.

Synthetic LXRs agonists function as insulin sensitizing agents in diverse obese and diabetic animal models. In diet-induced obesity in mice, TO901317 protected the animals against obesity and decreased insulin resistance and glucose intolerance, although the treatment increased liver fat accumulation (Gao and Liu, 2013; Grefhorst et al., 2005; Laffitte et al., 2003). LXR agonists also increased insulin sensitivity and stimulated insulin sensitivity, leading to improved carbohydrate metabolism, in genetically-induced obesity and insulin resistance (Grefhorst et al., 2005). Moreover, LXR activation increased insulin-mediated glucose uptake into the adipose tissue via upregulation of GLUT4 (Laffitte et al., 2003). GLUT4 plays a crucial role in insulin-mediated glucose uptake into

the adipose tissue and skeletal muscle and an impaired expression of GLUT4 has been linked to obesity and T2D. The GLUT4 gene is a direct transcriptional target for the LXR/RXR heterodimer and the ability of LXR ligands to induce GLUT4 expression is abolished in LXR-deficient mice (Dalen et al., 2003; Laffitte et al., 2003). In addition to the effects on GLUT4 expression, LXR agonists may also ameliorate insulin resistance by negatively regulating pro-inflammatory gene expression or by inducing the expression of anti-inflammatory genes (reviewed by Faulds, Zhao, and Dahlman-Wright 2010). In *ob*/*ob* mice, indeed, administration of GW3965 decreased adipose tissue inflammation and immune cell infiltration (Archer et al., 2013).

Another potential mechanism whereby LXR activity may impact hepatic glucose metabolism involves suppression of glucocorticoid signalling. TO901317 reduced hepatic expression of glucocorticoid receptor and 11β-hydroxysteroid dehydrogenase type 1 (the enzyme mediating the synthesis of active corticosterone from inactive 11- dehydrocorticosterone) in WT and *db*/*db* mice, but not in LXRα/β-deficient animals (Stulnig et al., 2002b). Thus, selective LXR agonists could have beneficial effects on insulin sensitivity by reducing local glucocorticoid activation through 11β-HSD-1 in insulin-responsive tissues.

However, despite the insulin sensitizing actions of the LXR pathway in general, it is important to highlight that the therapeutic use of currently available LXR agonists in obesity and diabetes is limited by the fact that activation of LXR promotes lipogenesis as described above (reviewed by Fiévet and Staels 2009).

4.2 LXR and immune system

Apart from their roles in the control of metabolic functions, LXRs play a crucial role in the regulation of inflammation through different mechanisms (Glaría et al., 2020). LXR agonists are capable of down-regulating gene expression independently of their binding to specific response elements, in part through a process known as transrepression. For example, activated LXRs inhibit transcription in response to TLR4 signalling in macrophages by antagonizing the transcription factor NFκB. The aforementioned mechanism involves nuclear receptor SUMOylation and subsequent prevention of corepressor release from inflammatory gene promoters (Huang and Glass, 2010). In addition, the activation of LXRs indirectly inhibits the binding of STAT1 to the regulatory regions of IFN-γ-responsive genes, such as those coding for nitric oxide synthase 2 (NOS2) and C-X-C motif chemokine

11 (CXCL11), without affecting STAT1 phosphorylation. In the case of at least the *Nos2* promoter, LXR-mediated transrepression required LXR SUMOylation but not the action of the corepressor NCoR (Pascual-García et al., 2013).

Reciprocally, several LXR-responsive genes are inhibited after the activation of macrophages by IFN-γ, including those coding for ABCA1 and SREBP1c, without affecting the ability of LXRs to bind to their regulatory regions. Overexpression of the coactivator CREB-binding protein (CBP) compensated the inhibitory effects of IFN-γ on the expression of the *Abca1* gene (Pascual-García et al., 2013).

Another mechanism contributing to the repression of inflammation by LXR agonists imply the increased transcription of the LXR target ABCA1. Changes in membrane cholesterol homeostasis as a consequence of ABCA1-mediated cholesterol efflux have been shown to disrupt the recruitment of key adaptor molecules to lipid rafts, thereby antagonizing TLR signalling (Ito et al., 2015). In addition, induction of the expression of SCD2 as part of the lipogenic program activated by LXRs (See *Section 4.1 LXR, Metabolism and Obesity*) is important for the conversion of fatty acids to derivatives with anti-inflammatory properties (Spann et al., 2012).

On the other hand, LXRs also exert important immunoregulatory functions in autoimmune diseases. A study in Korean patients associated genetic polymorphisms in LXRα with the susceptibility to systemic lupus erythematosus (SLE) (Jeon et al., 2014). Interestingly, pharmacologic activation of LXRs by GW3965 ameliorated the progression of lupus-like autoimmunity in mice (A-Gonzalez et al., 2009). In this work the authors identified MER proto-oncogene tyrosine kinase (MERTK) as a positive transcriptional target of LXRs. Since MERTK exerts important actions in the phagocytosis of apoptotic bodies, LXR-deficiency associates with an impaired clearance of apoptotic cells. As a consequence of these defects, LXRαβ-deficient mice manifested a breakdown in self-tolerance, developed autoantibodies and exhibited age-dependent autoimmune disease (A-Gonzalez et al., 2009; Bensinger et al., 2008). Moreover, the lack of functional LXRβ resulted in increased cellular cholesterol availability for cell division in lymphocytes (in line with decreased cholesterol efflux through ABCG1), which translated in splenomegaly (Bensinger et al., 2008).

Potent anti-inflammatory effects of LXR agonists have been demonstrated in inflammatory and autoimmune conditions, including experimental autoimmune encephalomyelitis (a model of multiple sclerosis in mice) and collagen-induced arthritis, in which Th17 cells contribute strongly (Cui et al., 2011; Huang et al., 2015b; Park et al., 2010; Secor McVoy et al., 2015). In this context, LXR activation suppressed Th17 differentiation indirectly through the induction of SREBP1 expression.

The mechanism underlying this effect involved the physical interaction of SREBP1 with the aryl hydrocarbon receptor on the *Il17* promoter, thus inhibiting Th17 cell differentiation (Cui et al., 2011). Moreover, LXR activation by TO901317 or GW3965 inhibited the differentiation and function of Th1, Th2 and Th17 cells through the modulation of the expression of the cytokines IFN-γ, IL-4 and IL-17, respectively (Solt et al., 2012). Interestingly, LXR suppressed pro-inflammatory T cell differentiation while reciprocally promoting Treg differentiation *in vitro* and inducing gut-associated Treg *in vivo* (Herold et al., 2017). Noteworthy, these LXR-activated Treg exhibited enhanced suppressive capacity.

Additionally, LXR activation modulated the microglial response through the inhibition of IL-1β production, NOS2 expression and nitric oxide synthesis, in part by histone deacetylation and impaired binding of NF-kB to the *Nos2* promoter (Secor McVoy et al., 2015). These effects also help explain the protective actions of GW3965 in experimental autoimmune encephalomyelitis.

Activation of LXRs by TO901317 alleviated ocular inflammation in a mice model of experimental autoimmune uveitis, improving the clinical and morphological scores and decreasing the expression of pro-inflammatory cytokines, including TNF-α, IL-1β, IL- 6, MCP-1, IFN-γ, and IL-17 in the retina. Moreover, the proportions of Th1 and Th17 cells expressing IFN-γ and IL-17 were also reduced in agonist-treated mice undergoing experimental autoimmune uveitis. The authors proposed that the alleviation of ocular inflammation could partially result from inhibition of the NF-kB signalling pathway (Yang et al., 2014a). Th17 cells also play an important role in two other intraocular inflammatory diseases, Behcet's disease and Vogt-Koyanagi-Harada syndrome, which are caused by impaired T cell responses (Wu et al., 2014). Patients with these diseases present decreased expression of LXRβ in peripheral blood mononuclear cells compared with healthy individuals. The stimulation of CD4+ T cells from these patients with GW3965 decreased the expression of IFN-γ and IL-17.

On the other hand, it has been demonstrated that B lymphocytes are important contributors to the pathogenesis of rheumatoid arthritis through autoantibody production, antigen presentation, cytokine release and T cell activation. In a model of collagen-induced arthritis, administration of GW3965 decreased B-lymphocyte stimulator (BLyS) production, which exerts an important role in the pathogenesis and progression of rheumatoid arthritis. In more detail, GW3965 suppressed the binding of NF-κB to the *BLyS* promoter region, and consequently, repressed IFN-γ- and TGF-β-induced BLyS production in B lymphocytes. These effects correlated with the protective actions of GW3965 in mice with collagen-induced arthritis (Huang et al., 2015b).

5. CD38

LXR induces the expression of the multifunctional enzyme CD38 (Matalonga et al., 2017). CD38 expression is also induced by inflammatory mediators (TNF-α and IFN-γ) or by bacterial LPS (Amici et al., 2018; Matalonga et al., 2017). CD38 is a 45 kDa transmembrane glycoprotein composed of a short cytoplasmic tail, a transmembrane domain and an extracellular domain (Malavasi et al., 2008). CD38 exerts both receptor- and enzyme-mediated functions. As a multi-functional (ecto)-enzyme, CD38 is involved in the catabolism of nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+) (Figure 9). At neutral pH, CD38 can convert NAD+ into cADPR through its ADPR cyclase activity. cADPR can then be hydrolyzed to generate ADP-ribose (ADPR). CD38 is also able to exert NAD glycolase activity to directly generate ADPR from NAD+ (Malavasi et al., 2008). Besides the generation of cADPR and ADPR, in acidic conditions, CD38 catalyzes the synthesis of nicotinic acid adenine dinucleotide phosphate (NAADP) from nicotinamide adenine dinucleotide phosphate (NADP+) (Lee, 2006). CD38 is also able to catalyze the degradation of the NAD precursor nicotinamide mono-nucleotide (NMN) into nicotinamide (Grozio et al., 2013). As a receptor, CD38 interacts with its ligand CD31, also known as platelet endothelial cell adhesion molecule-1 (PECAM-1) (Guerreiro et al., 2020; Horenstein et al., 1998). In addition, human CD38 can establish lateral associations with various membrane proteins/complexes, such as CD16 (in NK cells), the T cell receptor (TCR)/CD3 complex and CD4 (in T cells), membrane immunoglobulin (Ig) and the B cell co-receptor complex (CD19/CD81) (in B lymphocytes), and class II MHC (in monocytes) (reviewed by Glaría and Valledor, 2020)).

CD38 is considered the principal NAD+ regulator in mammalian cells. In fact, CD38 is a very inefficient cyclase and it must degrade nearly 100 molecules of NAD^+ to generate one molecule of cADPR (Aksoy et al., 2006a; Chini, 2009). Because of this inefficiency, activation of CD38 results in a strong decrease in NAD+ levels and mice deficient for CD38 show accumulation of NAD+ in many tissues (Aksoy et al., 2006a; Chini, 2009).

cADPR is a second messenger that regulates intracellular calcium homeostasis in many cell types. cADPR induces Ca^{2+} release from intracellular stores (sarcoplasmic reticulum and endoplasmic reticulum) by activating the ryanodine receptor 2 and enhancing store-operated Ca^{2+} entry (SOCE) (reviewed by Zuo et al. 2020). cADPR can also activate the transient receptor potential melastatin 2 (TRPM2) channel, inducing extracellular Ca^{2+} influx (Yu et al., 2019). In addition, NAADP can promote Ca^{2+} release from lysosomes via two-pore channels (TPCs) (Galione, 2019).

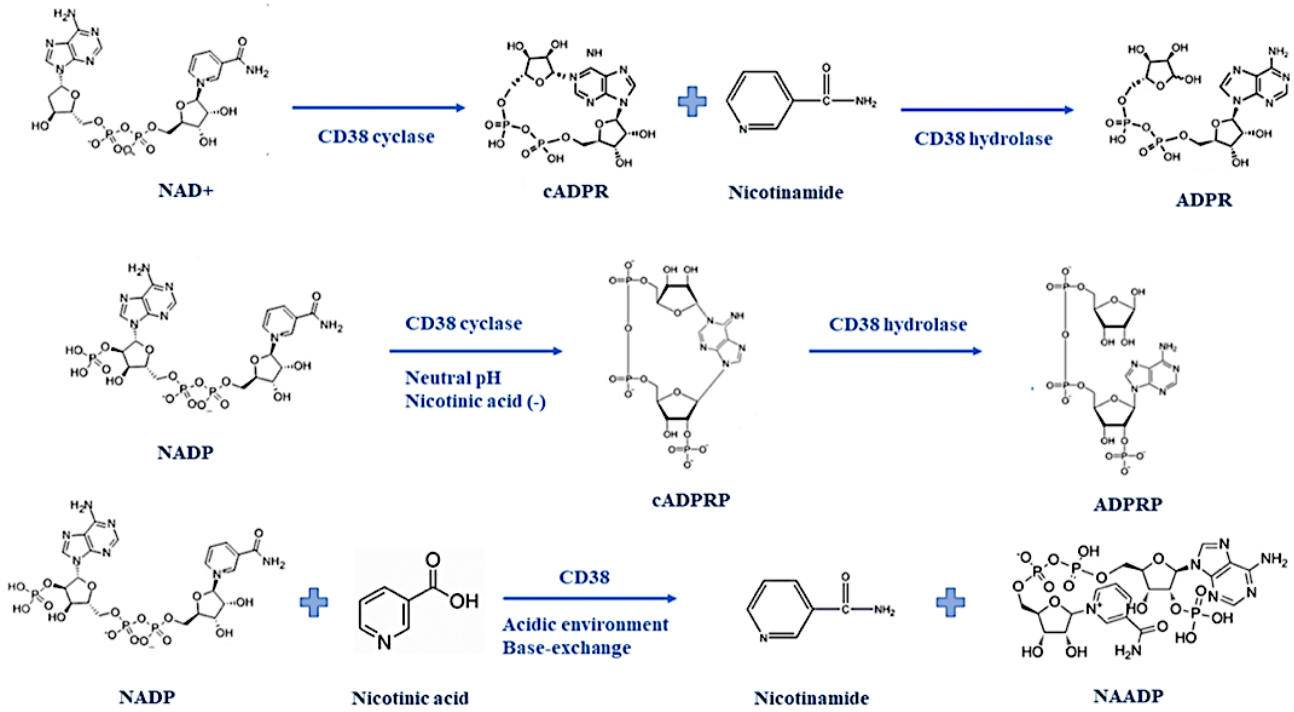


Figure 9. CD38 enzymatic activities. CD38 is an ectoenzyme that converts NAD+ and NADP+ into cADPR, ADPR and NAADP. The different enzymatic activities of CD38 are pH-dependent (Zuo et al., 2020).

5.1 CD38 and inflammation

In 1980 CD38 was originally identified as a lymphocyte activation marker (Reinherz et al., 1980). However, accumulated evidences indicate that CD38 is highly expressed in multiple immune cell populations, including T cells, NK cells, and dendritic cells (Malavasi et al., 2008). CD38 has been extensively studied for its role as a marker in haematological malignancies, including chronic lymphocytic leukaemia and multiple myeloma (Malavasi et al., 2011; Morandi et al., 2018). The use of CD38-deficient mice has demonstrated important roles of CD38 in dendritic cell and neutrophil migration, in T cell priming, and in humoral immunity (Beceiro et al., 2018; Cockayne et al., 1998; Partida-Sánchez et al., 2004). The aberrant expression or hyperactivity of CD38 has been shown to contribute to chronic inflammatory diseases, such as rheumatoid arthritis and asthma (Deshpande et al., 2017; Du et al., 2019; Postigo et al., 2012).

The study of CD38 as a receptor or co-receptor has shown discrepancies between humans and mice, in terms of its tissue distribution and signal transduction. Therefore, the knowledge derived from murine models needs to be validated in humans (Malavasi et al., 2008). In human T cells, ligation of CD38 with either agonistic antibodies or with its ligand CD31, triggered downstream effects that partially overlap with T cell receptor (TCR)/CD3 signalling (Zubiaur et al., 1997). CD38 ligation also

induced the expression of multiple cytokines such as IL-1β, IL-6, IL-10, and IFN-γ in both T cells and monocytes (Ausiello et al., 1995; Lande et al., 2002; Reiniš et al., 1997), thus suggesting that CD38 contributes to pro-inflammatory phenotypes in immune cells (reviewed by Glaría and Valledor 2020). Interestingly, it has been described that CD38 can indeed cooperate with TCR signalling as a consequence of the formation of supramolecular complexes localized in membrane lipid rafts (Muñoz et al., 2003; Zubiaur et al., 2002).

The possibility that CD38 acts as a coreceptor and/or benefits from signalling by other receptors has also been described in other cells of the immune system (de Weers et al., 2011). In B cells, signalling through CD38 required B cell receptor (BCR) expression, but the downstream effects differed based on differentiation stage: in mature splenic B cells, CD38 ligation promoted their proliferation whereas in immature B cells within the bone marrow, CD38 promoted their apoptosis (Funaro et al., 1997; Lund et al., 1996). In addition, CD38 ligation decreased the threshold for B cell activation via the BCR suggesting its participation in BCR signalling (Lund et al., 1996). In NK cells, signalling to CD38 ligation depends on the expression of CD16, and it triggers a cytotoxic response involving degranulation and the upregulation of inflammatory cytokines such as IFN-γ and TNF-α (Deaglio et al., 2002; Mallone et al., 2001; Sconocchia et al., 1999; de Weers et al., 2011). In dendritic cells, CD38 is expressed in association with CD83, and contributes to the activation of T cells. Additionally, CD38 can associate with other molecules involved in the dendritic cell migratory capacity, like CD11b and CCR7, thus modulating dendritic cell chemotaxis and trans-endothelial migration (Wang et al., 2004). Moreover, ligation of CD38 enhanced IL-12 production in synergy with IFNγ in human dendritic cells (Fedele et al., 2004).

Studies in a mouse model of rheumatoid arthritis, characterized by chronic inflammation, showed that the incidence of the disease was only slightly lower in CD38-deficient mice when compared to control mice. However, the severity of the disease was significantly lower in the absence of functional CD38 (Postigo et al., 2012). There was a lack of induction of iNKT cells in the spleen of CD38-deficient mice, as well as reduced percentages of Th1 cells in the draining lymph nodes. CD38-deficient dendritic cells derived from mice with collagen-induced arthritis had decreased expression levels of the transcription factor RelB, which is an important regulator of collagen-induced arthritis, and of MHCII, as well as reduced antigen presentation and phosphorylation of NF-κB (Du et al., 2019).

Studies in chronic lymphocytic leukaemia (CLL) patients demonstrated that CD38 expression significantly correlates with a more aggressive clinical behaviour and is a marker of poor prognosis

(Damle et al., 1999; Matrai, 2005). The comparison between CD38+ and CD38- CLL B cells showed that CD38+ cells had an augmented migratory capacity in response to the chemokine CXCL12, thus enhancing their homing to lymphoid tissues. In addition, these cells expressed higher levels of vascular endothelial growth factor (VEGF) and of the antiapoptotic factor induced myeloid leukaemia cell differentiation protein (MCL-1), suggesting that CD38+ CLL cells have an increased survival advantage within the microenvironment compared to CD38- clones (Pepper et al., 2008; Vaisitti et al., 2015).

The development of CD38-targeting antibodies has increased the therapeutic options for diseases in which target cells express high levels of CD38, such as in multiple myeloma. These antibodies have multiple mechanisms of action, including antibody-dependent cell killing, direct apoptotic activity, and immunomodulatory effects by the depletion of CD38+ immune suppressor cells. The enzymatic activity of CD38 may either be unaffected, e.g. daratumumab, or partially blocked by the antibodies, e.g. isatuximab (Deckert et al., 2014; Van De Donk et al., 2018). In more detail, daratumumab may cause a depletion of $CD38^{bright}$ Treg cells, while increasing the frequency and effector functions of helper and cytotoxic T cells (Krejcik et al., 2016). It has also been demonstrated that depletion of $CD38^{high}$ NK cells occurs in patients treated with daratumumab (Wang et al., 2018b). Compared to daratumumab, isatuximab displays stronger direct apoptotic activity mediated by the activation of caspases, lysosome permeabilization, and upregulation of reactive oxygen species (Moreno et al., 2019). Isatuximab was shown to cause a preferential depletion of Treg cells, which express high levels of CD38, through induction of apoptosis and inhibition of cell proliferation, not affecting other T cells (Feng et al., 2017). Anti-CD38 chimeric antigen receptor (CAR) T cells have also been developed with potent anti-tumour effects (Drent et al., 2017).

5.2 CD38, obesity and insulin resistance

Obese mice have been shown to express higher CD38 levels and CD38-deficient mice are protected against high fat diet-induced obesity, suggesting that CD38 plays a critical role in murine models of diet-induced obesity (Barbosa et al., 2007; Wang et al., 2018a). The expression of the adipogenic genes PPARγ, fatty acid binding protein 4 (FABP4) and CCAAT-enhancer-binding protein alpha (C/EBPα) in adipose tissues was significantly attenuated in CD38–deficient mice fed with a high fat diet as compared with WT mice, suggesting that the protective phenotype of CD38-deficient mice

during obesity may be mediated by the inhibition of adipocyte differentiation in adipose tissues (Wang et al., 2018a).

Controversial observations have been reported regarding the role of CD38 in obesity-associated insulin resistance. On one side, CD38-deficient mice (in C57BL/6 background) have been shown to be protected against high-fat diet-induced glucose intolerance, liver steatosis and metabolic syndrome, and they have an enhanced energy expenditure (Barbosa et al., 2007). These beneficial effects seem to be mediated by a mechanism that is sirtuin (SIRT)1-dependent. Of note, SIRT1 is a NAD-dependent deacetylase with important actions in metabolism (Zhou et al., 2018). Mice deficient for CD38 have higher NAD^+ levels and increased SIRT1 activity compared with WT mice (Aksoy et al., 2006a, 2006b). Recently, a number of molecules involved in the regulation of SIRT activity and/or NAD levels have been associated with early (SIRT4, SIRT7, nicotinate phosphoribosyltransferase (NAPRT)1 and nicotinamide nucleotide adenylyltransferase (NMNAT)2) and late phases (NMNAT3, nicotinamide riboside kinase (NMRK)2, ABCA1 and CD38) of glucose intolerance (Drew et al., 2016).

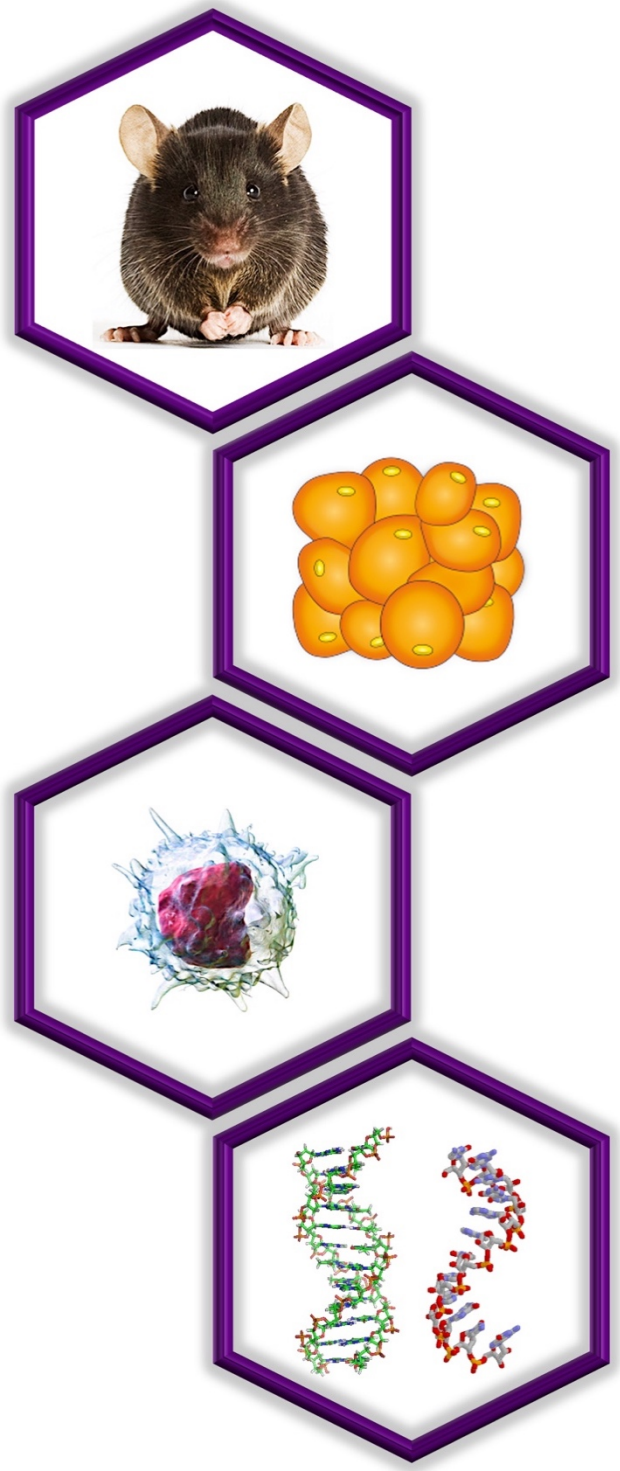
On the other side, CD38-deficient mice in an ICR background displayed reduced insulin secretion from pancreatic islets and increased glycemia (Kato et al., 1999), whereas mice overexpressing a CD38 transgene were protected from diabetes (Kato et al., 1995). Moreover, increased expression of CD38 in selective tissues has been proposed to play important roles in glucose tolerance and in insulin secretion (Malavasi et al., 2008). In adipocytes, for example, CD38 may contribute to mediate the translocation of GLUT4 to the plasma membrane in response to insulin and PPARγ activation (Song et al., 2012). In addition, CD38-deficient pancreatic β-cells (in C57BL/6 background) presented altered Ca^{2+} homeostasis and reduced responsiveness to insulin, but not to glucose (Johnson et al., 2006). Moreover, pancreatic islets from CD38-deficient mice were more susceptible to apoptosis compared with islets from WT mice, raising the possibility that CD38 plays an antiapoptotic role without directly controlling glucose signalling in these cells.

Interestingly, anti-CD38 antibodies have been described in the sera of Japanese and Italian cohorts of T2D patients (Ikehata et al., 1998; Pupilli et al., 1999), which is in line with the recent consideration that autoimmune events may contribute to T2D. However, the Japanese study reported a stimulatory effect of anti-CD38 antibodies on insulin secretion in rat islets while the Italian study associated these antibodies with inhibitory effects in human islets.

In a model of atherosclerosis induced by feeding LDLR-deficient mice with a Western diet, the mice that had been transplanted with bone marrow cells deficient for CD38 showed a significant

reduction in the atherosclerotic burden and in the macrophage infiltration within atherosclerotic lesions in comparison with the mice receiving WT bone marrow cells. The defective chemotactic capacity of CD38-deficient myeloid cells and the importance of mononuclear cell infiltration during early atherosclerosis development helped explain this phenotype (Beceiro et al., 2018).

As described in *Section 3. PHARMACOLOGIC TREATMENT OF INSULIN RESISTANCE AND TYPE 2 DIABETES AND THEIR EFFECTS IN METAFLAMMATION*, most of the traditional drugs to treat T2D are directed to decrease hyperglycemia either by inhibiting glucose hepatic production or glucose uptake by the kidneys, or by increasing insulinemia. However, these strategies result in numerous undesired side effects. Nowadays, compelling evidence has shown that several members of the nuclear receptor family improve insulin sensitivity in an agonist-dependent manner. A comprehensive analysis of the actions of LXRs, which are deeply involved in the control of metabolism and the inflammatory response, may reveal general and tissue-restricted mechanisms to control obesity-induced insulin resistance.

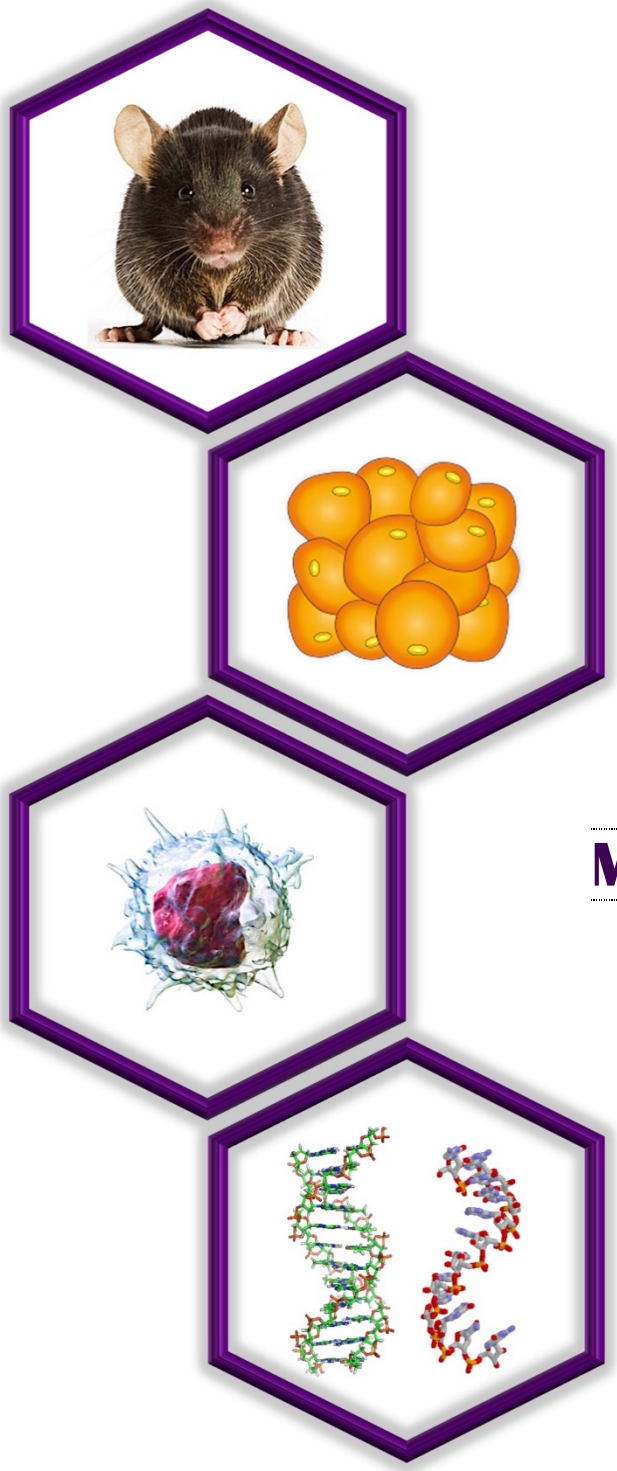


OBJECTIVES

We hypothesize that specific mechanisms of cross-talk between different pro-inflammatory pathways and LXRs may be responsible for the tissue-specific and systemic control of insulin sensitivity by these nuclear receptors. In particular, the role of CD38 in the insulin-sensitizing actions of LXR agonists deserves investigation. For this purpose, we proposed to develop the following two major objectives:

1. To characterize novel mechanisms of cross-talk between LXRs and the IFN-γ-signaling pathway.

2. To study the relevance of CD38 in the insulin-sensitizing actions of LXRs.



MATERIALS AND METHODS

Reagents.

Recombinant murine interferon-gamma (IFN-γ) was purchased from Pierce Biotechnology (Rockford, IL). The LXR agonists TO901317 (T1317) and GW3965 were purchased from Cayman Europe (Estonia) and Chemilieva (China), respectively.

Animals.

C57BL/6J mice were purchased fom Envigo Laboratories. STAT1-deficient mice were kindly donated by Anna Planas (IDIBAPS, Barcelona, Spain). CD38-deficient mice are in C57BL/6J background (>10 generations) were used in collaboration with Jaime Sancho (CSIC, Granada). All mice used in these studies were maintained at the animal facilities of *Universitat de Barcelona,* at 21°C with free access to water and, unless otherwise indicated, a standard chow diet. All the protocols requiring animal manipulation have been approved by the ethical committees for experimentation with animals from *Parc Científic de Barcelona* and *Universitat de Barcelona*. Bone marrows from IRF1-deficient mice were kindly donated by Lionel Apetoh (INSERM, Dijon, France).

Bone marrow-derived macrophages.

Bone marrow-derived macrophages were obtained from the tibiae and femurs of six to ten-week-old C57BL/6J mice as described (Valledor et al., 2000). Bone marrow precursors were differentiated in DMEM (PAA Laboratories, Velizy-Villacoublay, France), supplemented with 10% heat inactivated fetal bovine serum (FBS) (PAA Laboratories) and 30% L-cell conditioned medium as a source of M-CSF. L-cell conditioned media was obtained as the supernatant from L929 cells (mouse fibroblast cell line) grown in DMEM supplemented with 10% heat inactivated FBS. In some experiments, the cells were replated and incubated in DMEM supplemented with 1% FBS before activation of the LXR pathway.

Diet-induced obesity mouse model.

Four-week-old male mice of the indicated genotypes were fed either the standard normal diet (Bioserv F4031, 63% energy from carbohydrate, Frenchtown, NJ, USA) or a high fat diet (Bioserv S3282, 60% energy from fat, Frenchtown, NJ, USA) for 10 weeks. Body weight were measured weekly. The development of obesity and insulin resistance was evaluated by following the increase in body weight weekly and by performing a glucose tolerance test (GTT) and an insulin tolerance test (ITT) at the end of the experiment (see below), respectively. Two weeks before the euthanasia, GW3965 (20 mg/Kg of body weight/day) or vehicle, each one suspended in carboxymethylcellulose (0.05 % in PBS), were administered daily by oral gavage. Four and two days before euthanasia, the

mice were subjected to a GTT and an ITT (see below), respectively. After 6 h of fasting, half of the animals in each group were intra-peritoneally injected with insulin (0.5 UI/kg body weight in PBS) 15 min before euthanasia. Afterwards, the mice were anesthetized, and blood samples were collected by heart puncture in 1 mL EDTA Tri-K tubes. Then, the mice were sacrificed by cervical dislocation and tissues were collected. Blood samples were centrifuged at 1000xg for 10 min and the supernatants (plasma) were transferred to new tubes. Plasma and tissue samples were stored at -80ºC until further processing for analysis.

Irradiation and bone marrow transplantation

Eight-week-old C57BL/6J males were sub-lethally irradiated with two sessions of 4.5 Gy separated by 4hr. The animals were then injected with $3x10^6$ bone marrow cells from either WT or CD38-deficient male donors. Eight weeks after the bone marrow transfer, the mice were subjected to diet-induced obesity and to treatments with GW3965 or vehicle as described above.

Glucose tolerance test (GTT) and insulin tolerance test (ITT)

The mice were fasted for 6 h and then intra-peritoneally injected with glucose (2 g/kg body weight in PBS) or insulin (0.5 UI/ kg body weight in PBS). Glycemia was measured from the tail tip using a Glucocard SM (Menarini Diagnostics) before and at 30, 60 and 90 min after the injection of glucose or insulin.

Plasma insulin determination

Insulinemia was determined in the plasma from blood samples collected by intracardiac puncture (see above) or from the tip of the tails using Microvette® tubes (Sarstedt Inc). An ELISA assay (Crystal Chem, #90080) was performed to measure plasma insulin levels according to the manufacturer's guidelines.

Pancreatic Insulin Content by acid ethanol extraction

Pancreas were isolated, weighted and incubated overnight at -20 ºC in 5 ml of cooled acid ethanol (70% ethanol and 1.5 % HCl). Afterwards, the samples were homogenized using a Polytron homogenizer and centrifuged at 2150 rpm for 15 min at 4 ºC. For each sample, the aqueous solution was transferred to a new 15ml conical tube. Acid ethanol extracts (100 uL) were neutralized with 100 uL Tris 1M pH7.5 and diluted 1/2500 with Crystal Chem Kit Diluent. Finally, the insulin content was measured using an ELISA assay (Crystal Chem, #90080) following the manufacturer's recommendations.

Pancreatic islet enrichment

Pancreatic islet isolation was performed according to (Villarreal et al., 2019). After locating the pancreas in the abdominal cavity, the common bile duct was carefully clamped using a haemostatic forceps. The ampulla of Vater (a small area joining the pancreatic duct and the common bile duct) was injected with 3 mL of a solution containing collagenase P (Roche Diagnostics, #11249002001) until the head, neck, body, and tail regions of the pancreas were all fully inflated. The inflated pancreas was carefully dissected out and placed in 3 mL of ice-cold collagenase P solution. After chopping the pancreas for 3–5 s, the samples were incubated 15 min in a 37ºC shaker at 100–120 rpm for digestion. Then, the samples were placed on ice and 40 mL of ice-cold STOP solution were added to each sample to terminate the enzymatic digestion. The samples were then centrifuged at 300xg for 30 s. The sediments were washed three times with ice-cold STOP solution and then three times with ice-cold HBSS, and finally suspended by vortex in 5 mL of Histopaque®-1077 (Sigma Aldrich) at room temperature. Next, sequential addition of 5 mL of Histopaque®-1077 and 10 mL of HBSS was performed at room temperature. After forming density gradients, the tubes were centrifuged at 1700xg for 15 min. The islets in the gradient were recovered, washed three times with ice-cold HBSS, and suspended in 1 ml of Tri Reagent (Sigma Aldrich) and used for total RNA extraction as described below.

RNA extraction and cDNA synthesis.

Total RNA was extracted using Tri Reagent (Sigma Aldrich) following the manufacturer's recommendations. Total RNA was quantified using Nanodrop (ThermoScientific, Waltham, MA). For cDNA synthesis, 1 µg of total RNA was subjected to reverse transcription using 200U of Moloney murine leukaemia virus (M-MLV) reverse transcriptase RNase H Minus, Point Mutant (Promega), M-MLV reaction buffer, 10 mM dNTPs (Promega), and oligo-dT (20mM) following the manufacturer's recommendations.

Quantitative real-time PCR analysis.

Quantitative real time (qRT-PCR) was performed using the Power SYBR Green Reagent Kit (Applied Biosystems, Darmstadt, Germany) following the manufacturer's recommendations. The primer sequences used for qRT-PCR analysis are shown in Table 1. Real-time monitoring of PCR amplification was performed using the CFX384 Real Time System (BioRad) (95 ^{o}C 10 min, followed by 35 cycles of 95 ^{o}C 30 s, 60 ^{o}C 30 s, 72 ^{o}C 30 s). Each determination was performed in triplicates.

The data were expressed as the ratio of the expression level of the target mRNA normalized to ribosomal L14 and referred as relative expression.

Table 1. Primers used for qRT-PCR analysis.

Molecule	Gen ID	Forward primer sequence 5'-3'	Reverse primer sequence 5'-3'
11bhsd1	*Hsd11b1*	ttattgtcaaggcgggaaag	gcaatcataggctgggtcat
Abca1	*Abca1*	gcgagggctcatcgacat	gaagcggttctccccaaac
Adiponectin	*Adipoq*	agccgcttatgtgtatcgctca	tgccgtcataatgattctgttgg
Aim	*Cd5l*	gttggatcgtgtttttcaga	tcccactagctgcactttggt
Ccl19	*Ccl19*	aggacatctgagcgattccag	agtcttccgcatcattagcac
Ccl2	*Ccl2*	Aggtccctgtcatgcttctg	gctgctggtgatcctcttgt
Ccl20	*Ccl20*	cgactgttgcctctcgtaca	aggaggttcacagccctttt
Ccl21	*Ccl21*	gactgctgccttaagtacagcc	tctgttcagttctcttgcagccc
Ccl5	*Ccl5*	Ctgctgctttgcctacctct	tccttcgagtgacaaacacg
Ccr5	*Ccr5*	caacattgtcctcctcctgacc	gagtctctgttgcctgcatgg
Ccr6	*Ccr6*	ttgtcctcaccctaccgttc	agggcttgagatgatgatgg
Ccr7	*Ccr7*	ccaggaaaaacgtgctggtg	ggccaggttgagcaggtagg
Cd11c	*Itgax*	aaaatctccaacccatgctg	caccaccagggtcttcaagt
Cd38	*Cd38*	gcatgttcaagctcctcctt	tcagtccaggctacaggtga
Cd68	*Cd68*	cttcccacaggcagcacag	aatgatgagaggcagcaagagg
Chrebp	*Mlxipl*	gcatcctcatccgaccttta	gatgcttgtggaagtgctga
Cox2	*Ptgs2*	attctttgcccagcacttca	gggatacacctctccaccaa
Cxcl11	*Cxcl11*	aatttacccgagtaacggctg	attatgaggcgagcttgcttg
F4/80	*Adgre1*	ctttggctatgggcttccagtc	gcaaggaggacagagtttatcgtg
Foxp3	*Foxp3*	cccatccccaggagtcttg	accatgactaggggcactgta
Gip	*Gip*	tggctttgaagacctgctc	ttgttgtcggatcttgtcca
Glp1	*Gcg*	ggcacattcaccagcgactac	caatggcgacttcttctggg
Glut4	*Slc2a4*	gctttgtggccttctttgag	cggcaaatagaaggaagacg
Hmox1	*Hmox1*	gccgagaatgctgagttcatg	tggtacaaggaagccatcacc
Idol	*Mylip*	atgctgtgctatgtgacgagg	tcgatgatccctagacgcctg
Ifng	*Ifng*	actggcaaaaggatggtgac	tgagctcattgaatgcttgg
Il10	*Il10*	agccttatcggaaatgatccagt	ggccttgtagacaccttggt
Il17	*Il17*	tccagaaggccctcagacta	ctcgaccctgaaagtgaagg
Il18	*Il18*	actttggccgacttcactgt	gggttcactggcactttgat
Il1b	*Il1b*	tgggcctcaaaggaaagaat	caggcttgtgctctgcttgt
Il6	*Il6*	ccagagatacaaagaaatgatgg	actccagaagaccagaggaaat
Irf1	*Irf1*	ttggcatcatggtggctgt	aaggaggatggtcccctgttt
L14	*Rpl14*	tcccaggctgttaacgcggt	gcgctggctgaatgctctg
Leptin	*Lep*	aagaagatcccagggaggaa	tgatgagggttttggtgtca
Leptinr	*Lepr*	cctcttgtgtcctactgctcg	gaaattcagtccttgtgcccag
Lxra	*Nr1h3*	ccttcctcaaggacttcagttacaa	catggctctggagaactcaaagat
Lxrb	*Nr1h2*	cattgcgactccaggacaaga	cccagatctcggacagcaag
Mertk	*Mertk*	gactccctatcccggagttc	ctgcagcctcaacacagaga
Mgl1	*Clec10a*	tgagaaaggctttaagaactggg	gaccacctgtagtgatgtggg
Mgl2	*Mgl2*	aggcaccctaagagccattt	ccctcttctccagtgtgctc
Nos2	*Nos2*	gccaccaacaatggcaaca	cgtaccggatgagctgtgaatt
Perilipin1	*Plin1*	agcgtggagagtaaggatgtc	cttctggaagcactcacagg
Resistin	*Retn*	ttccttgtccctgaactgct	tgctgtccagtctatccttg
Sirt1	*Sirt1*	agctggggtttctgtctcctg	gggaagtccaccgcaaggcg
Srebp1c	*Srebp1c*	aggccatcgactacatccg	atccatagacacatctgtgcctc
Stat1	*Stat1*	tcacagtggttcgagcttcag	gcaaacgagacatcataggca
Tgfb1	*Tgfb1*	gagcccgaagcggactacta	tggttttctcatagatggcgttg
Tnfa	*Tnf*	ccagaccctcacactcagatc	cacttggtggtttgctacgac
Visfatin	*Nampt*	attcaaggagatggcgtgga	cttctgtagcaaagcgccac

Protein extraction and immunoblot analysis.

Tissues samples were homogenized in extraction lysis buffer (20 mM sodium 4-(2-hydroxyethyl)-1-piperazineethanesulfonate (HEPES), pH 7.5, 10 mM ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 2.5 mM MgCl2, 40 mM 2-glycerophosphate, and 1 % NP-40) using a Polytron, and supplemented with 2 mM sodium orthovanadate, 1 mM dithiothreitol (DTT), 0.5 mM phenylmethyl sulfonyl fluoride (PMSF), 1 µg/mL aprotinin, and 1 µg/mL leupeptin. Insoluble material was removed by centrifugation at 13,000xg for 10 min at 4 ^{o}C. Cell lysates (80 µg) were boiled at 95 ^{o}C for 5 min in Laemli SDS-loading buffer, separated by SDS-PAGE and electrophoretically transferred to PVDF membrane through iBlot® 2 Dry Blotting System (Thermo Fisher Scientific). The membranes were blocked in 5 % fat free-milk (for total AKT or GAPDH analysis) or 5 % bovine serum albumin (for phosphorylated-AKT analysis) in TBS-0.1 % Tween 20 (TBS-T) for 1 h and then incubated overnight with rabbit anti-AKT (Sant Cruz Biotechnology, sc-1619), anti-phosphoSer473-AKT (P-AKT) (Abcam, ab81283) or anti-GAPDH (St John's Laboratory Ltd, STJ97090) at 4 ^{o}C. The membranes were washed three times in TBS-T and then incubated for 1 h with peroxidase-conjugated anti-rabbit IgG (Jackson Laboratory, 115-095-003). After three washes of 10 min with TBS-T, enhanced chemiluminescence reaction was performed (SuperSignal West Pico Plus Chemiluminescent Substrate, Thermo Scientific) and detected by exposing the membranes to X-ray films (Fujifilm, Tokyo, Japan).

Analysis of adipose tissue macrophages by flow cytometry

Flow cytometry analysis of adipose tissue macrophages was performed according to (Cho et al., 2014). The epididymal adipose tissue was isolated and weighted, minced in small pieces, and incubated with collagenase type 2 (C6885, Sigma Aldrich) at 37ºC for 30 min with vigorous shaking. After digestion, EDTA was added to a final concentration of 10mM, and the samples were incubated at 37ºC for 5 min. The digested samples were filtered using a 100-mm nylon filter. Subsequently, the cell slurries were centrifuged at 500xg for 10 min at 4 ºC to separate adipocyte cells and stromal vascular cells (SVCs). The adipocytes were collected (upper layer) and kept for mRNA expression analysis, whereas the middle layer was discarded. The SVC-containing pellets (bottom layer) were suspended in 1 ml of red blood cell (RBC) lysis buffer and incubated 5 min at room temperature with occasional gently shaking. The RBC lysis buffer was neutralized, and the SVC were pelleted and suspended in 5 ml cooled FACS buffer (PBS with 1 mM EDTA, 25 mM HEPES, and 1 % heat inactivated FBS). SVCs (10^6 cells) were suspended in 100 uL of FACs buffer and incubated on ice for 30 min with 1 ug of Fc-block (anti-CD16/32, BD Biosciences). Next, 100 ul of antibody cocktail were added to

each sample (Figure 10a). The cells were incubated 60 min at 4 ºC protected from light, washed twice with FACS buffer and resuspended in 400 uL of PBS. DAPI was added (0.1 ug/mL) right before analysing the cells by flow cytometry. Cell populations were analysed using a FacsAria (BD Biosciences). The gating strategy (Figure 10b) included the identification of general CD45+ leukocytes, macrophages (CD45+ CD11b^{high} F4/80^{high}) and non-macrophage myeloid cells (CD45+ CD11b+/- F4/80^{negative}).

a

Antibody	Clone	Supplier
F4/80 PE	BM8	eBioscience
CD11b APC eFluor 780	M1/70	eBioscience
CD11c PE-Cy7	N418	eBioscience
CD45.2 PerCP-Cy5.5	104	eBioscience
CD301 Alexa Fluor 647	ER-MP23	AbD Serotec

b

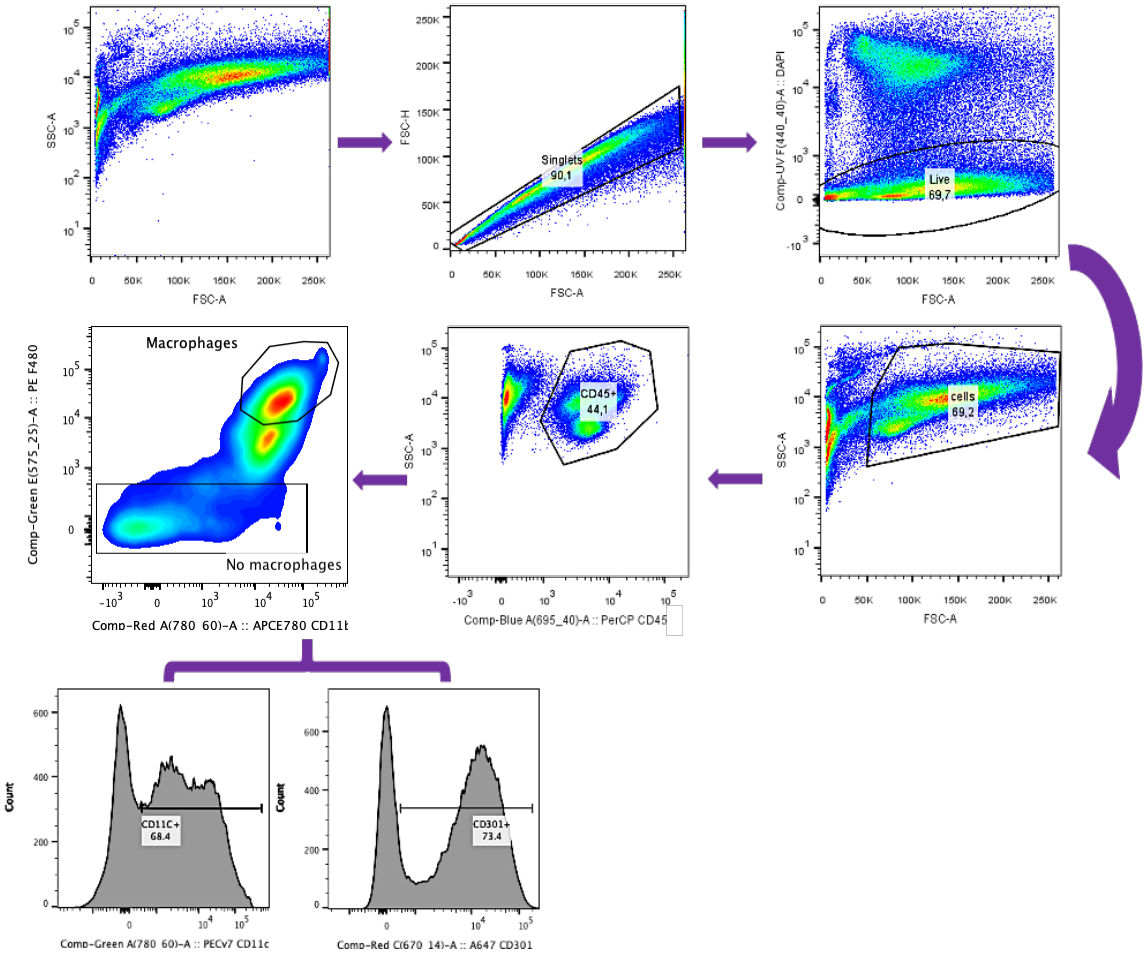


Figure 10. Antibodies and gating strategy used for flow cytometry analysis. a) Antibodies used for flow cytometry analysis. b) Gating strategy. Singlets were gated in an FSC-A *versus* FSC-H plot. Within this gate, live cells were selected as DAPI negative cells and, later, the population of interest was defined using a FSC-A *versus* SSC-A plot. Next, adipose tissue leukocytes (CD45+) were selected. Adipose tissue macrophages from both lean and obese mice co-express F4/80 and CD11b. Therefore, within the CD45+ population, macrophages were gated as CD11b^{high} F4/80^{high} cells and non-macrophage myeloid cells were gated as CD11b+/- F4/80^{negative}. Finally, the levels of surface expression of CD11c (M1 marker) and CD301 (M2 marker) were analysed separately in viable macrophages and non macrophage myeloid cells from both lean and obese mice.

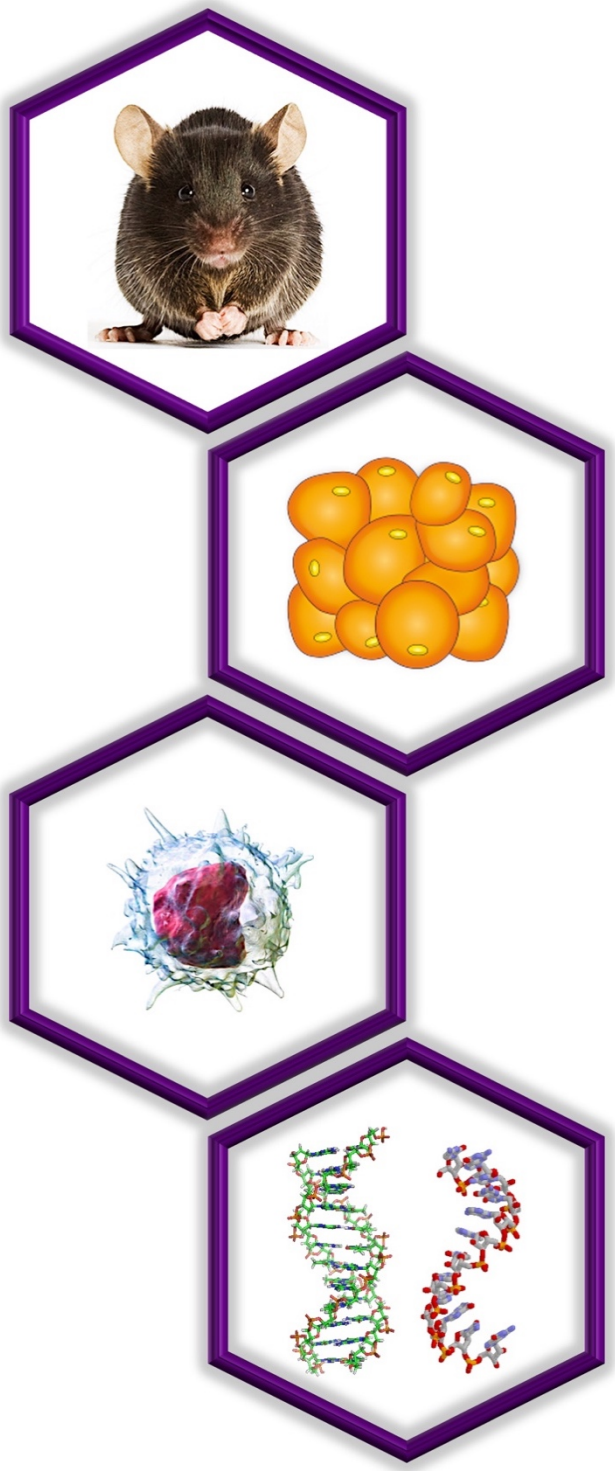
Alternatively, SVC-containing pellets were lysed in Tri reagent and used for total RNA extraction as described above.

Cytokine measurements through Procartaplex Immunoassay

Whole blood samples were collected by heart puncture in 1 mL EDTA Tri-K tubes. Samples were subsequently centrifuged at 1000g and stored at −80 ◦C until analysis. Plasma cytokine levels were assessed by using the customized five-plex ProcartaPlex™ Mouse Panel (leptin, IFN-g, IL-6, IL-10 and TNF-a), according to manufacturer's protocol (ProcartaPlex™ Multiplex Immunoassays, eBioscience, San Diego, CA). Cytokine concentrations were determined by means of analyte-specific capture beads coated with target-specific capture antibodies using Luminex technology. The samples were reading in MAGPIX™ system by duplicate. Because for IFN-g, IL-6, IL-10 and TNF-a values were undetectable, we later used a High Sensitivity three-plex ProcartaPlex™ Mouse Panel (IFN-g, IL-6, and TNF-a) to measure the concentration of these cytokines in the same samples analyzed previously.

Statistical analysis

Statistical analyses were performed with Graph Pad Prism Software. For data with normal distribution, a one-way ANOVA (for one factor) or a two-way ANOVA (for two factors) test were used. In many cases, the analysis was complemented with a Student T-test for selected comparisons. For non-parametric data, a Kruskall Wallis-Dunn's test or a U Mann-Whitney test were used. We used Grubbs' test (GraphPad) to determine significant outliers. Differences were considered significant when $p < 0.05$.



RESULTS

1. To characterize novel mechanisms of cross-talk between LXR and the IFN-γ-signaling pathway.

Our group had previously demonstrated reciprocal negative cross-talk between LXRs and IFN-γ signalling in macrophages (Pascual-García et al., 2013). Functional STAT1 is required for the negative effects mediated by IFN-γ on the expression of a specific subset of LXR target genes involved in metabolic responses. We now explored whether the transcription factor IRF1, which is induced downstream of STAT1 activation, plays a role in this cross-talk. For this purpose, WT and IRF1-deficient bone marrow-derived macrophages were stimulated with IFN-γ and/or LXR agonists and the expression of selected IFN-γ or LXR target genes was evaluated.

Among the genes that are part of the macrophage transcriptional response to IFN-γ, we selected classical pro-inflammatory mediators previously shown to be repressed by LXR agonists (Pascual-García et al., 2013). The contribution of IRF1 to the transcriptional induction of the selected genes showed gene-specificity. For instance, the induction of *Ccl5* and *Nos2* expression in response to IFN-γ was blunted in the absence of functional IRF1 expression, whereas partial IRF1 contribution was observed on *Cox2* and *Cxcl11* expression (Figure 11). LXR activation by GW3965 was able to downregulate the induction of all these genes in response to IFN-γ in WT macrophages, at least at the 6 h time point in which maximal induction was observed. However, the capability of the LXR agonist to repress the IFN-γ-induced expression of *Cox2* and *Cxcl11* was impaired in IRF1-deficient macrophages (Figure 11), suggesting that the negative cross-talk between LXRs and IRF1 is important for the repression of these genes in response to the LXR agonist.

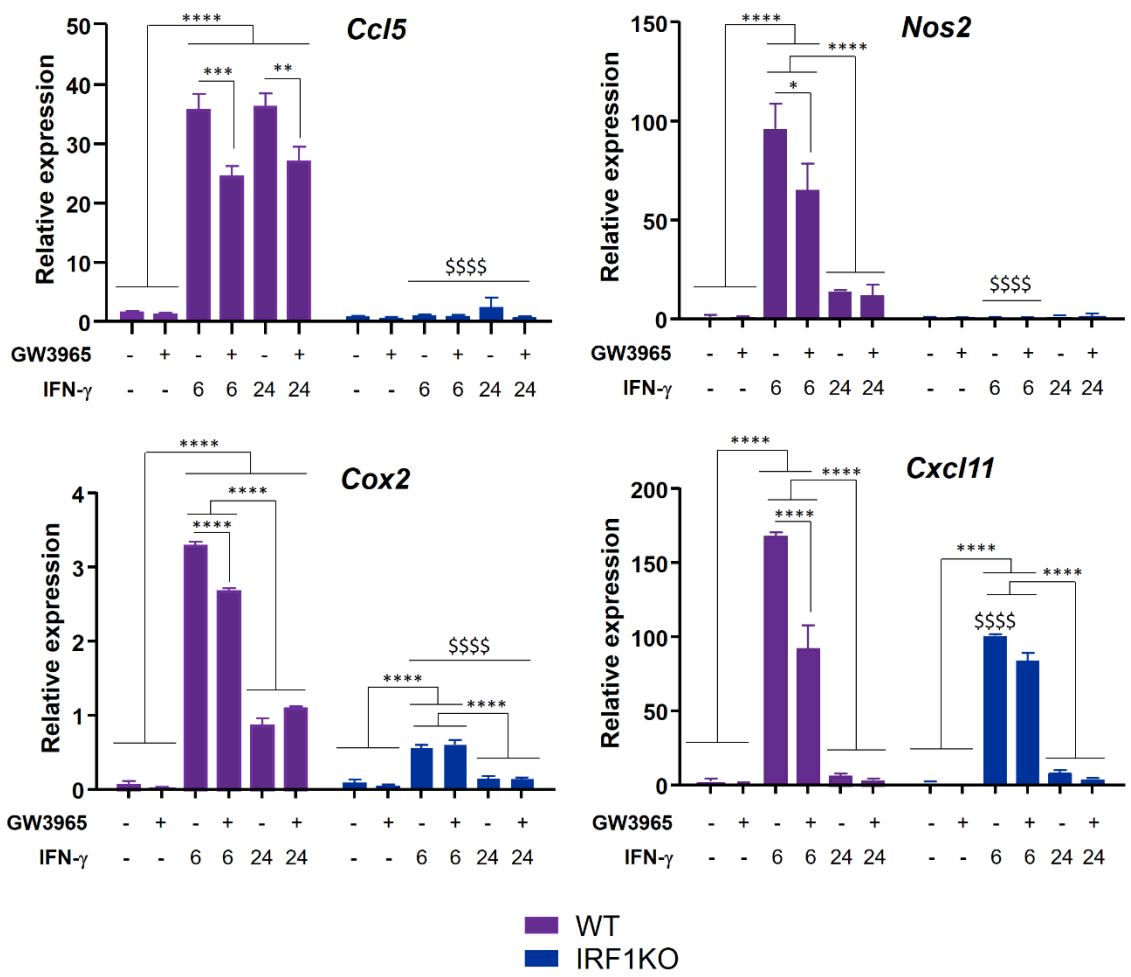


Figure 11. IRF1 was required for the full induction of several macrophage pro-inflammatory genes in response to IFN-γ. Bone marrow-derived macrophages from WT or IRF1-deficient (IRF1KO) mice were placed in DMEM-1% FBS and treated with the LXR agonist GW3965 (1 µM) or vehicle for 18h and then stimulated with IFN-γ (5ng/ml) for 6 or 24h. Relative expression levels were analysed by qRT-PCR (normalized to L14 expression levels). Mean ± SEM from n=3 independent experiments. Two-way ANOVA-Tukey posthoc, *p <0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 (selected comparisons within a given genotype), $$$$ p < 0.0001 (comparisons of a given experimental condition between IRF1KO and WT).

Notably, however, the induction of IRF1 expression was not inhibited upon LXR activation (Figure 12), which suggests that the cross-talk between LXR and IRF1 is exerted at the post-transcriptional level.

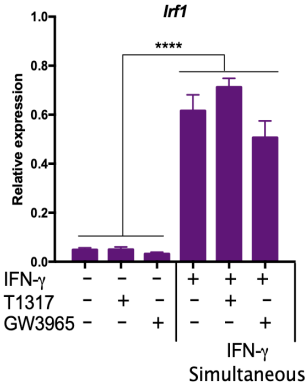


Figure 12. IRF1 expression was not inhibited upon LXR activation. Bone marrow-derived macrophages from WT mice were stimulated simultaneously with the LXR agonists T1317 or GW3965 (1 μM each) and IFN-γ (5ng/ml) for 24h (in DMEM-1% FBS). Control cells were either left untreated or treated with T1317 or GW3965. Relative expression levels were analyzed by qRT-PCR (normalized to L14 expression levels). Mean ± SEM from n=4 independent experiments. One-way ANOVA-Tukey posthoc, ****p < 0.0001.

On the other hand, IFN-γ was able to downregulate the induction of several LXR target genes, namely *Abca1*, *Idol* and *Aim* (Figure 13), thus validating our previous observations (Pascual-García et al., 2013). IRF1 deficiency resulted in selective effects on LXR target gene expression. IRF1 partially contributed to the negative effects that IFN-γ signalling exerts on the expression of *Abca1 and Idol*. Interestingly, IRF1 was required for basal expression and for LXR-mediated induction of *Aim*, an anti-apoptotic factor secreted by macrophages (Figure 13).

However, not all the actions of the IFN-γ signalling pathway resulted in negative effects on LXR target genes. In particular, LXR agonists and IFN-γ signalling cooperated for the induction of *Mertk*, which encodes a protein involved in the phagocytosis of apoptotic cells, and of CD38, a multifunctional enzyme that regulates the intracellular levels of NAD. Interestingly, while functional IRF1 was necessary for these cooperative actions on *Mertk* expression, the levels of induction of *Cd38* by IFN-γ were higher in IRF1-deficient cells than in WT cells (Figure 13).

Our next step was to test the effect of STAT1 deficiency on the induction of *Cd38* expression in macrophages and compare it with the effects observed in the context of IRF1 deficiency. As a control, we validated that STAT1 expression is required for the inhibitory effects of IFN-γ on *Abca1* induction by LXR agonists (Figure 14) (Pascual-García et al., 2013). In addition, functional STAT1 was required for the cooperative actions of LXR agonists and IFN-γ on *Mertk* and *Cd38* expression. Interestingly, the de-repression of *Cd38* observed in IRF1-deficient cells was not observed in Stat1-deficient macrophages (Figure 14), which suggests that IRF1 may act directly or indirectly as a repressor of *Cd38* expression and thereby play a fine-tuning role on the expression of this gene during inflammation.

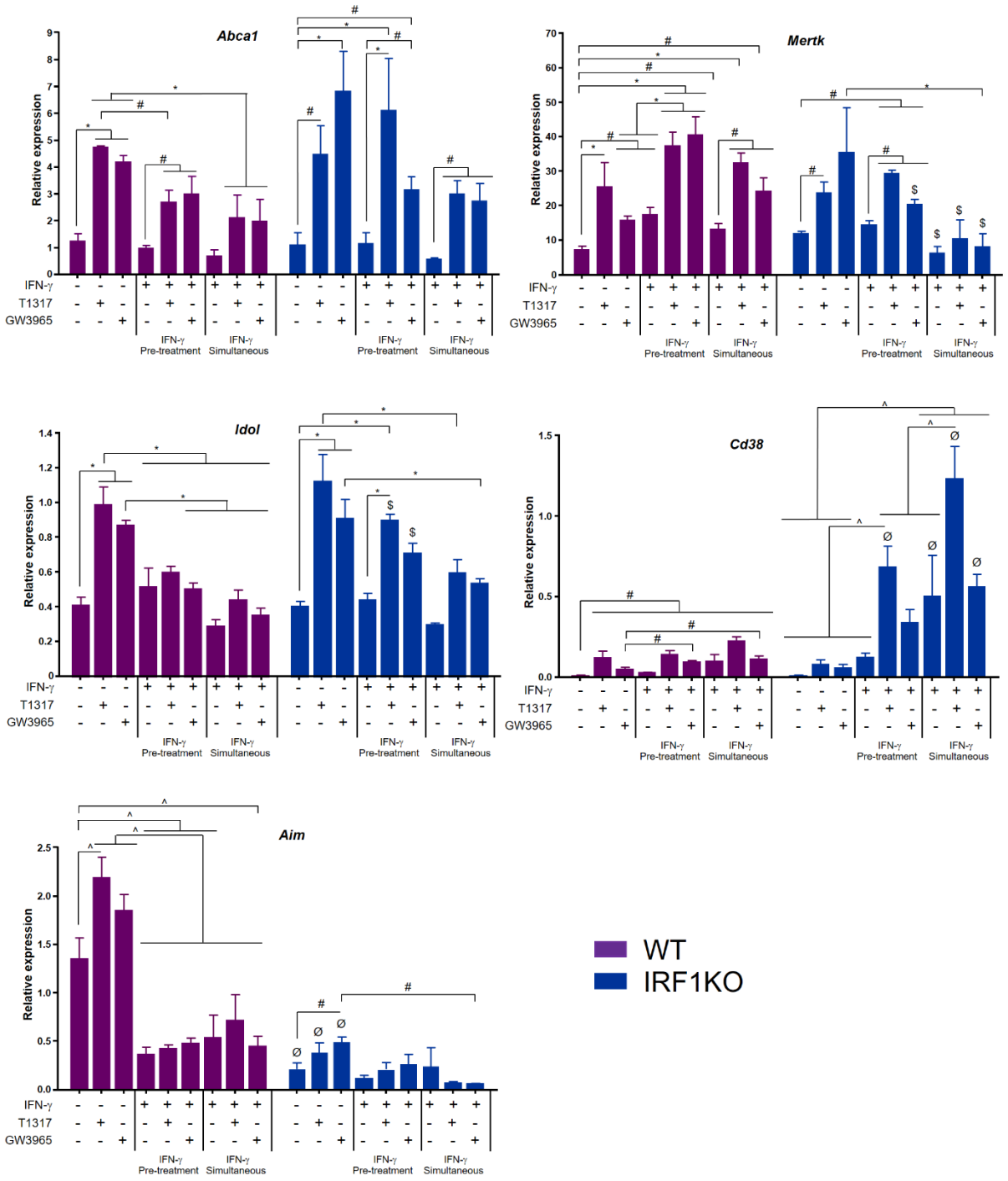


Figure 13. IRF1 exerted selective effects on LXR target gene expression. Macrophages were obtained from WT and IRF1-deficient (IRF1KO) mice and placed in DMEM-1% FBS. Two different types of stimulations were performed in these studies. On one side, the cells were pre-treated for 18h with IFN-*γ* (5ng/mL) or vehicle before the stimulation with the LXR agonists T1317 or GW3965 (1μM each, 24h) (indicated as IFN-*γ* Pre-treatment). On the other side, the cells were simultaneously treated with IFN-*γ* and T1317 or GW3965 (indicated as IFN-*γ* simultaneous). Relative expression levels of LXR target genes were determined using qRT-PCR (normalized to L14 expression levels). Mean ± SEM, n=3-5 mice/experimental group. For *Abca1*, *Mertk* and *Idol*, a two-way ANOVA test with Tukey posthoc did not render significant differences between genotypes. The data within each genotype was then analysed by one-way ANOVA-Tukey posthoc and relevant comparisons are displayed, *$p < 0.05$. In addition, $ ($p < 0.05$, T-test) reflects statistically significant differences in a given experimental condition between IRF1KO and WT. For *Cd38* and *Aim*, two-way ANOVA-Tukey posthoc, ^$p < 0.05$ (for relevant comparisons within a given genotype), Ø $p < 0.05$ (for differences in a given experimental condition between IRF1KO and WT). For all the genes analysed, selected experimental conditions were also compared with a T-test, #$p < 0.05$.

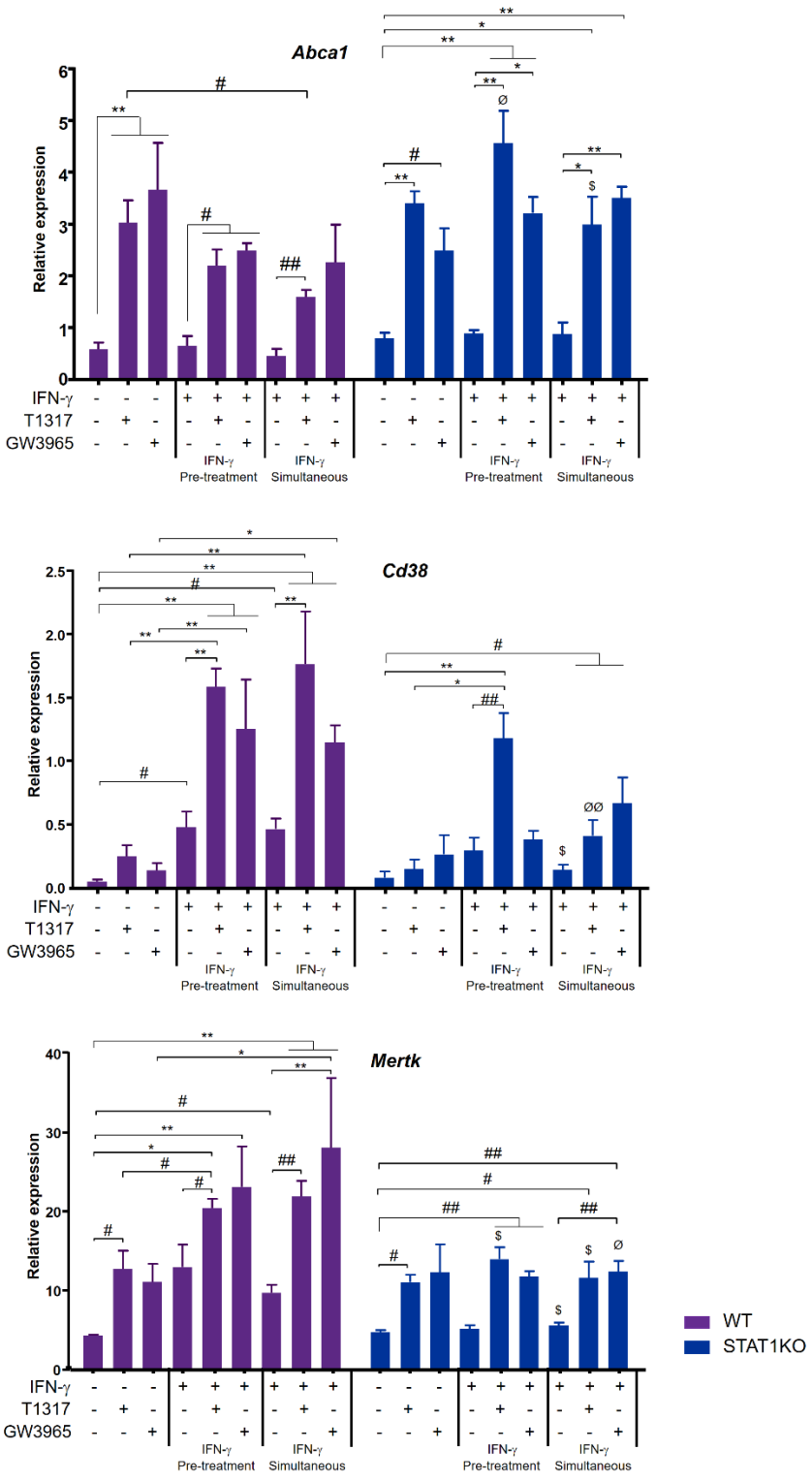


Figure 14. The induction of CD38 by IFN-γ was not de-repressed in STAT1-deficient macrophages.
Macrophages were obtained from WT and STAT1-deficient (STAT1KO) mice and placed in DMEM-1% FBS. Two different types of stimulations were performed in these studies. On one side, the cells were pre-treated for 18h with IFN-γ (5ng/mL) or vehicle before the stimulation with the LXR agonists T1317 or GW3965 (1µM each, 24h) (indicated as IFN-γ Pre-treatment). On the other side, the cells were simultaneously treated with IFN-γ and T1317 or GW3965 (indicated as IFN-γ simultaneous). Relative expression levels of LXR target genes were determined using qRT-PCR (normalized to L14 expression levels). Mean ± SEM from n=4 independent experiments. Two-way ANOVA-Tukey posthoc, *$p < 0.05$, **$p < 0.01$ (selected comparisons within a given genotype); Ø $p < 0.05$, ØØ $p < 0.01$ (comparisons of a given experimental condition between STAT1KO and WT). Selected experimental conditions were also compared using a T-test, # $p < 0.05$, ## $p < 0.01$ (within a genotype) and $ $p < 0.05$ (between genotypes).

2. To study the relevance of the LXR-CD38 axis in the insulin-sensitizing actions of LXR agonists.

2.1.- The improvement of systemic insulin sensitivity upon LXR activation requires functional CD38 expression.

As mentioned in the Introduction and in the previous section, several evidences indicate that CD38 is an LXR target gene in macrophages and dendritic cells (Beceiro et al., 2018; Matalonga et al., 2017). In the present work we further characterized the induction of CD38 by an LXR agonist in different tissues in the context of obesity. WT mice were fed with either a standard chow diet or with a high fat diet for 10 weeks. The high fat diet group was further divided into two subgroups receiving either vehicle or the LXR agonist GW3965 (20 mg/kg/day) daily by oral gavage throughout the last 14 days of the experiment (starting at week 8). The expression of CD38 was analysed in several tissues (Figure 15). Significant induction of CD38 upon LXR activation was observed in epididymal white adipose tissue, skeletal muscle and spleen.

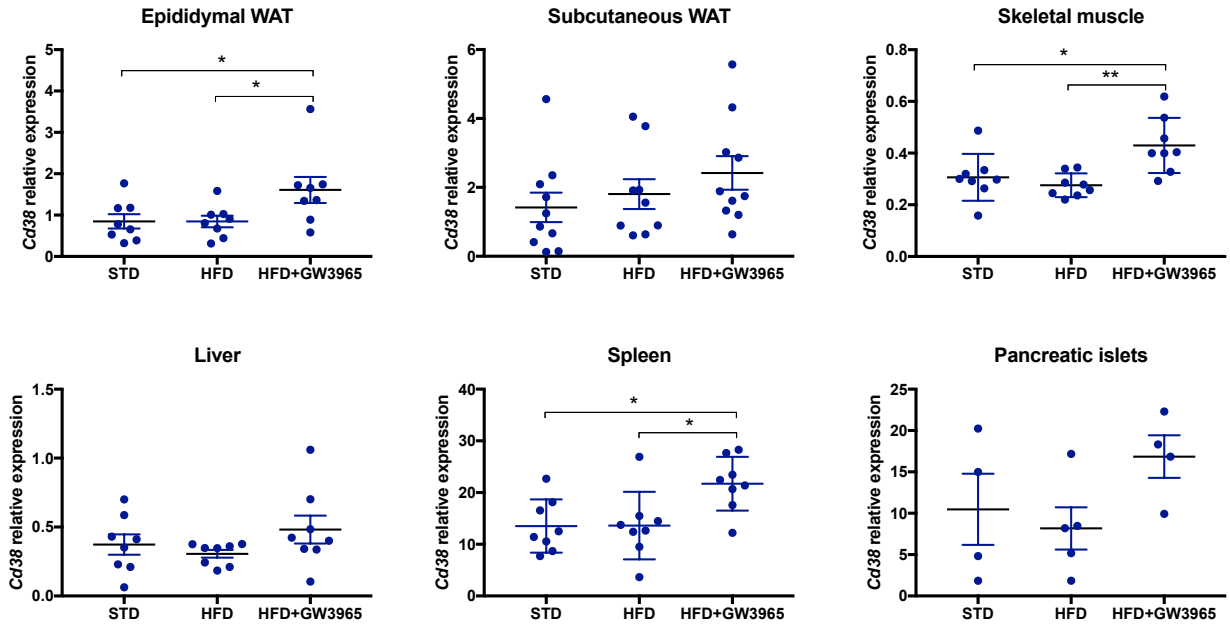


Figure 15. LXR activation upregulated the expression of CD38 in several tissues in obese mice. WT mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. Relative expression levels of CD38 were determined using qRT-PCR (each point was performed in triplicates). Mean values of normalized relative expression ± SEM. n=4-10 mice/group (depending on the tissue analysed). One-way ANOVA-Tukey posthoc, *$p < 0.05$, **$p < 0.01$. WAT, white adipose tissue.

Since CD38 expression was induced in several metabolic tissues in obese mice undergoing pharmacologic LXR activation, we investigated the role of CD38 in the insulin-sensitizing actions of the LXR agonist. WT or CD38-deficient mice were fed either a standard or a high fat diet and treated with vehicle or GW3965 as described above. Body weight was measured weekly. All the mice receiving a high fat diet underwent a higher increase in their weight than the mice fed a standard diet. However, as reported previously (Barbosa et al., 2007), WT mice gained more weight than CD38-deficient mice in response to the high fat diet (Figure 16a-b). GW3965 administration induced a loss of weight in all the mice exposed to the high fat diet, regardless of the genotype (Figure 16a and c). On the other hand, the animals fed with a high fat diet were characterized by increased levels of basal glycemia after 6 h of fasting, and GW3965 administration decreased glycemia independently of CD38 (Figure 16d).

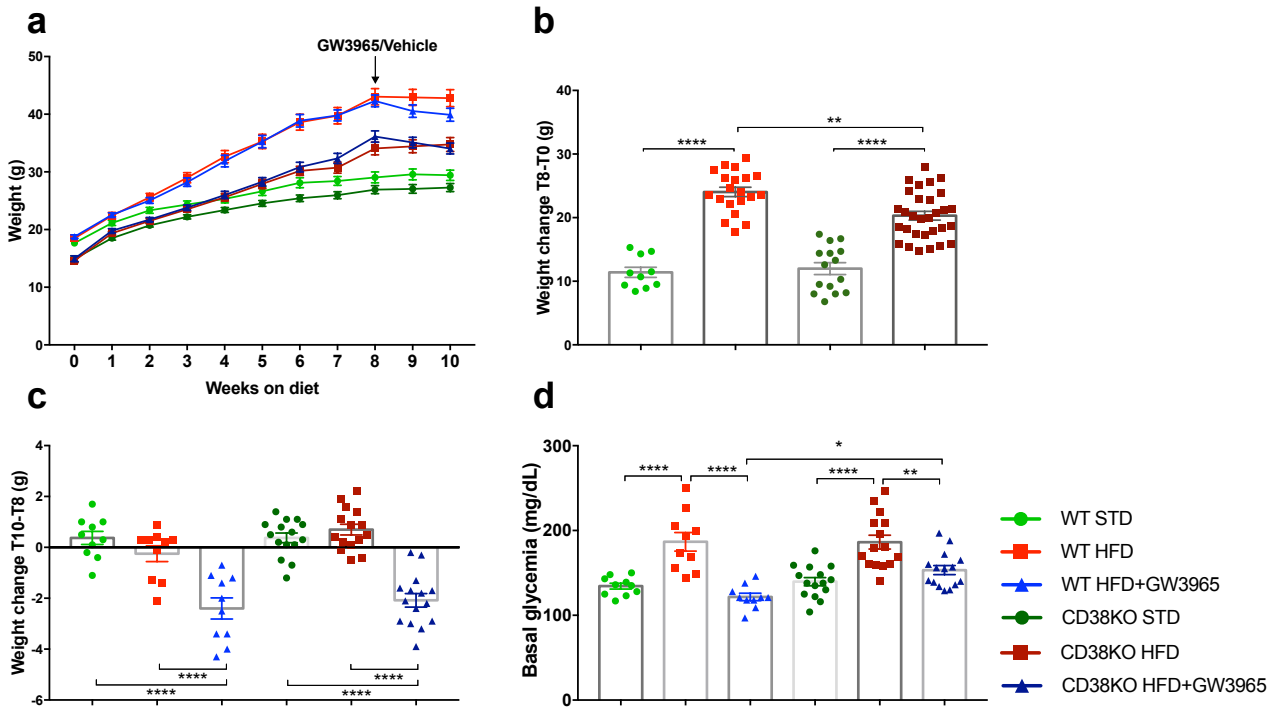


Figure 16. GW3965 decreased body weight and basal glycemia in diet-induced obese mice in a CD38-independent manner. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. **a**) Growth curve along the weeks of diet intervention. **b**) Weight change during the first 8 weeks on diet. **c**) Weight change during the last two weeks. **d)** Six-hour fasted glycemia at week 10. Mean ± SEM (n=10-15 mice/group). Two-way ANOVA-Tukey posthoc, *p < 0.05, **p < 0.01, ****p < 0.0001. Irrelevant comparisons have been omitted from the graphic.

After 10 and 12 days of GW3965 treatment, the mice were fasted for 6 h and then subjected to a GTT or an ITT, respectively. The GTT analysis showed that, under standard diet conditions, CD38-deficient mice have a slightly reduced capacity to decrease glycemia after glucose injection than WT mice (Figure 16a-b). In this same analysis, both WT and CD38-deficient animals developed glucose intolerance upon a high fat diet, although the level of glucose intolerance was slightly higher in CD38-deficient mice (Figure 17a-b). Administration of the LXR agonist GW3965 efficiently alleviated this condition in both WT and CD38-deficient mice, reaching the levels of their respective control groups on standard diet (Figure 17a-b).

The results of the ITT analysis showed that, under standard conditions, CD38-deficient mice perform better in response to insulin injection than their WT counterparts (Figure 17c-d). The high fat diet reduced insulin sensitivity in both genotypes, although CD38-deficient mice, again, displayed increased insulin sensitivity in comparison to WT animals. Interestingly, in response to GW3965 treatment, obese WT mice recovered insulin sensitivity to the levels observed in mice on standard diet, while this improvement was drastically impaired in the absence of functional CD38 expression (Figure 17c-d).

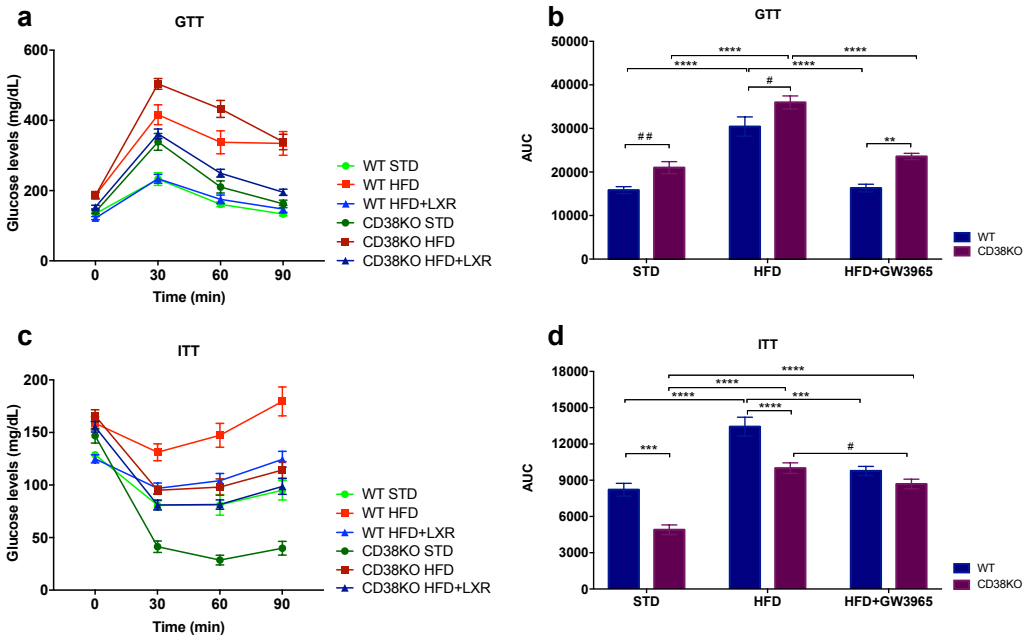


Figure 17. The improvement in systemic insulin sensitivity after LXR activation required functional CD38 expression. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. After 10 and 12 days of GW3965 (or vehicle) treatment, the mice were fasted for 6 h and then subjected to a GTT (a-b) or an ITT (c-d), respectively. The area under the curve (AUC) from 0 to 90 min is represented for both tests, GTT (b) and ITT (d). Mean ± SEM (n=10-15 mice/group). Two-way ANOVA-Tukey posthoc, **p < 0.01, ***p < 0.001 ****p < 0.0001. In addition, selected conditions were compared using a T-test, # p < 0.05, ## p < 0.01. Irrelevant comparisons have been omitted from the graphic.

Altogether, these results suggest that CD38-deficient mice are more glucose intolerant than WT mice in all the conditions evaluated. Despite this fact, CD38-deficient mice showed an increased insulin sensitivity. Administration of the LXR agonist GW3965 improved glucose tolerance in high fat diet-fed mice regardless of the genotype. However, the insulin sensitizing action of GW3965 was severely impaired in CD38-deficient mice. These results suggest that the effects of the LXR agonist on systemic insulin sensitivity require CD38 expression. To corroborate this hypothesis, we evaluated the in vivo response of the InR pathway by measuring insulin-induced AKT phosphorylation in the skeletal muscle, the tissue that contributes more importantly to glucose uptake in response to this hormone. In these assays, the mice were stimulated or not with an intraperitoneal injection of insulin 15 min before the euthanasia (Figure 18). In line with the ITT, when this response to insulin was analysed in the context of a standard diet, higher levels of AKT phosphorylation were observed in CD38-deficient mice than in the WT counterparts. The high fat diet reduced the response to insulin in both WT and CD38-deficient mice, although the response to insulin, again, was higher in CD38-deficient mice than in WT mice. In other words, despite the high fat diet, the CD38-deficient mice remained more sensitive to insulin than the WT counterparts. Interestingly, GW3965 administration to high fat diet-fed mice was able to restore the response to insulin in skeletal muscle only in WT animals, whereas no effect of the LXR agonist was observed in CD38-deficient mice. In conclusion, LXR activation restored skeletal muscle InR signalling in obese mice in a CD38-dependent manner.

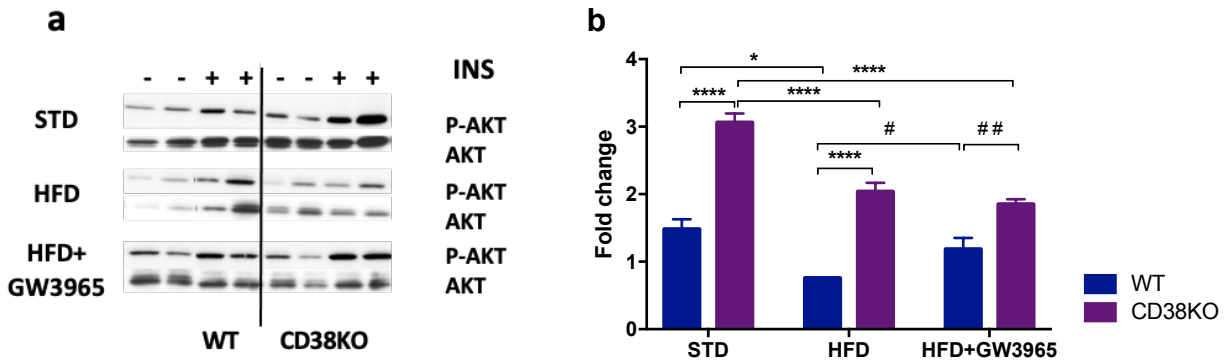


Figure 18. High fat diet induced insulin resistance in skeletal muscle of WT and CD38-deficient mice and GW3965 administration restored the normal response only in WT animals. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. The mice were fasted for 6 h and then, half of them were injected intraperitoneally with 0.5UI/kg of insulin 15 min before the euthanasia. Skeletal muscle was collected from each mouse and whole tissue extracts were prepared as described in Methods. **a**) Representative immunoblots of phosphorylated AKT (P-AKT) and total AKT in skeletal muscle of the indicated animals and conditions. **b**) For each sample, the P-AKT values were normalized to the total AKT values. The graphic represents the effect of insulin (in fold change) on the P-AKT/total AKT ratio in insulin treated (+INS) versus non-treated mice (-INS) for each experimental group. Mean ± SEM (n=6 animals/group). Two-way ANOVA-Tukey posthoc *p < 0.05, ****p < 0.0001. Selected conditions were also compared with a T-test, #p<0.05, ##p<0.01. Irrelevant comparisons have been omitted from the graphic.

We next evaluated the effects of the high fat diet and the LXR agonist on the systemic levels of insulin (insulinemia) in WT and CD38-deficient mice. As shown in Figure 19a, increased basal insulinemia was observed in WT mice exposed to a high fat diet, in comparison with the levels under a standard diet. The treatment of WT mice with GW3965 diminished the insulinemia in response to the high fat diet. In CD38-deficient mice, the high fat diet induced lower levels of insulinemia than in WT mice. Importantly, however, GW3965 did not efficiently decrease insulinemia in CD38-deficient animals (Figure 19a), in line with the lack of effects of the LXR agonist on systemic insulin sensitivity in these mice (Figure 17c-d).

Considering the changes in basal insulinemia described above, we decided to evaluate the increase in insulinemia in response to glucose. For this purpose, insulin levels were measured in blood samples obtained from the tail vein at different time points within the first 30 minutes after an intraperitoneal injection of glucose. In WT mice, obesity induced an increase in insulinemia in response a glucose challenge in comparison with the response in lean animals (Figure 19b-c). A tendency for lower insulinemia was observed in response to the LXR agonist treatment in obese WT mice, although these differences did not reach statistical significance in this study. The glucose-stimulated increase in insulinemia in CD38-deficient mice was markedly lower for all the

experimental conditions, compared with their WT counterparts (Figure 19b-c). In line with the ITT data, these results support the idea that CD38-deficient mice have increased insulin sensitivity and, consequently, require a smaller increase in insulinemia to restore glycemia than WT mice.

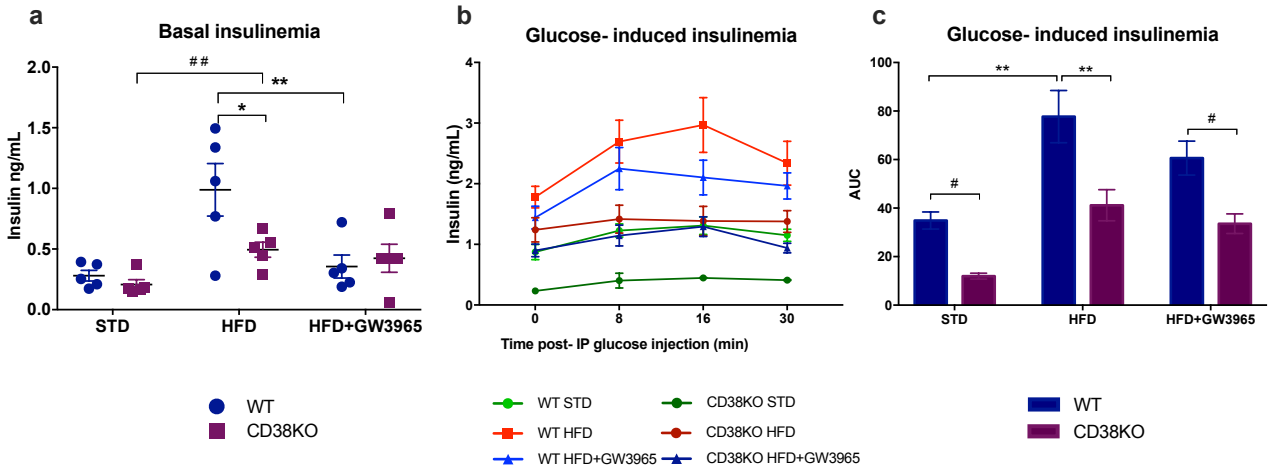


Figure 19. GW3965 administration in high fat diet-fed mice ameliorated hyperinsulinemia in WT but not in CD38-deficient mice. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. The mice were fasted for 6 h. **a)** Basal insulinemia measured by ELISA at day 10 of GW3965 administration. Mean ± SEM (n=5-6 mice/group). In **b-c**, blood samples from the tail vein were collected at the indicated time points after intraperitoneal (IP) glucose injection. **b**) Glucose-induced insulinemia measured by ELISA. **c)** The area under the curve (AUC) is represented for each experimental group in **b**. In **b-c**, mean ± SEM (n=3-7 animals/group). Two-way ANOVA-Tukey posthoc, **p < 0.01. Selected experimental conditions were also compared using a T-test, # p < 0.05, ## p < 0.01.

In addition, we studied the pancreatic insulin content in our experimental settings using an acid ethanol extraction method and measuring the insulin levels by ELISA (Figure 20). WT animals fed with a high fat diet presented higher pancreatic insulin contents compared with the standard diet group, and LXR agonist administration ameliorated this increase. In contrast, CD38-deficient animals did not show a significant increase in the pancreatic levels of insulin in response to the high fat diet and the levels of insulin remained lower than in the WT counterparts for all the conditions tested.

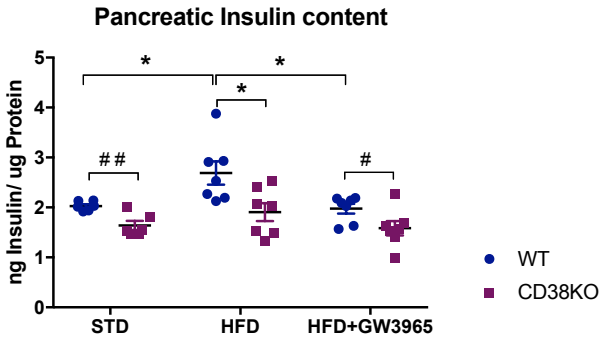


Figure 20. The pancreatic insulin content was increased by a high fat diet and reduced by GW3965 in WT but not in CD38-deficient mice. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. The pancreas samples were collected from mice fasted for 6 h, incubated for 16h in cold acid ethanol and processed for insulin determination as described in Methods. Pancreatic insulin levels were measured by ELISA and normalized to the overall protein content measured by the Bradford method. Mean ± SEM. n=6-7 animals/group. Two-way ANOVA-Tukey posthoc, *$p < 0.05$. Selected experimental conditions were also compared using a T-test, # $p < 0.05$, ## $p < 0.01$. Irrelevant comparisons have been omitted from the graphic.

Taking together the data presented in this section, CD38-deficient mice have increased insulin sensitivity than WT mice. As a consequence, both the insulin demand and the pancreatic insulin content are reduced in CD38-deficient mice. In addition, the ameliorating effects of the LXR agonist GW3965 on diet-induced insulin resistance are highly dependent on functional expression of CD38. These actions are consistent with predominant effects in insulin peripheral target tissues, although we cannot exclude that the LXR agonist can also downregulate insulin secretion through a direct action in pancreatic islets.

2.2.- Effects of LXR activation on genes with relevant roles in metabolic homeostasis

Once we had a general picture of the systemic effects of the LXR agonist on glucose tolerance and insulin sensitivity, we decided to analyze the expression of selected genes coding for important mediators of metabolic homeostasis. Their expression was analysed in insulin target tissues and in the spleen from WT and CD38-deficient mice.

First, we evaluated the expression levels of the adipokine leptin in the white adipose tissue. Leptin levels increase during obesity due to the development of leptin resistance, a condition that usually parallels obesity-induced insulin resistance (Ghadge and Khaire, 2019). Hence, the expression of leptin was upregulated in epididymal and subcutaneous white adipose tissues in both WT and CD38-deficient mice fed with a high fat diet (Figure 21a-b). LXR activation led to a significant decrease in leptin levels in the subcutaneous adipose tissue and a tendency for such downregulation in the epididymal adipose tissue in WT mice (Figure 21a-b).

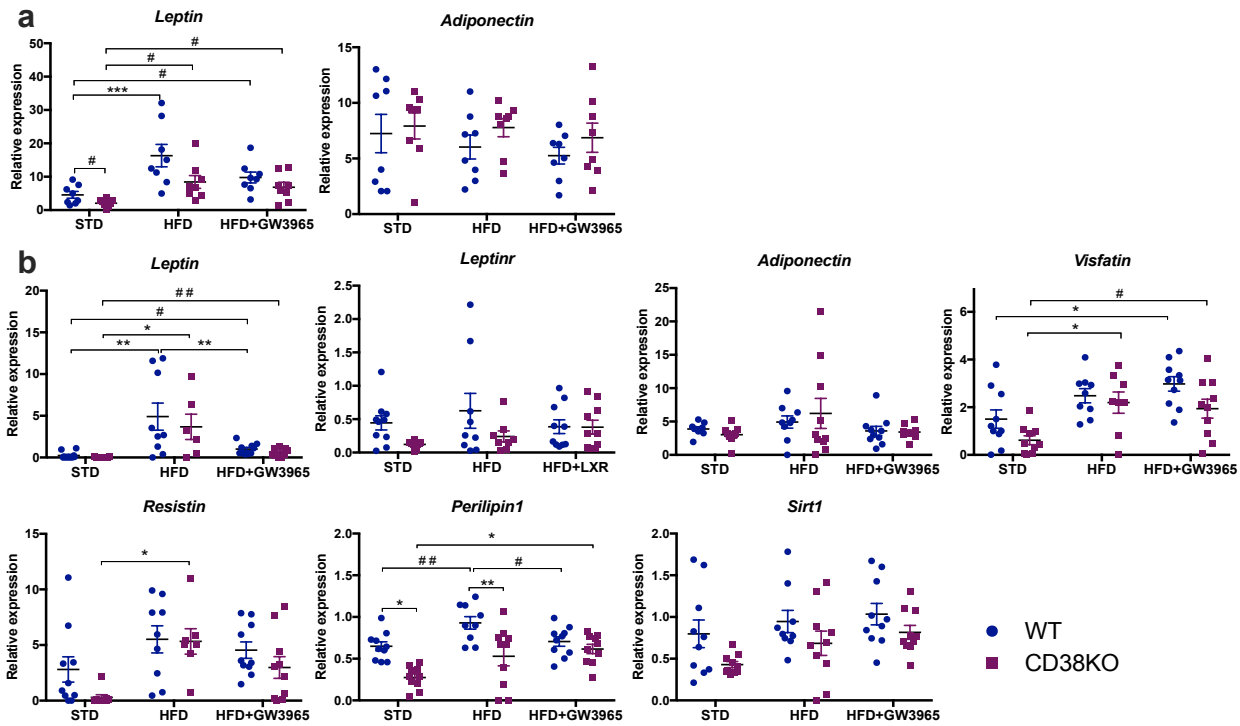


Figure 21. Effects of high fat diet and GW3965 on the expression of a cluster of genes associated with metabolic homeostasis in white adipose tissues. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. In each mouse, the epididymal (a) and subcutaneous (b) white adipose tissues were isolated and the relative expression levels of selected genes were determined using qRT-PCR (normalized to the expression levels of L14). Mean ± SEM, n = 8-10 mice/group. Two-way ANOVA-Tukey posthoc, *p < 0.05, **p < 0.01, ****p < 0.0001. Selected experimental conditions were also compared using a T-test, # p < 0.05, ## p < 0.01. Irrelevant comparisons have been omitted from the graphic. *Leptinr*, leptin receptor; *Sirt1*, sirtuin 1.

In addition to leptin, the expression of the following genes associated with metabolic homeostasis in white adipose tissues was analysed in this study: the leptin receptor; sirtuin 1 (an NAD+-dependent histone/protein deacetylase whose expression can be induced as a response to leptin) (Song et al. 2018); the lipid droplet-associated protein perilipin 1, which controls adipocyte lipid metabolism (Greenberg et al., 1991); and the adipokines adiponectin, which is involved in regulating glucose levels as well as fatty acid breakdown (Yanai and Yoshida, 2019), visfatin (the extracellular form of the nicotinamide phosphoribosyltransferase enzyme which also exerts a role as a cytokine) (Chini et al. 2014), and resistin (a controversial candidate potentially linking energy homeostasis and inflammation) (Park et al. 2017). In our model, we observed a significant increase in the expression of perilipin1 in response to the high fat diet in WT mice, which was inhibited by the LXR agonist. The levels of perilipin1 in CD38-deficient mice were significantly lower than in WT animals, and a tendency for induction was observed in response to the diet, although it was not suppressed by GW3965. In contrast to WT mice, visfatin and resistin were upregulated by the high fat diet in CD38-deficient mice, without any suppressive effect of the LXR agonist (Figure 21b). No significant changes in adiponectin, leptin receptor and sirtuin 1 were detected in these experimental conditions.

We further explored whether the diet or the presence of functional CD38 affected the expression of leptin in a different anatomic location, the spleen (Figure 22). Of note, the overall levels of leptin in the spleen are more than two orders of magnitude lower than in the white adipose tissue (compare the values of relative expression in Figures 21 and 22). In this context, the high fat diet resulted in only a tendency for increased leptin expression in this organ in WT mice (Figure 22), with no significant effect of the LXR agonist. As in the white adipose tissue no significant changes in splenic adiponectin expression were observed in any of the conditions evaluated (Figure 22).

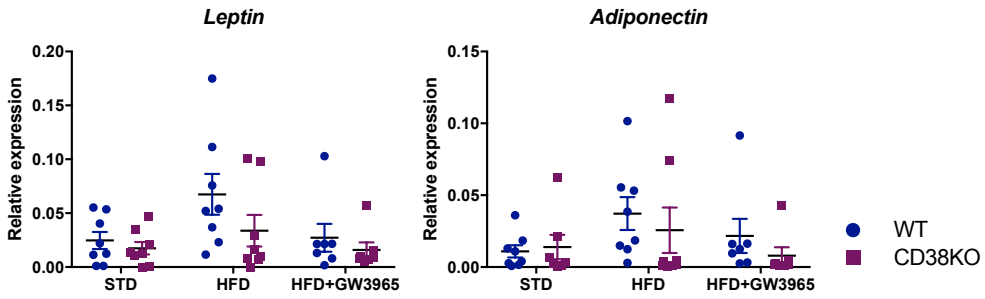


Figure 22. Effects of the high fat diet or LXR activation on the expression of leptin and adiponectin in the spleen. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. In each mouse, the spleen was isolated and the relative expression levels of selected genes was determined using qRT-PCR (normalized to the expression levels of L14). Mean ± SEM, n = 8 mice/group. No significant differences were detected using a Two-way ANOVA-Tukey or individual T-tests.

We next evaluated if the effects of LXR translated into changes in the circulating levels of leptin (leptinemia) (Figure 23). In general, leptinemia was higher in WT than in CD38-deficient mice. The high fat diet increased leptinemia in both genotypes, but LXR activation significantly decreased it only in WT mice (Figure 23). These observations suggest that the LXR-mediated downregulation of leptinemia depend on the functional expression of CD38.

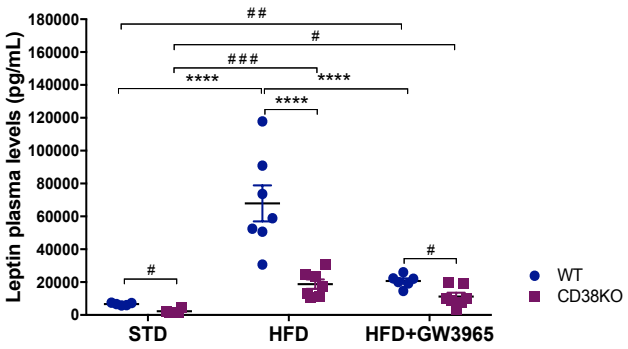


Figure 23. High fat diet-induced hyperleptinemia was decreased by LXR activation in WT mice. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. The levels of leptin in plasma were determined using a Procartaplex Immunoassay. Mean ± SEM. N=4-6 mice/group. Two-way ANOVA-Tukey posthoc, ***p < 0.001. Selected conditions were also compared with a T-test, # p < 0.05, ## p < 0.01, ### p < 0.001.

Several reports have proposed that intestinal factors secreted during the meal potentiate insulin secretion (Vilsbøll et al., 2002). GLP-1 and GIP (gastric inhibitory polypeptide) are secreted by intestinal L cells (whose abundance increases toward the ileum and the colon) and K cells (present mainly in the duodenum and the upper jejunum), respectively. The development of pharmacologic therapy of T2D by incretins focused on GLP-1 was based on the observation that administration of GLP1 agonists reduced glycemia in patients with T2D (Vilsbøll et al., 2002), while GIP may worsen it when administered acutely (Nauck et al., 1993; Vilsbøll et al., 2002). However, the in vivo administration in rodents of a truncated GIP analogue improved glucose tolerance and insulin secretion, reduced β-cell apoptosis, and increased pancreatic islet and β-cell area, as well as pancreatic insulin content (Hasib et al., 2017; Widenmaier et al., 2010). Based on these observations, we analysed whether LXR activation affected the expression of the genes encoding GLP-1 and GIP in the colon and duodenum, respectively. As shown in Figure 24, the expression levels of GLP-1 and GIP did not change in response to either the high fat diet or the LXR agonist in the duodenum. However, a slight induction of Glp1 expression was observed in WT mice exposed to the combination of the high fat diet and the LXR agonist.

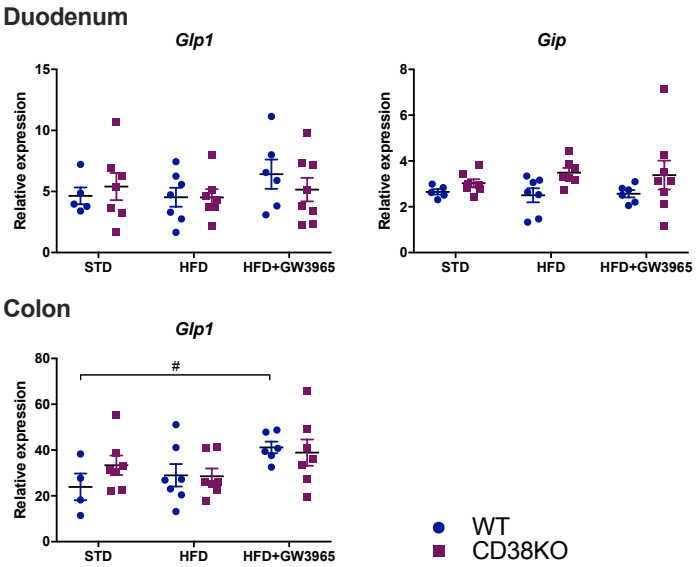


Figure 24. Effects of the high fat diet or the administration of GW3965 on the expression levels of *Glp1* or *Gip*. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. Colon and duodenum were isolated. The expression levels of GLP-1 and GIP were determined by qPCR. Mean ± SEM. N=4-8 mice/group. No significant differences were detected upon analysis by two-way ANOVA-Tukey. Selected comparisons were further analyzed using a T-test, # $p < 0.05$.

Next we examined the expression of well-established LXR target genes involved in lipid and glucose homeostasis. The expression of *Abca1* and *Srebp1c* was induced in several metabolic tissues and in the spleen of WT mice exposed to the combination of the high fat diet and the LXR agonist (Figures 25 and 26). Interestingly, the capability of GW3965 to induce the expression of *Abca1* and *Srebp1c* in several tissues was impaired in CD38-deficient mice, which suggests that several aspects in LXR signalling beyond the effects on insulin sensitivity are affected by the lack of functional CD38 expression.

In contrast, no effect of the diet or the LXR agonist was observed on *Glut4* or *Chrebp* expression in adipose tissues of WT mice (Figure 25). Of note, in CD38-deficient mice, the expression of Glut4 in the subcutaneous adipose tissue was augmented in response to the high fat diet, and the LXR agonist inhibited this increase despite the presence of an LXR response element in the *Glut4* promoter (Laffitte et al., 2003)(Figure 25).

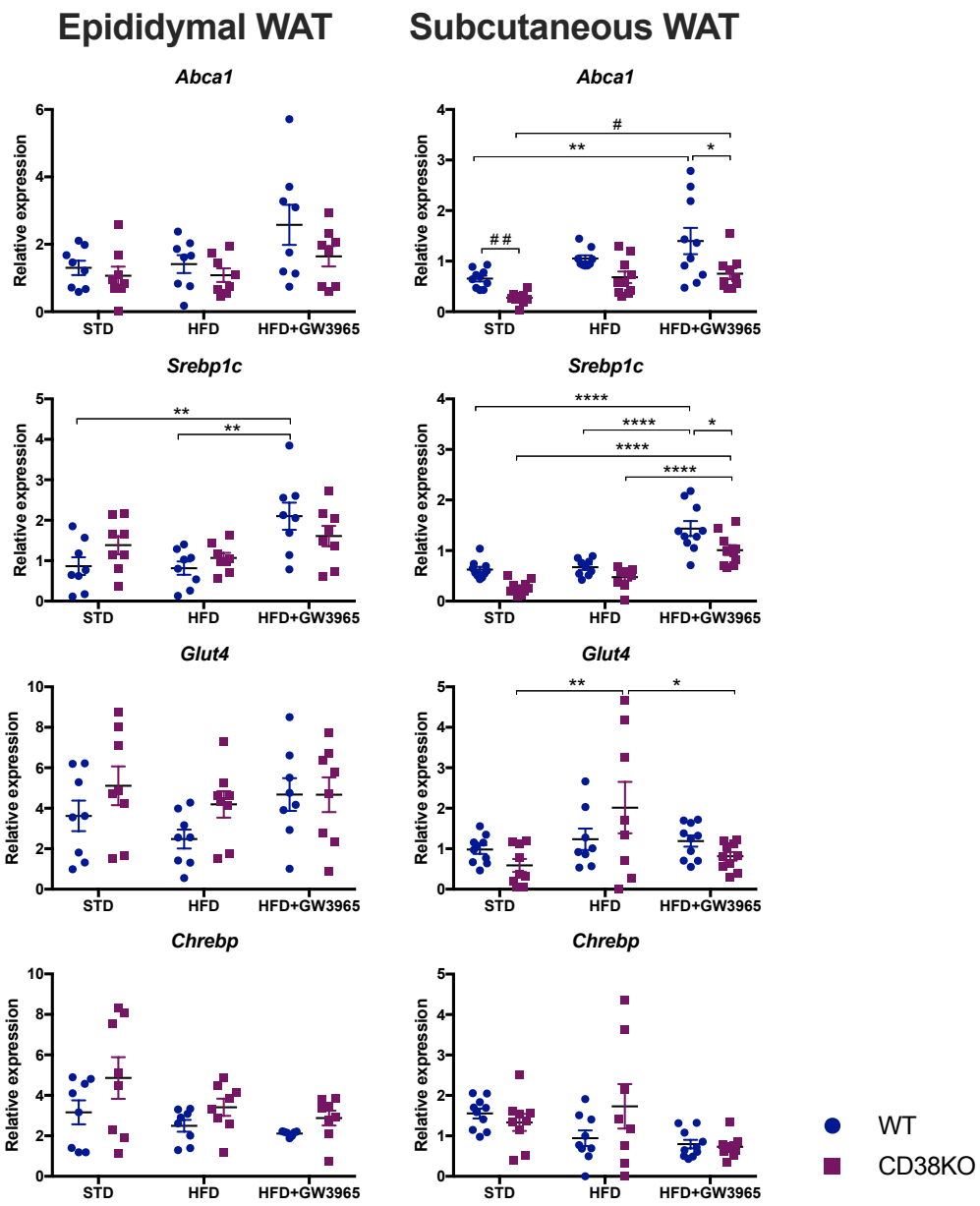


Figure 25. Effects of a high-fat diet and GW3965 administration on the expression of selected LXR target genes in adipose tissues. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. Relative expression levels of LXR target genes were determined using qRT-PCR in epididymal and subcutaneous white adipose tissues. Mean ± SEM. N=8 mice/group. Two-way ANOVA-Tukey posthoc, *p < 0.05, **p < 0.01, ****p < 0.0001. Selected conditions were also compared with a T-test, # p < 0.05, ## p < 0.01.

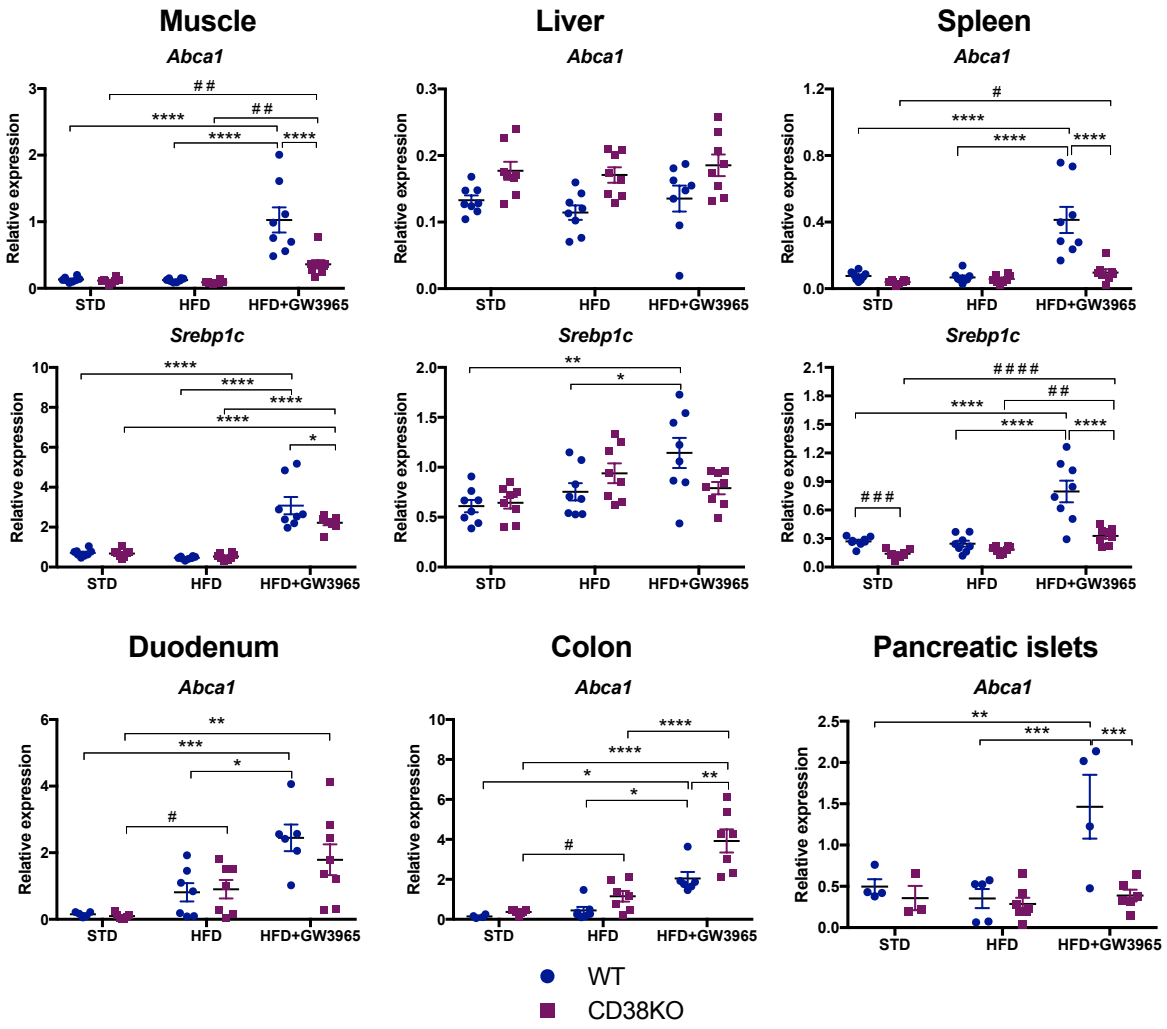


Figure 26. Effects of a high-fat diet and GW3965 administration on the expression of selected LXR target genes in non-adipose tissues. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. Relative expression levels of LXR target genes were determined using qRT-PCR in the indicated tissues. Mean ± SEM. N=7-8 mice/group. Two-way ANOVA *p < 0.05, **p < 0.01, ****p < 0.0001. Selected conditions were also compared with a T-test, # p < 0.05, ## p < 0.01, ### p < 0.001, #### p < 0.0001.

As a summary, two main conclusions can be taken from this section. First, leptinemia is negatively controlled by the LXR pathway and this effect requires functional CD38 expression. Second, CD38 also contributes to full induction of two canonical LXR targets (ABCA1 and SREPB1c) in several tissues, suggesting that the activities mediated by CD38 may influence the transcriptional program of the LXR pathway.

2.3.- Effects of LXR activation on metaflammation-associated gene expression

As described in the Introduction, increased adiposity promotes the production of inflammatory molecules by both adipocytes and infiltrated immune cells. Since LXR exerts a diversity of repressive actions on inflammation in other models of disease we explored the effects of the LXR agonist on the expression of mediators of metaflammation. We initially focused these studies in the epididymal white adipose tissue, as it is the anatomic location in which robust metaflammation is induced during obesity. Based on the fact that IFN-γ contributes to the pathogenesis of obesity-associated insulin resistance (Wang et al. 2019), and considering the repressive effects of LXR agonists on IFN-γ-induced gene expression in macrophages, as shown in *Results, Section 1* and in our previous report (Pascual-García et al., 2013), our screening included (although it was not restricted to) important mediators of the physiological response to IFN-γ.

In the epididymal white adipose tissue of WT mice, the expression of the genes encoding the cytokines TNF-α and IL-6 was significantly induced by the high fat diet (Figure 27). In addition, there was a tendency for increased IL-1β expression under those conditions, although it should be noted that the general levels of expression of this cytokine in the adipose tissue were very low (Figure 27). In contrast, the levels of *Il18*, *Il17* and *Ifng* were not affected by the diet. Consistently, the high fat diet did not induce the expression of the genes coding for the IFN-γ-signalling mediators STAT1 and IRF1 or for the enzymes NOS2 and COX2 in the epididymal adipose tissue (Figure 28). In general, we observed a tendency for the diet-induced expression of TNF-α and IL-1β in CD38-deficient mice, but these effects were not significant. Remarkably, LXR activation selectively reduced the high fat diet-induced expression of *Tnfa* in the epididymal adipose tissue of WT mice, but not in CD38-deficient mice (Figure 27).

Paradoxically, despite the fact that the adipose tissue contributes to obesity-associated metaflammation through the production of pro-inflammatory cytokines such as TNF-α and IL-6, increased mRNA levels of the anti-inflammatory cytokines IL-10 and TGF-β were also detected in the epididymal adipose tissue of both WT and CD38-deficient mice fed a high fat diet (Figure 27). These observations reflect a complex scenario that combines both pro- and anti-inflammatory signals within the adipose tissue as a result of obesity. Nevertheless, administration of the LXR agonist did not alter the expression of IL-10 and TGF-β in the epididymal adipose tissue regardless of the genotype (Figure 27).

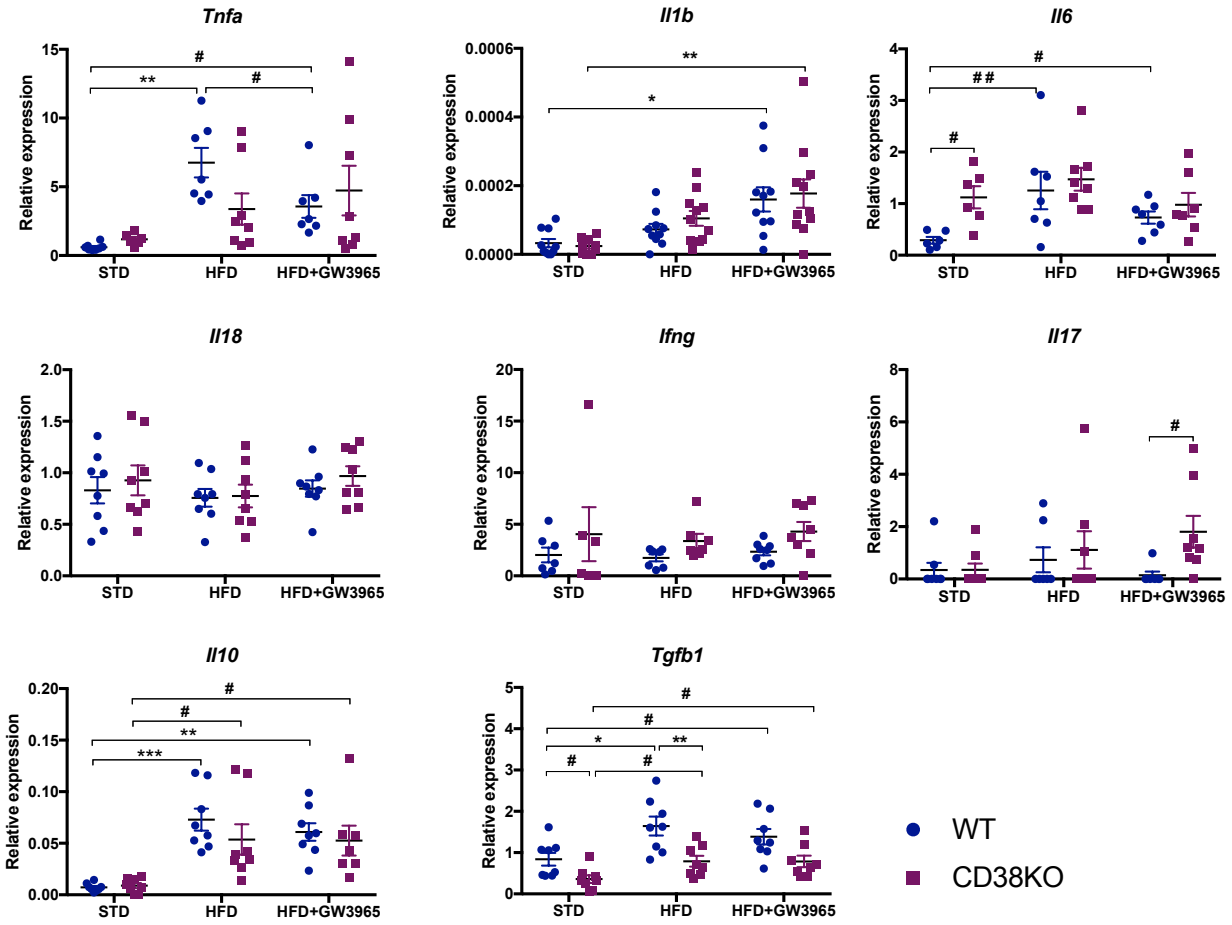


Figure 27. Effects of high-fat diet and GW3965 administration on the mRNA expression of pro-inflammatory and anti-inflammatory cytokines in the epididymal adipose tissue. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. Relative expression levels of selected cytokines were determined using qRT-PCR in the epididymal adipose tissue. Mean ± SEM. N=8 mice/group. Two-way ANOVA-Tukey *p < 0.05, **p < 0.01, ***p < 0.001. Selected experimental conditions were also compared through a Student T-test or a Mann-Whitney test (for *Il17*), # p < 0.05, ## p < 0.01.

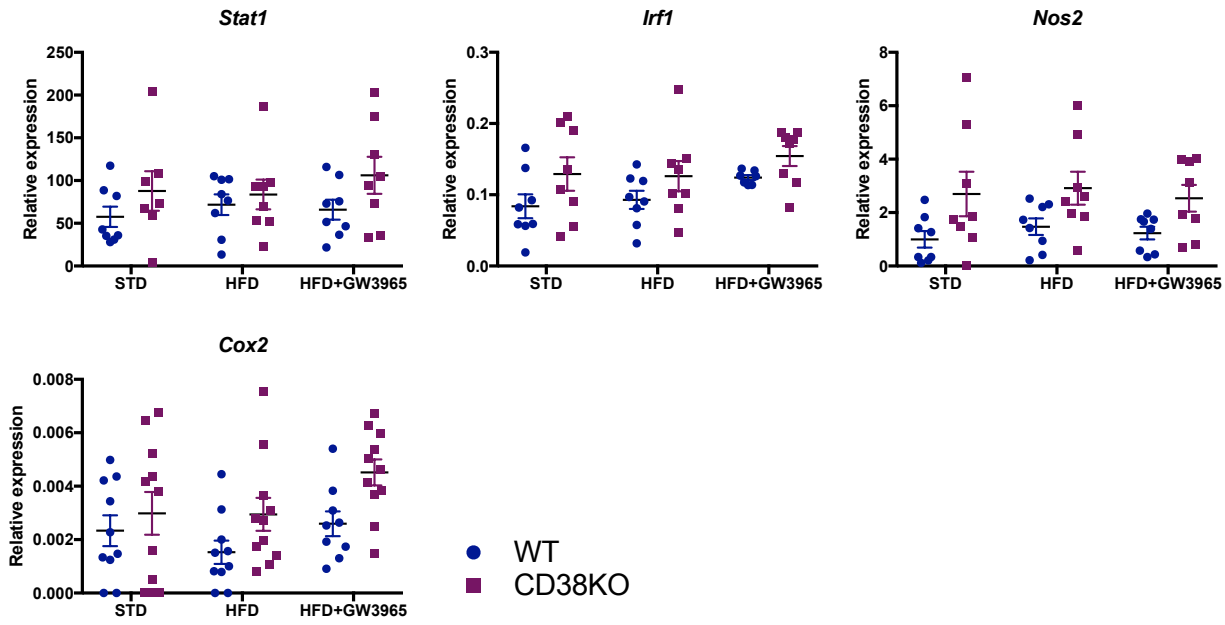


Figure 28. The high fat diet or LXR activation did not affect the expression of key molecules in the IFN-γ signaling pathway in the epididymal white adipose tissue. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. Relative expression levels of selected cytokines were determined using qRT-PCR in the epididymal adipose tissue. Mean ± SEM. N=8 mice/group. No significant differences were detected by Two-way ANOVA-Tukey or T-test between the experimental conditions evaluated.

We next evaluated whether the effects of the high fat diet or the LXR agonist translated into changes in the circulating cytokine levels of TNF-α, IFN-γ and IL-6 using a Procartaplex Immunoassay. As shown in Figure 29, no changes of the systemic levels of these cytokines were detected in response to the diet. These observations suggest that in our model of obesity-associated insulin resistance there are local signs of metaflammation in the white adipose tissue, which are not accompanied by increased circulating levels of the cytokines explored here, an aspect that has been also reported by other authors (Xu et al., 2002). Of note, however, obese WT mice undergoing GW3965 treatment showed increased IL-6 levels in the blood. Whether this increase is caused by the LXR agonist alone or the combination of agonist and diet needs to be determined.

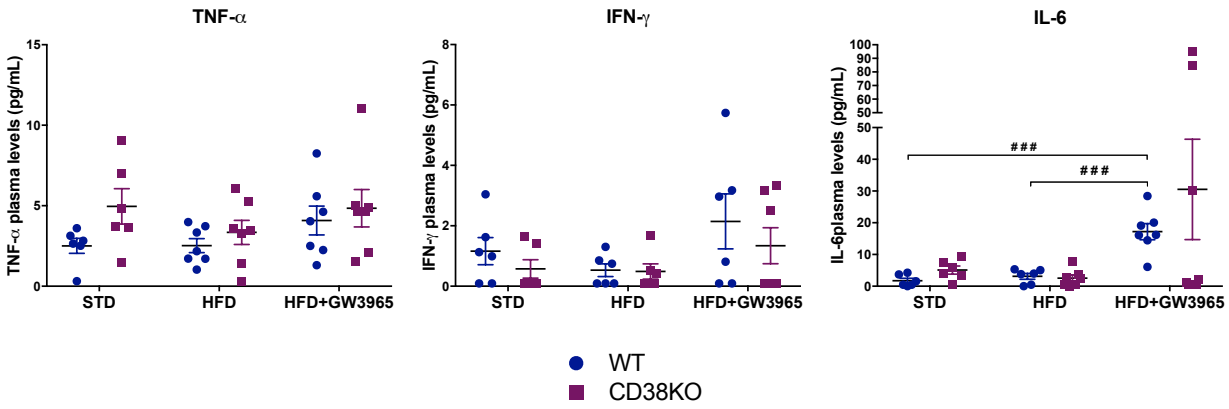


Figure 29. The systemic levels of TNF-α, IFN-γ or IL-6 were not increased by the high fat diet. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. The determination of TNF-α, IFN-γ and IL-6 levels in plasma samples was performed using a Procartaplex Immunoassay. Mean ± SEM. N=6 animals/group. No significant differences were detected by Two-way ANOVA-Tukey. Selected experimental conditions were further analysed by T-test. $^{###}p < 0.001$.

We further extended our analysis of the expression of IFN-γ to other tissues: the skeletal muscle, the liver, the spleen, and pancreatic islets (Figure 30). However, no effect of the diet was observed in any of the tissues analysed. Interestingly, higher levels of *Ifng* expression were detected in liver and spleen of CD38-deficient mice, in comparison with WT animals (Figure 30). Based on the fact that obesity promotes the expansion of Th17 cells and that IL-17 is expressed at elevated levels in obese individuals (Lee et al. 2017; Ahmed and Gaffen 2010), we also analysed the expression IL-17 in skeletal muscle, liver, and spleen. Higher *Il17* expression was detected in the liver of CD38-deficient mice (in comparison to WT mice) and a tendency for increased expression of this cytokine was observed in response to the high fat diet in the skeletal muscle and spleen of both WT and CD38-deficient mice. However, activation of the LXR pathway did not repress *Ifng* or *Il17* expression (Figure 30).

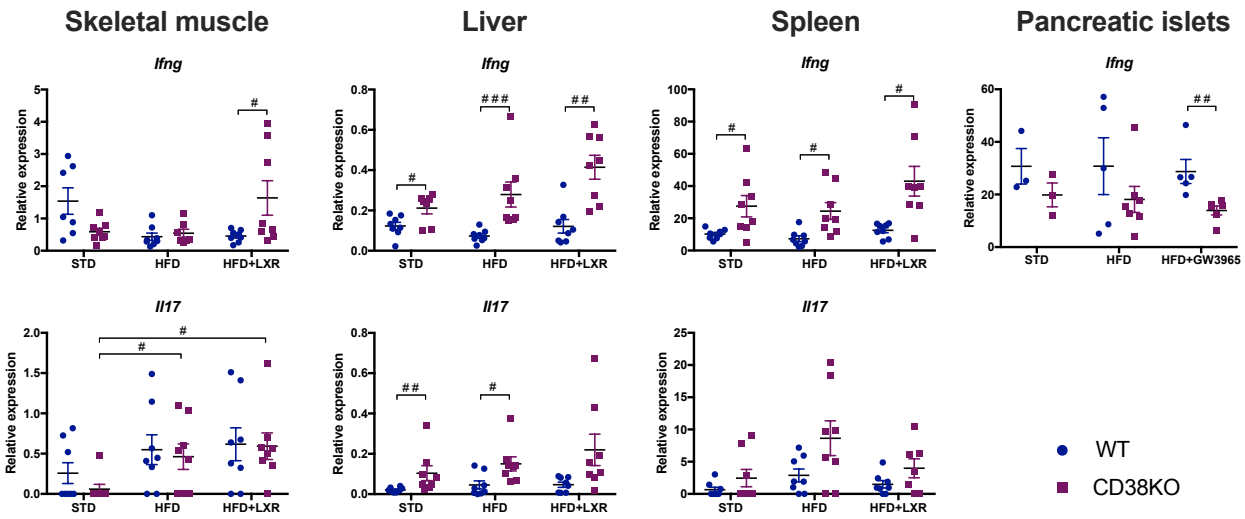


Figure 30. Effects of the high fat diet and GW3965 administration on IFN-γ and IL-17 expression in several tissues. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. Relative expression levels of *Ifng* and *Il17* were determined using qRT-PCR in the indicated tissues. Mean ± SEM. N=3-8 animals/group. No significant differences were detected by Two-way ANOVA-Tukey or Kruskal Wallis-Dunn's tests. Selected experimental conditions were compared by T-test (for parametric datasets) or Mann-Whitney test (for non parametric datasets), # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$.

Based on the fact that the epididymal white adipose tissue is the tissue in which more prominent induction of inflammatory markers was detected in response to the diet in our model of obesity-associated insulin resistance, the expression of selected chemokines and chemokine receptors that are important for immune cell recruitment was also evaluated in this tissue. The high fat diet resulted in significant increased expression of CCR5 and CCR7 and a tendency for upregulation in CCR6 expression, which might influence immune cell recruitment. However, LXR activation did not counteract these effects (Figure 31). In addition, significant increased expression of Ccl2 and Ccl20 was detected selectively in CD38-deficient mice in response to the high fat diet or to the combination of high fat diet and the LXR agonist, respectively (Figure 31).

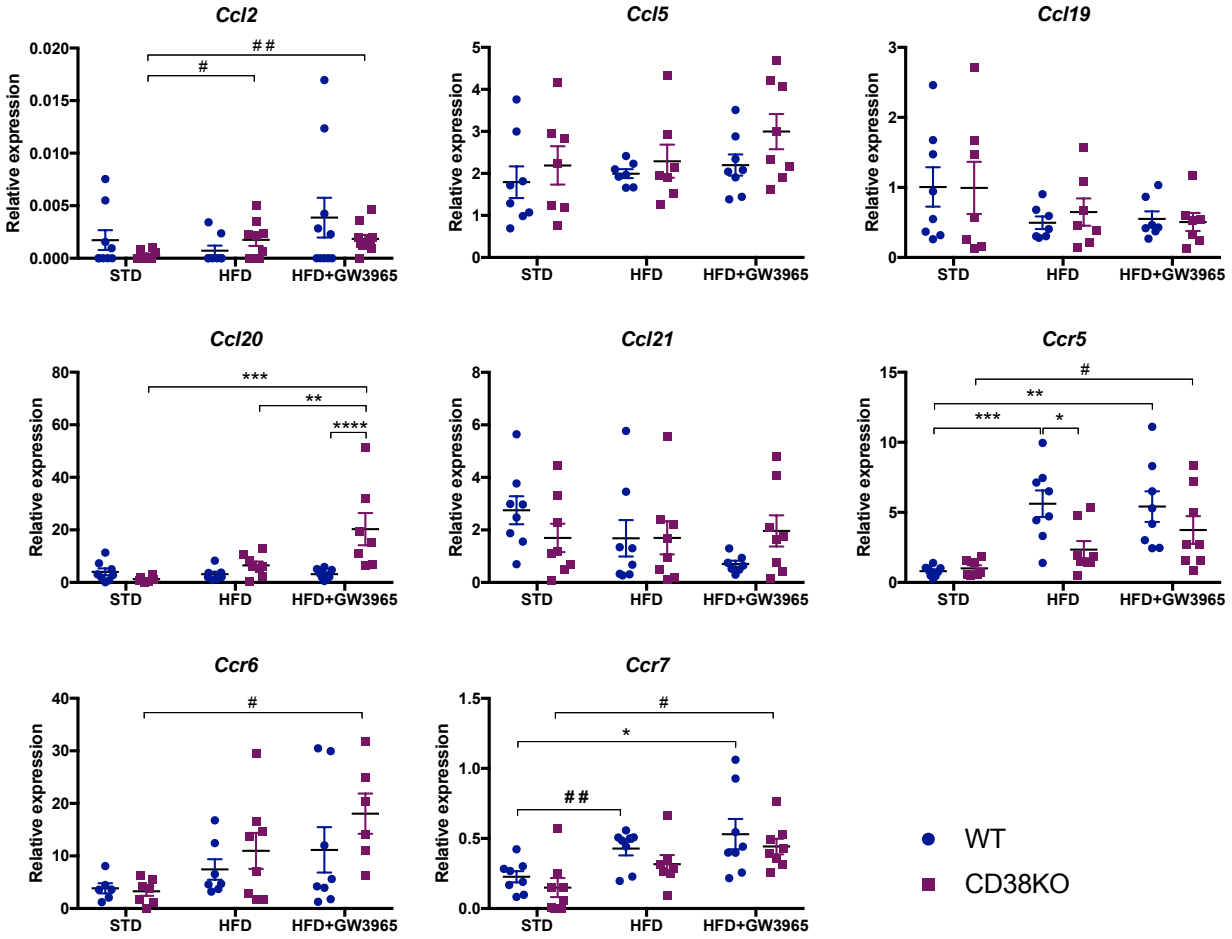


Figure 31. Effects of high-fat diet and GW3965 administration on the mRNA expression of selected chemokines and chemokine receptors in epididymal white adipose tissue. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. Relative expression levels of selected genes were determined using qRT-PCR in the epididymal white adipose tissue. Mean ± SEM. N=8-10 animals/group. Two-way ANOVA-Tukey, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Selected conditions were also compared using a T-test, # p < 0.05, ## p < 0.01.

In humans, insulin resistance and obesity have been associated with an increase in the levels of heme-oxygenase 1 (Hmox1) and a downregulation of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in white adipose tissue in comparison with healthy individuals (Halleux et al., 2001; Jais et al., 2014). Hmox1 catabolizes free heme into biliverdin, ferrous iron and carbon monoxide, and the latter plays anti-inflammatory roles by multiple mechanisms including the upregulation of IL-10 (Canesin et al., 2020). 11β-HSD1 reduces cortisone to the active hormone cortisol, which exerts anti-inflammatory actions via activation of glucocorticoid receptors (Stulnig et al., 2002b). In our model, we corroborated the induction of *Hmox1* expression in the adipose tissue in response to a high fat diet (Figure 32). However, LXR activation did not significantly inhibit its expression. In

addition, inhibited expression of *11bHsd1* was observed in the adipose tissue in response to the high fat diet. Interestingly, the LXR agonist raised the expression levels of *11bHsd1* to those in the standard group in both WT and CD38-deficient animals (Figure 32).

An additional analysis of the aforementioned genes was performed in the spleen (Figure 32). The expression levels of *Hmox1* in the spleen were upregulated in response to GW3965 administration in WT but not in CD38-deficient mice. On the other hand, *11bHsd1* expression was not affected by the diet or the LXR agonist, but lower levels of expression of this gene were observed in the spleen of CD38-deficient mice (in comparison with WT mice).

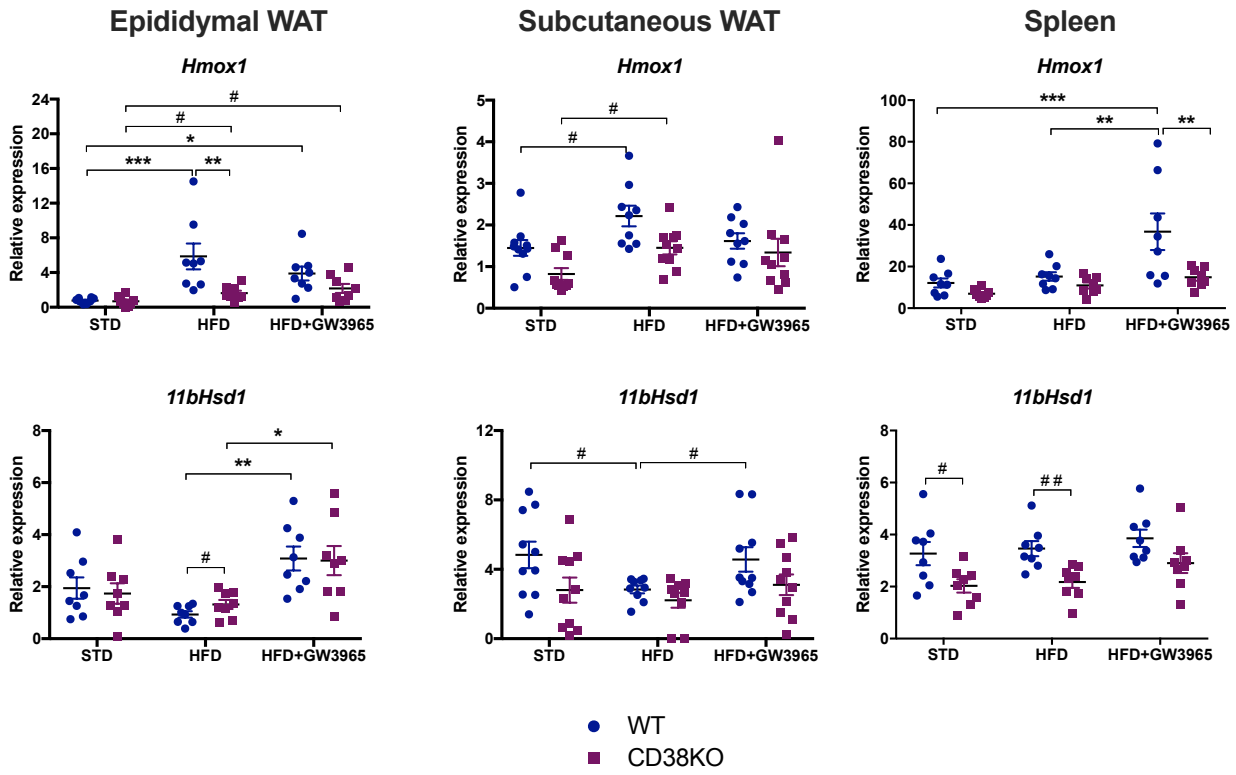


Figure 32. Effects of high-fat diet and GW3965 administration on the mRNA expression of *Hmox1* and *11bHsd1* in adipose tissues. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. Relative expression levels of selected genes were determined using qRT-PCR in the epididymal or subcutaneous white adipose tissue (WAT) or in the spleen. Mean ± SEM. N=8-10 animals/group. Two-way ANOVA-Tukey $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Selected conditions were also compared using a T-test (for parametric datasets) or a Mann-Whitney test (for non parametric datasets), # $p < 0.05$, ## $p < 0.01$.

To summarize, the results obtained in these studies indicate that relevant pro-inflammatory markers, such as TNF-α, IL-6, CCR5 and CCR7, are increased in the epididymal adipose tissue in WT mice exposed to a high fat diet, although this increase does not translate into higher levels of TNF-α in the blood. Importantly, activation of the LXR pathway results in significant downregulation of TNF-α expression in the epididymal white adipose tissue and this effect requires functional CD38 expression. In addition, the expression of the anti-inflammatory mediators IL-10 and TGFβ is also promoted by the high fat diet, however the LXR agonist does not inhibit such effects.

2.4.- Effects of LXR activation on macrophage infiltration to epididymal white adipose tissue

Considering the well described roles of LXRs as negative regulators of inflammation, we studied the expression levels of molecules that serve as markers for myeloid cell infiltration in the white adipose tissue. F4/80 and CD68 are considered as conventional tissue macrophage markers. In addition, infiltrated adipose tissue macrophages and dendritic cells also express CD11c, whereas resident macrophage populations express CD301 (encoded by two homologue genes *Mgl1* and *Mgl2* in mice). In our model, the high fat diet increased the levels of F4/80, CD68, CD11c and Mgl1 in the epididymal white adipose tissue of WT mice. The levels of some of these markers (F4/80, CD68 and CD11c) also raised in the epididymal adipose tissue of CD38-deficient animals fed with a high fat diet, but to a lower extent than in WT mice. Notably, LXR activation selectively decreased the high fat diet-induced CD11c levels in epididymal white adipose tissue from WT mice, but not in CD38-deficient mice (Figure 33).

As a control, we also explored whether the LXR agonist affected these myeloid cell markers in the spleen (Figure 33). Interestingly, splenic F4/80 mRNA levels were increased by the combination of the high fat diet and GW3965 in WT mice, but not in CD38-deficient mice. No effect of the diet alone was observed on any of the markers tested in the spleen. Importantly, the LXR agonist did not alter the levels of CD11c in this organ, suggesting that the potential inhibitory action of LXRs on CD11c levels is tissue-specific.

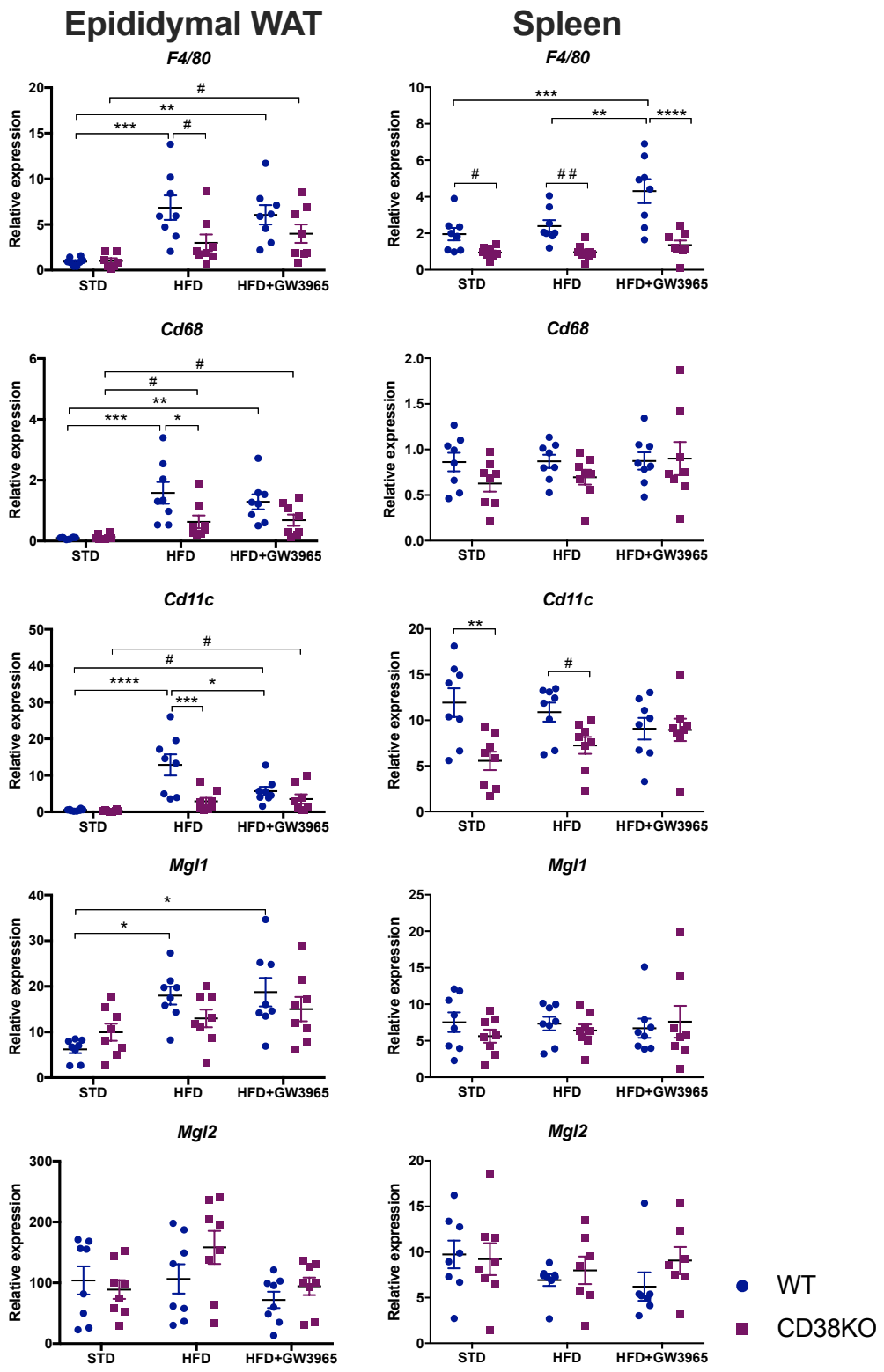


Figure 33. Effects of the high fat diet and LXR activation on the expression of markers of tissue infiltrated macrophages. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. Relative expression levels of selected genes were determined using qRT-PCR in the epididymal white adipose tissue (WAT) or in the spleen. Mean ± SEM. N=7-8 animals/group. Two-way ANOVA-Tukey. $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Selected conditions were also compared using a T-test, # $p < 0.05$, ## $p < 0.01$.

We hypothesized that the local effects of LXR activation on CD11c levels could be due to either decreased macrophage or dendritic cell infiltration to the adipose tissue or to decreased CD11c gene expression in macrophage/dendritic cells within this tissue. We therefore performed flow cytometry analysis to determine the effects of the LXR agonist on the frequency of macrophages within the adipose tissue and on the levels of expression of surface CD11c in these cells. For this purpose, the epididymal adipose tissue was isolated and the adipocyte and SVC fractions were separated. The SVC fractions were processed for flow cytometry and the gating strategy (Figure 10) allowed for the identification of CD45+ leukocytes, macrophages (CD45+ CD11b^{high} F4/80^{high}) and non-macrophage myeloid cells (CD45+ CD11b+ F4/80-).

The high fat diet increased the frequency of CD45+ leukocytes in the epididymal white adipose tissue of WT mice (Figure 34), although the administration of GW3965 did not change significantly the overall frequency of these cells. In the case of CD38-deficient mice, similar frequencies of CD45+ leukocytes were observed under standard diet conditions in comparison to WT mice, but the high fat diet did not significantly increase the percentage of these cells.

Next, we calculated the frequency of myeloid cells within the singlets gate. The high fat diet induced a significant increase in the percentage of macrophages in both mice groups, with no effects on the frequency of non-macrophage myeloid cells (which includes dendritic cells) (Figure 34). Treatment with the LXR agonist resulted in reduced frequencies of macrophages in CD38-deficient mice and a trend for such reduction in WT mice (Figure 34).

The analysis of infiltrated *versus* resident immune cells (based on the expression of CD11c and CD301, respectively) showed that the high fat diet increased the percentage of CD11c+ cells in leukocytes and macrophages in WT mice and, to a lesser extent, in CD38-deficient mice (Figure 34). In addition, an increase in CD301+ cells was observed in response to the high fat diet in both mice groups (Figure 34). Importantly, a trend for reduced percentages of CD11c+ or CD301+ cells was observed in the epidydimal adipose tissue of obese animals upon administration of the LXR agonist (Figure 34).

Mean Fluorescence Intensity (MFI) is often used to compare the expression levels of proteins of interest between different cell populations or experimental conditions. When the MFI for CD11c staining was analysed in the three cell populations object of study, a tendency for increased levels of surface CD11c expression was observed in total leukocytes from both mice groups (Figure 35). However, the LXR agonist did not reduce the levels of surface expression of CD11c.

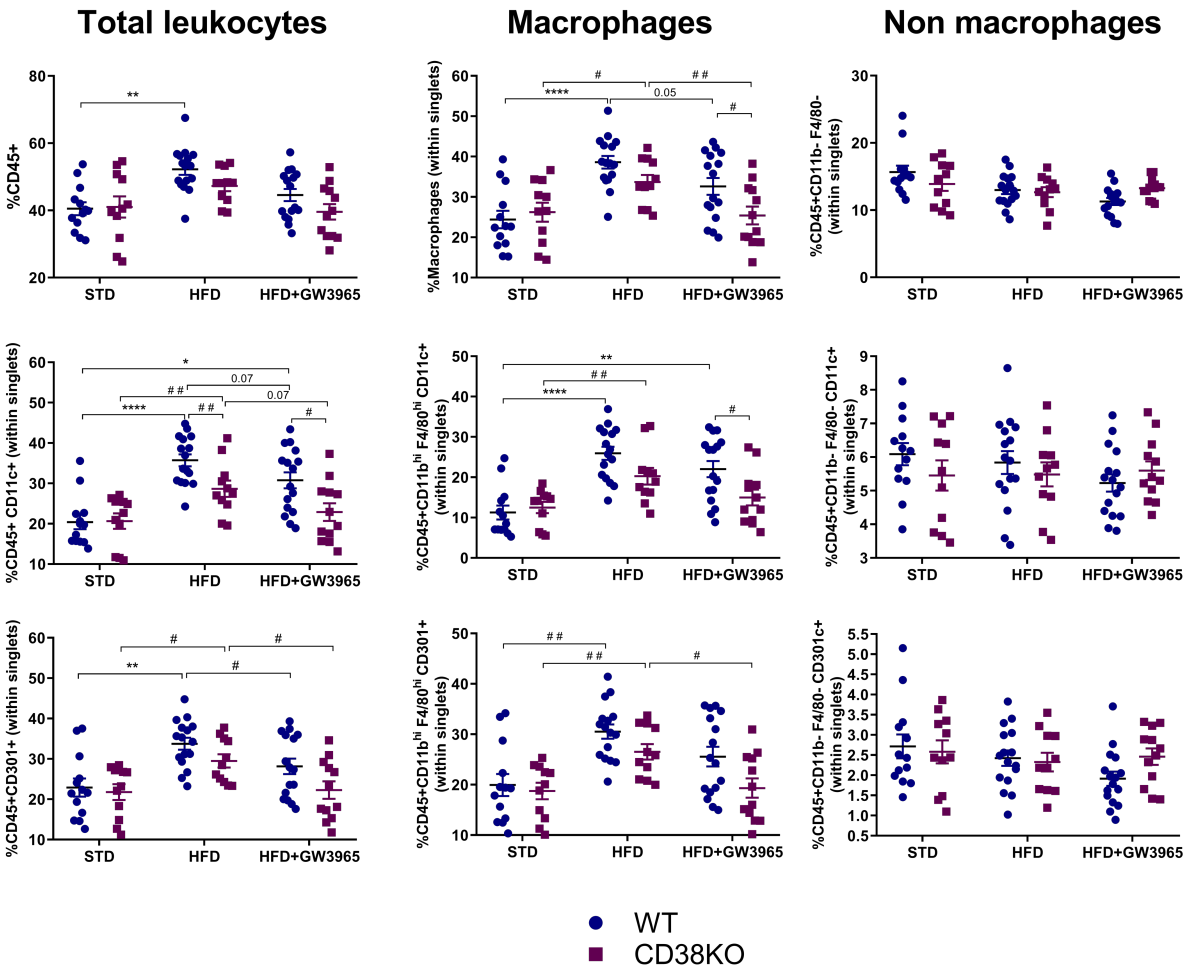


Figure 34. Effects of the high fat diet and LXR activation on the frequency of several immune cell populations in the SVC fraction of the epididymal white adipose tissue. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. The epididymal white adipose tissue was digested and the adipocyte and SVC fractions were separated. The SVC fractions were stained and analysed by flow cytometry as described in Materials and Methods. The graphics display the percentage of total CD45+ leukocytes (left), of macrophages (CD45+ CD11b^{high} F4/80^{high}) (centre) and of non-macrophage myeloid cells (CD45+ CD11b+ F4/80-) (right) within the singlets gate. In addition, the percentage of CD11c+ or CD301+ cells was determined for each of the cell populations described above (total leukocytes, macrophages and non-macrophage myeloid cells) within the singlets gate. Mean ± SEM. N=11-16 animals/group. Kruskal-Wallis-Dunn's test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Selected conditions were also compared using a Mann-Whitney test, # p < 0.05, ## p < 0.01. Non relevant comparisons have been omitted from the graphics.

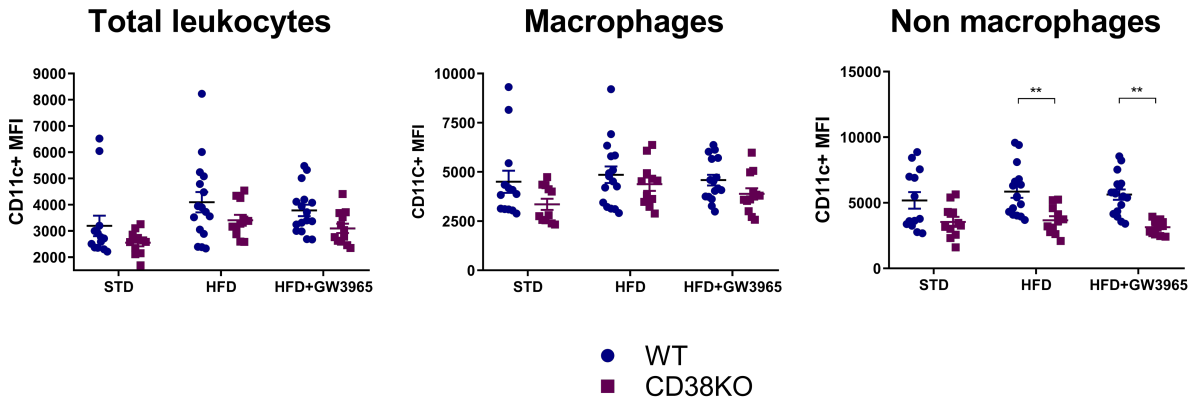


Figure 35. GW3965 administration did not alter the surface expression levels of CD11c in total leukocytes (CD45+) or myeloid cell populations within the SVC of the white adipose tissue. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. The epididymal white adipose tissue was digested and the adipocyte and SVC fractions were separated. The SVC fractions were stained and analysed by flow cytometry as described in Materials and Methods. For each cell population analysed, total CD45+ leukocytes (left), macrophages (CD45+ CD11b^{high} F4/80^{high}) (centre) and non-macrophage myeloid cells (CD45+ CD11b+/- F4/80-) (right), the mean fluorescence intensity (MFI) for CD11c+ staining was determined (within the gate of CD11c+ cells). Mean ± SEM. N=11-16 animals/group. Two-way ANOVA-Tukey. *p < 0.05, **p < 0.01. Selected conditions were also compared using a T-test. #p < 0.05.

In summary, while the high fat diet promotes the accumulation of infiltrated macrophages in the epididymal white adipose tissue, LXR activation slightly decreased the frequency of these cells in this tissue without affecting the surface expression levels of CD11c. The fact that the LXR agonist inhibited the mRNA expression levels of CD11c in whole adipose tissue may, therefore, be a consequence of the tendency for decreased frequencies of infiltrated CD11c+ immune cells in response to the LXR agonist. We cannot discard, though those different experimental approaches used to isolate the samples for these analyses (total epididymal adipose tissue for mRNA determination *versus* SVC fraction for immunostaining) have an impact on the effects of the LXR agonist on CD11c expression. For this reason, we determined the mRNA expression of *Cd11c* in cells also in the SVC fraction. As shown in Figure 36, under these experimental conditions the expression of this marker was induced in response to the high fat diet, but no inhibitory effect was observed upon pharmacological LXR activation, thus contrasting with the data shown in Figure 33. Although we do not have a clear explanation, it is possible that the procedure used to generate the SVC fractions might attenuate the effect of the LXR agonist on *Cd11c* expression.

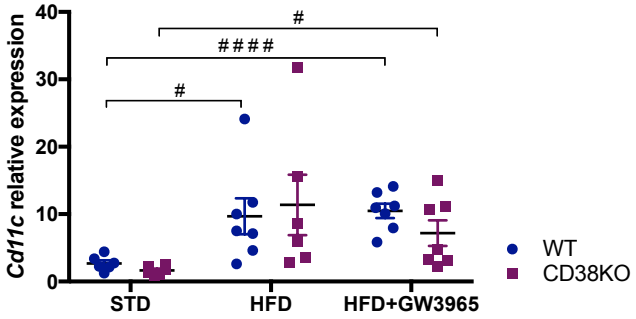


Figure 36. GW3965 administration did not ameliorate the *Cd11c* expression levels induced by high fat diet in SVC from white adipose tissue. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. The epididymal white adipose tissue was digested and the adipocyte (AD) and SVC fractions were separated. The expression of selected genes was analysed by qRT-PCR. Mean values of normalized relative expression ± SEM. n=5-7 mice/group. Selected conditions were compared using a T-test. #$p < 0.05$, ####$p < 0.0001$.

2.5.- Effects of LXR activation on metabolic and metaflammation gene expression in adipocytic and SVC fractions of the epididymal white adipose tissue

Although LXR activation did not impact strongly the frequency of macrophages within the epididymal white adipose tissue, we were interested in exploring whether the changes in gene expression shown in Sections 2.2 and 2.3 are effects of the diet or the LXR agonist in adipocytes and/or in stromal cells. For this reason, the expression of selected genes was analysed in both the SVC and the adipocyte cell fractions of the epididymal white adipose tissue (Figure 37).

The expression of the canonical LXR target gene *Abca1* was induced in both cell fractions in WT and CD38-deficient animals exposed to the combination of the high fat diet and LXR agonist, in agreement with the results previously shown for different tissues (Figures 25 and 26) and indicating that LXR activation is effective in both cell fractions.

As expected, adipocytes are the major contributors to leptin expression in the epididymal adipose tissue and are responsible for the induction of this gene in response to the high fat diet (Figure 37). In line with the data shown in Figure 21A for the whole epididymal white adipose tissue, the LXR agonist significantly inhibited the expression of leptin only in WT mice (Figure 37).

In WT mice, the proinflammatory cytokines TNF-α and IL-6 were induced in both metabolic and stromal compartments in response to the high fat diet (Figure 37). Of note, although higher levels of expression of these cytokines were detected in the SVC fraction, their upregulation in response to the diet was more pronounced in adipocytes. No significant inhibition of these cytokines was observed in the mice treated with the LXR agonist, in contrast with the effects described for *Tnfa* in whole epididymal adipose tissue (Figure 27). In both the SVC and adipocytic fractions from CD38-deficient mice, a tendency for increased expression of *Tnfa* and *Il6* was detected under standard diet conditions, with no effect of the high fat diet alone or in combination with the LXR agonist (Figure 37). This trend had been already shown for *Il6* in whole epididymal adipose tissue (Figure 27).

Hmox1 expression was induced by the high fat diet in metabolic cells of WT mice, with no significant effect of the LXR agonist (Figure 37). No clear effect of the diet or the LXR agonist was observed in CD38-deficient adipocytes (Figure 37). This profile recapitulates the data obtained in whole epididymal adipose tissue (Figure 32). On the other hand, we did not observe changes on the

expression of 11βHSD1 in the SVC or adipocyte fractions from WT or CD38 deficient mice (Figure 37), in contrast with the results shown for the whole epididymal adipose tissue (Figure 32).

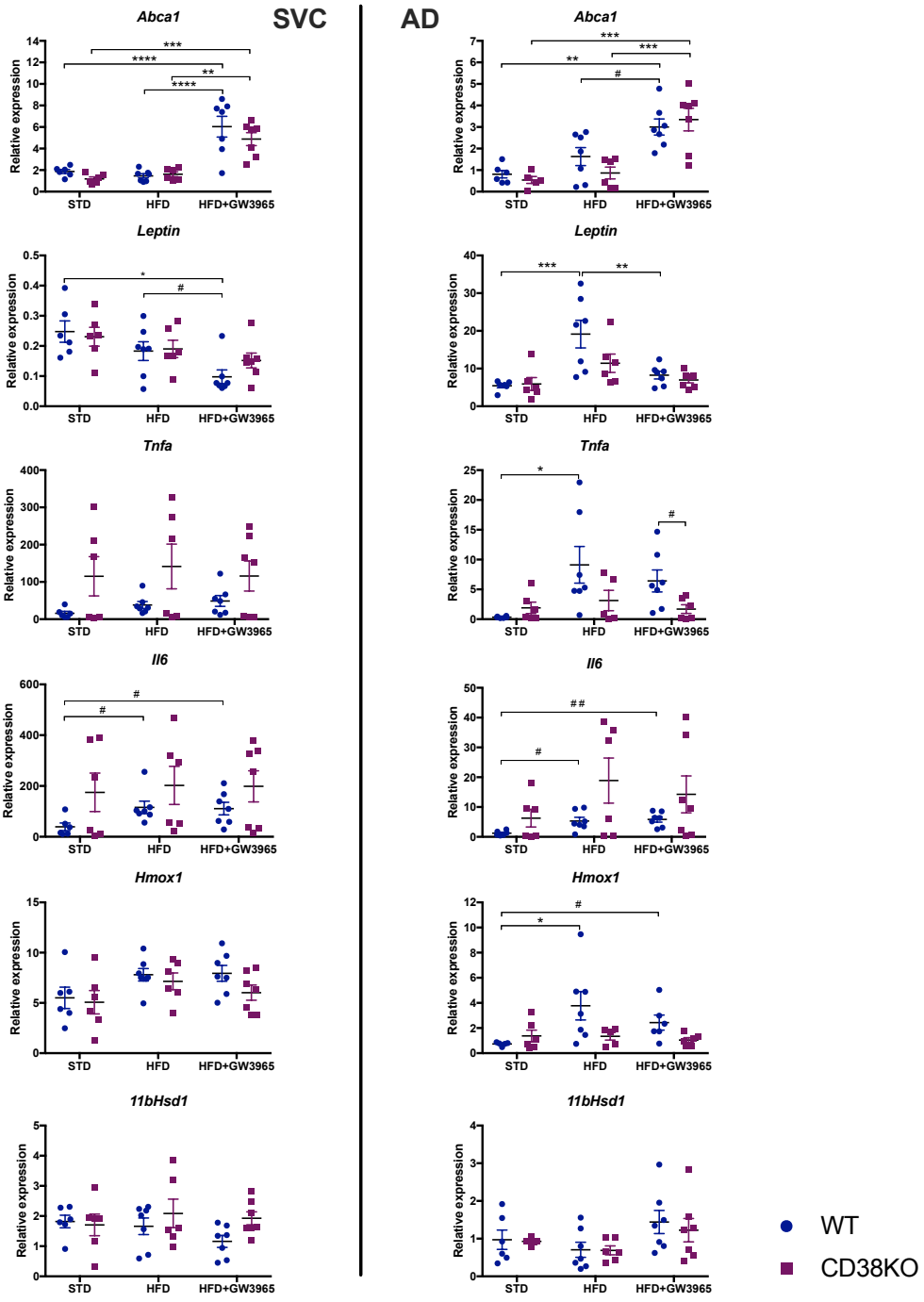


Figure 37. Effects of a high fat diet and LXR activation on the expression of selected genes in adipocytic and SVC fractions from the epididymal white adipose tissue. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. The epididymal white adipose tissue was digested and the adipocyte (AD) and SVC fractions were separated. The expression of selected genes was analysed by qRT-PCR. Mean values of normalized relative expression ± SEM. n=5-7 mice/group. Two-way ANOVA-Tukey. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Selected conditions were also compared using a T-test. #p < 0.05, ##p < 0.01. Non relevant comparisons have been omitted from the graphic.

In the SVC fractions we also analysed the levels of the cytokines IFN-γ and IL-17 and of FOXP3 (as a marker of Treg cells). No significant changes were observed for IFN-γ or IL-17 under the conditions analysed (Figure 38), in agreement with the results in whole epididymal adipose tissue (Figure 27). Notably, a tendency for increased FOXP3 expression was observed in high fat diet-fed CD38-deficient mice (but not in WT mice), which became significant upon the combination of the diet and the LXR agonist (Figure 38).

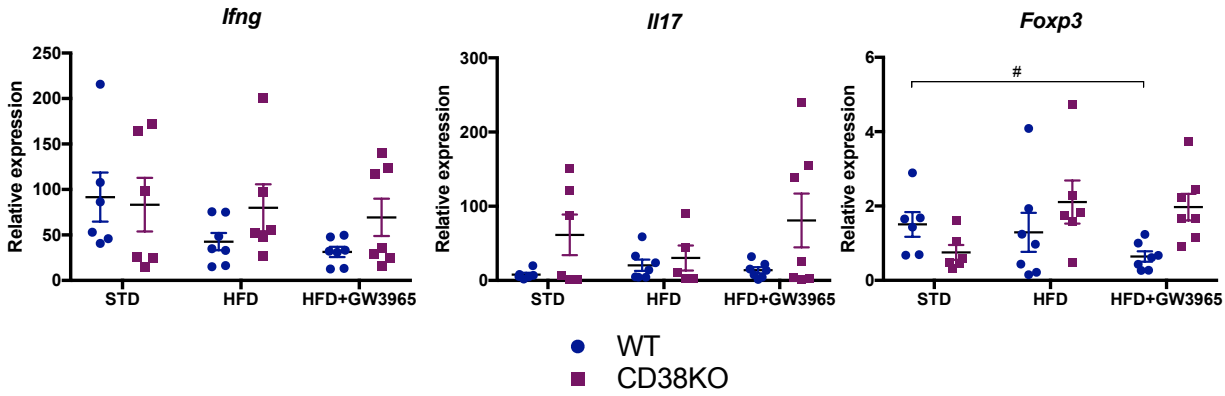


Figure 38. Effects of a high fat diet or GW3965 administration on the expression levels of IFN-γ, IL-17 or FOXP3 in the SVC fraction of the epididymal white adipose tissue. WT and CD38-deficient (CD38KO) mice were fed for 10 weeks either a standard chow diet (STD) or a high fat diet (HFD). The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) (HFD group) or the LXR agonist GW3965 (20 mg/kg/day) (HFD+GW3965 group) daily by oral gavage during the last 14 days. The epididymal white adipose tissue was digested and the SVC fractions were isolated. The expression of selected genes was analysed by qRT-PCR. Mean values of normalized relative expression ± SEM. n=6-7 mice/group. Two-way ANOVA-Tukey did not detect significant differences. Selected conditions were also compared using a T-test. #$p < 0.05$.

As a summary, in this section we have focused our attention on a few selected genes that were regulated by the high fat diet and/or the LXR agonist in whole white adipose tissue. The expression analysis performed separately in adipocytes and stromal cells has allowed us to better understand which compartment contributes to the overall effects of the diet on each of the genes evaluated. In general, despite the fact that both cell fractions contribute to the diet-induced increase in TNF-α and IL-6, the metabolic compartment seems to better recapitulate the effects of the diet, the LXR agonist and the CD38 deficiency in whole adipose tissue, at least for the group of genes analysed here.

2.7.- The expression of CD38 in bone marrow-derived cells is not required for the insulin-sensitizing actions of LXRs.

To elucidate if the expression of CD38 specifically in bone marrow-derived immune cells is relevant for the effects of the LXR agonist in metaflammation and insulin resistance we performed a bone marrow transfer assay. Eight-week-old C57BL/6J male mice were sub-lethally irradiated and then injected with $3x10^6$ bone marrow cells obtained from either WT or CD38-deficient male donors. This way, a group of WT mice harboured CD38-deficient bone marrows (CD38KO>WT), whereas control mice harboured WT bone marrows (WT>WT). Eight weeks after the bone marrow transfer, the mice were placed on either a standard chow diet or a high fat diet for 12 weeks. As in previous experiments, the groups on a high fat diet were further subdivided in two groups receiving either vehicle or the LXR agonist GW3967 (20mg/kg/day) daily by oral gavage during the last 14 days of the experiment (starting at week 10).

Control experiments demonstrating that this protocol results in efficient engraftment of a CD38-deficient hematopoietic system had been previously performed (Matalonga et al., 2017). In addition, we checked if the bone marrow transfer affected the overall expression of CD38 in epidydimal and subcutaneous white adipose tissues (Figure 39). In epididymal white adipose tissue, lower expression levels of *Cd38* were detected in the samples derived from the mice recipient of CD38-deficient bone marrows (Figure 39). Of note, the induction of *Cd38* by the LXR agonist was reduced in these mice, compared to the control mice (Figure 39), thus suggesting that bone marrow-derived cells are major contributors to the upregulation of CD38 in response to the LXR agonist in this tissue. In contrast, the overall expression of *Cd38* in the subcutaneous white adipose tissue was not largely affected by the genotype of the bone marrow, which raises the possibility that most of the expression of CD38 in this tissue derives from adipocytes. Indeed, higher levels of CD38 were observed in the subcutaneous white adipose tissue from mice fed with a high fat diet, but no induction by the LXR agonist was detected (Figure 39). As a control, we also analysed the expression levels of LXR isoforms in these mice (Figure 39). However, the expression of LXRα and LXRβ was not affected by any of the experimental conditions used in these experiments (Figure 39).

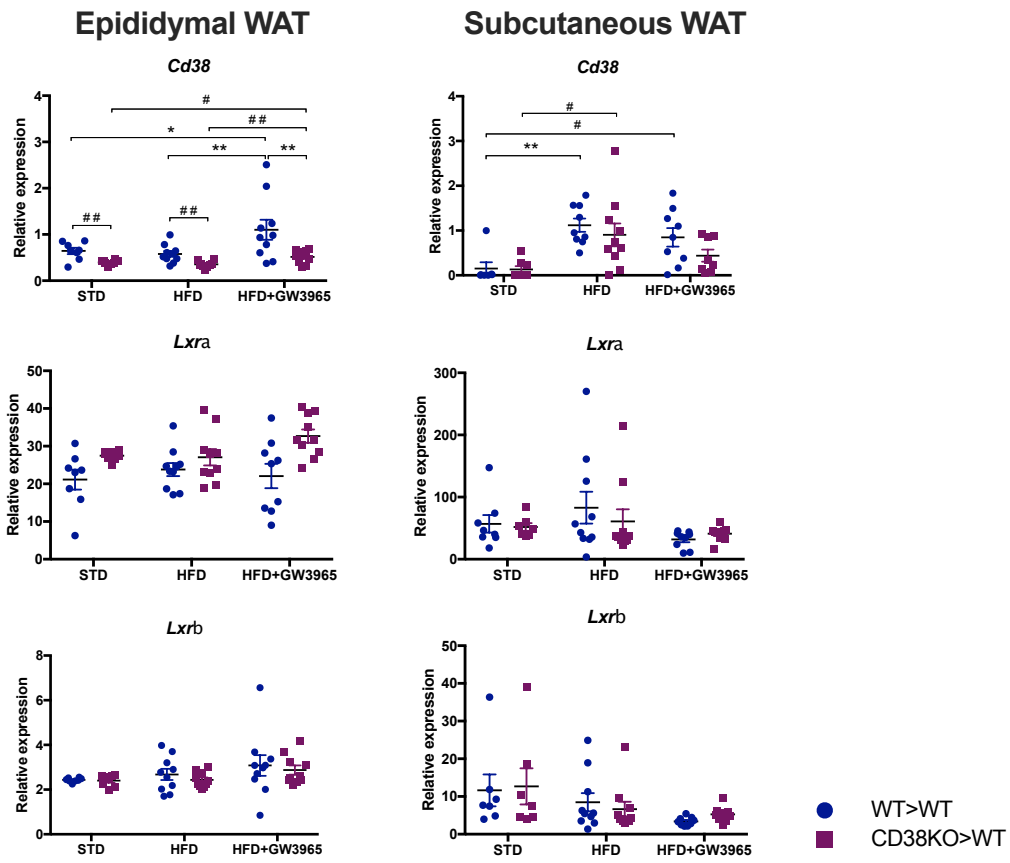


Figure 39. Reconstitution of WT mice with a CD38-deficient bone marrow impacts the expression profile of CD38 in the epididymal white adipose tissue. Eight-week-old C57BL/6J male mice were sub-lethally irradiated and then injected with 3x106 bone marrow cells from either WT (WT>WT) or CD38-deficient (CD38KO>WT) male donors. Eight weeks after the bone marrow transfer, the mice were fed either a standard chow diet (STD) or a high fat diet (HFD) for 12 weeks. The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) or GW3965 (20 mg/kg/d, HFD+GW3965) daily by oral gavage, throughout the last 14 days of the experiment. Epididymal and subcutaneous white adipose tissues (WAT) were isolated. The relative expression levels of Cd38, Lxra and Lxrb were determined using qRT-PCR. Mean values of normalized relative expression ± SEM. Two-way ANOVA-Tukey. *p < 0.05, **p < 0.01. Selected conditions were also compared using T-test, #p < 0.05, ##p < 0.01.

Despite the fact that the diet intervention started at an older age (16 weeks), the mice reconstituted with WT bone marrows underwent significant weight gain in response to the high fat diet and a loss in weight upon LXR activation (Figure 40a), thus recapitulating the results shown for control WT mice in experiments starting the diet intervention at 4 weeks of age (Figure 16). In addition, mice reconstituted with CD38-deficient bone marrows were not protected from the development of adiposity in response to the high fat diet (Figure 40a-b), which contrasts with the phenotype observed in full CD38-deficient mice (Figure 16).

Interestingly, the absence of functional CD38 expression in bone marrow-derived cells did not influence the weight gain in response to the high fat diet (Figure 40a-b) or the loss of weight upon LXR activation (Figure 40a-c). In the same line, the mice reconstituted with CD38-deficient bone marrows showed similar changes in basal glycemia as control mice in response to the high fat diet or the LXR agonist (Figure 39d).

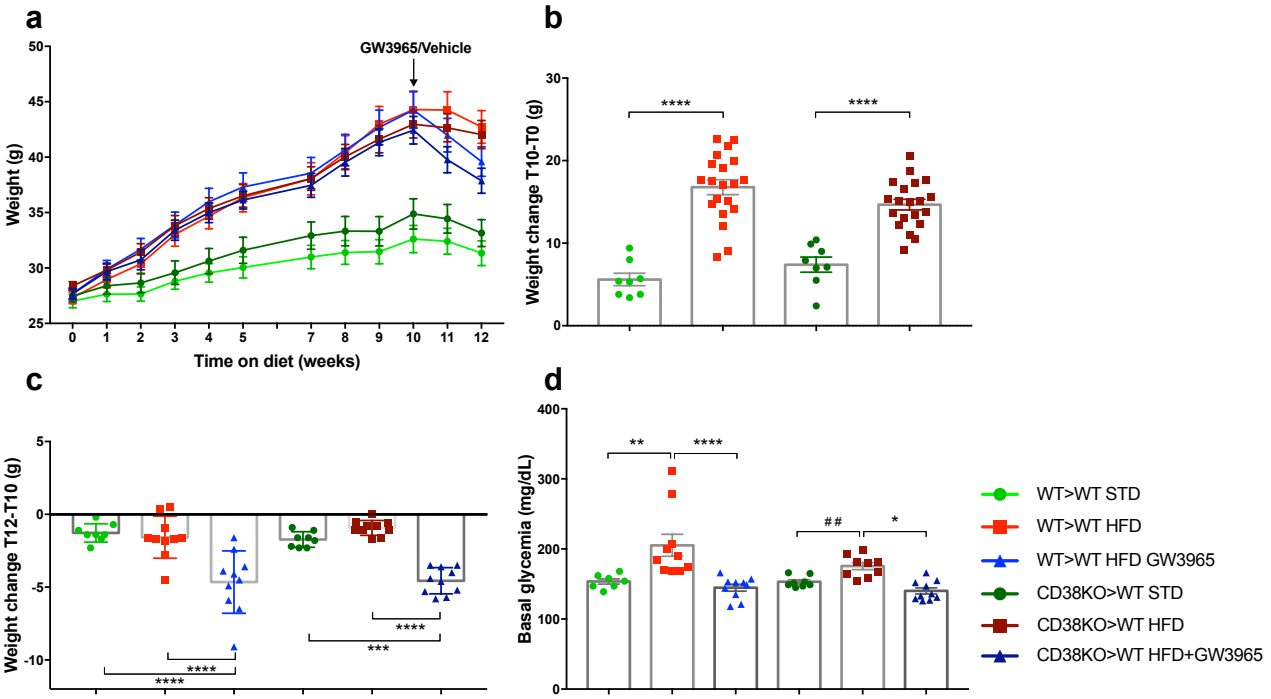


Figure 40. CD38 deficiency in bone marrow-derived cells is not sufficient to protect against the development of adiposity. Eight-week-old C57BL/6J male mice were sub-lethally irradiated and then injected with 3x106 bone marrow cells from either WT (WT>WT) or CD38-deficient (CD38KO>WT) male donors. Eight weeks after the bone marrow transfer, the mice were fed either a standard chow diet (STD) or a high fat diet (HFD) for 12 weeks. The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) or GW3965 (20 mg/kg/d, HFD+GW3965) daily by oral gavage, throughout the last 14 days of the experiment. a) Growth curve along the weeks of diet intervention. b) Weight change during the first 10 weeks on diet. c) Weight change during the last two weeks. d) Six-hour fasted glycemia at week 12. Mean ± SEM (n=8-10 mice/group). Two-way ANOVA-Tukey, **p < 0.01, ***p < 0.001, ****p < 0.0001. Selected conditions were also compared with a T-test, ##p < 0.01. Irrelevant comparisons have been omitted from the graphic.

GTT and ITT tests were also performed to further characterize glucose homeostasis in the mice used in these studies. In the GTT test, the mice harbouring a CD38 deficiency in bone marrow-derived cells performed similarly than the control mice (Figure 41a-b). These results contrast with the glucose intolerant phenotype developed by systemic CD38-deficient mice (Figure 17a-b), thus indicating that the CD38 deficiency in the hematopoietic system does not mediate this trait. In the ITT analysis, the mice with a CD38 deficiency in bone marrow-derived cells performed slightly worse than control mice under standard diet conditions and, in response to the high fat diet, showed a

higher degree of insulin resistance than the control mice. However the administration of the LXR agonist fully restored insulin sensitivity in these animals (Figure 41c-d). These observations contrast, again, with the phenotype observed in systemic CD38-deficient mice, which showed higher insulin sensitivity than WT mice, but an impaired capacity to improve insulin sensitivity upon LXR activation (Figure 17c-d). Overall, these results suggest that the insulin-sensitizing effects of the LXR agonist do not depend on CD38 expression in bone marrow-derived cells.

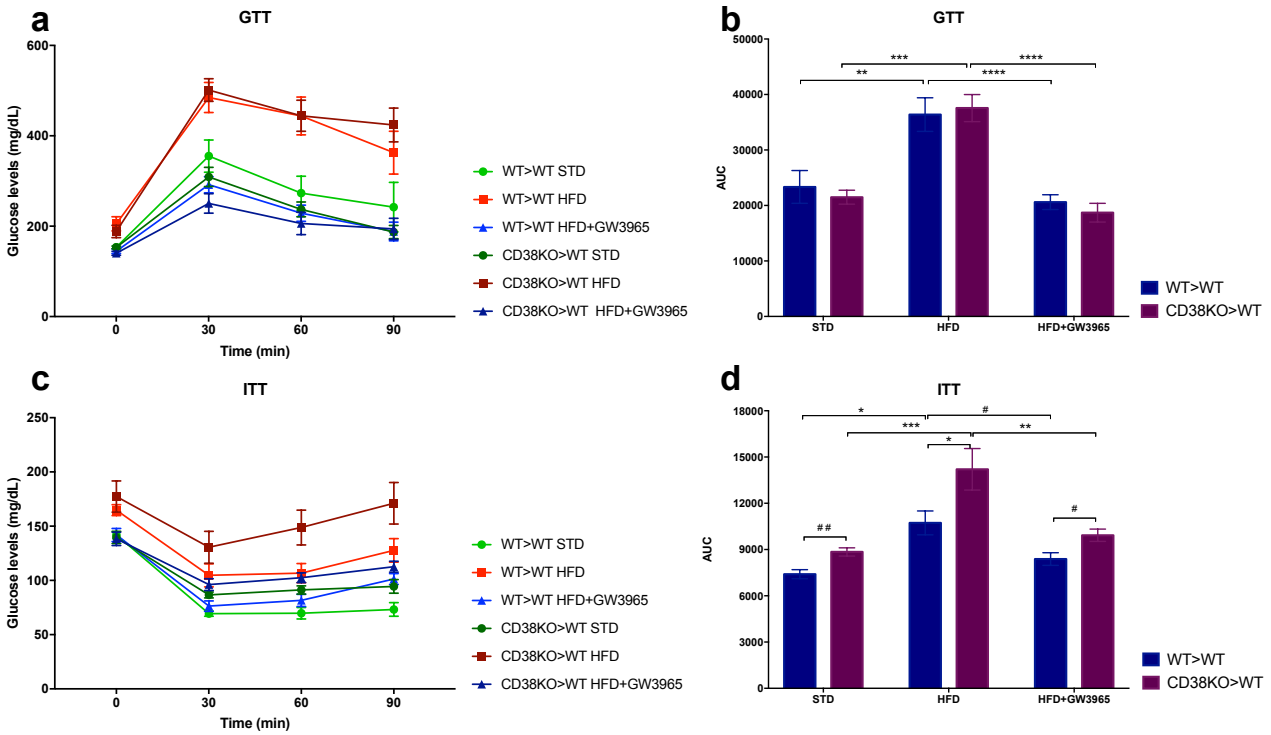


Figure 41. The insulin-sensitizing effects of the LXR agonist do not depend on CD38 expression in bone marrow-derived cells. Eight-week-old C57BL/6 male mice were sub-lethally irradiated and then injected with 3x106 bone marrow cells from either WT (WT>WT) or CD38-deficient (CD38KO>WT) male donors. Eight weeks after the bone marrow transfer, the mice were fed either a standard chow diet (STD) or a high fat diet (HFD) for 12 weeks. The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) or GW3965 (20 mg/kg/d, HFD+GW3965) daily by oral gavage, throughout the last 14 days of the experiment. After 10 and 12 days of GW3965 (or vehicle) treatment, the mice were fasted for 6 h and then subjected to a GTT (a-b) or an ITT (c-d), respectively. The area under the curve (AUC) from 0 to 90 min is represented for both tests, GTT (b) and ITT (d). Mean ± SEM (n=8-10 mice/group). Two-way ANOVA-Tukey, *p < 0.05, **p < 0.01, ***p < 0.001 ****p < 0.0001. In addition, selected conditions were compared using a T-test, # p < 0.05, ## p < 0.01. Irrelevant comparisons have been omitted from the graphic.

When InR signalling in the skeletal muscle was compared in both groups of mice, no differences were observed in insulin-induced AKT phosphorylation under standard conditions nor on the reduction of this response by the high fat diet (Figure 42). Remarkably, administration of the LXR agonist restored InR signalling in both types of mice (Figure 42). These results contrast with the lack of restoration of InR signalling in response to the LXR agonist in systemic CD38-deficient mice (Figure 18).

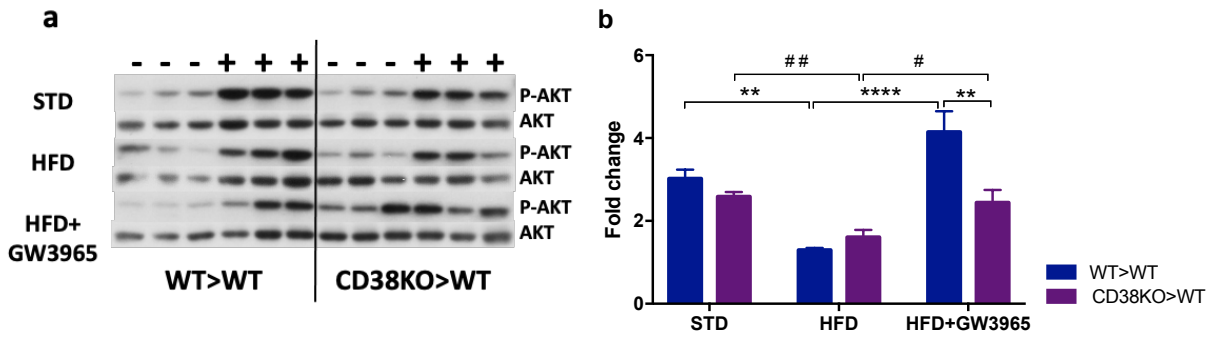


Figure 42. CD38 expression in bone marrow-derived cells is not required for the restoration of InR signalling in response to the LXR agonist. Eight-week-old C57BL/6 male mice were sub-lethally irradiated and then injected with 3x106 bone marrow cells from either WT (WT>WT) or CD38-deficient (CD38KO>WT) male donors. Eight weeks after the bone marrow transfer, the mice were fed either a standard chow diet (STD) or a high fat diet (HFD) for 12 weeks. The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) or GW3965 (20 mg/kg/d, HFD+GW3965) daily by oral gavage, throughout the last 14 days of the experiment. The mice were fasted for 6 h and then, half of them were injected intraperitoneally with 0.5UI/kg of insulin 15 min before the euthanasia. Skeletal muscle was collected from each mouse and whole tissue extracts were prepared as described in Methods. a) Representative immunoblots of phosphorylated AKT (P-AKT) and total AKT in skeletal muscle of the indicated animals and conditions. b) For each sample, the P-AKT values were normalized to the total AKT values. The graphic represents the effect of insulin (in fold change) on the P-AKT/total AKT ratio in insulin treated (+INS) versus non-treated mice (-INS) for each experimental group. Mean ± SEM (n=3-5 animals/group). Two-way ANOVA-Tukey posthoc **p < 0.01, ****p < 0.0001. In addition, selected conditions were compared using a T-test, # p < 0.05, ## p < 0.01. Irrelevant comparisons have been omitted from the graphic.

Following the rationale applied in previous sections, we also analysed the expression of selected genes coding for relevant mediators of metabolic homeostasis in white adipose tissues. In this sense, in epididymal white adipose tissue, the high fat diet induced leptin expression in mice transplanted with either WT or CD38-deficient bone marrows, and administration of GW3965 inhibited leptin expression in both groups of mice, reaching the levels observed under standard diet conditions (Figure 43a). An increase in leptin expression was also observed in the subcutaneous white adipose tissue in response to the high fat diet, but this increase was higher in the mice recipient of WT bone marrows (Figure 43a), which differs from the equivalent induction of leptin levels either in the epidydimal white adipose tissue of both groups of mice (Figure 43a) or in the subcutaneous white adipose tissue of WT and full CD38-deficient mice (Figure 21b). Moreover, the LXR agonist only significantly inhibited diet-induced leptin expression in mice recipient of WT bone marrows (Figure 43b), again in contrast with the results obtained in the epididymal white adipose tissue of these same groups of mice (Figure 43a).

On the other hand, adiponectin expression was clearly reduced in the epididymal white adipose tissue of both groups of mice in response to the high fat diet, with no effect of the LXR agonist (Figure 43a). These results contrast with the data shown in Figure 21a, in which adiponectin expression was not affected by the high fat diet in the epididymal white adipose tissue, but are in agreement with the results from the literature (Halleux et al., 2001; Marinho et al., 2020). It is possible that differences in the age of the mice and/or on the number of weeks on diet intervention are responsible for these contrasting results.

In subcutaneous white adipose tissue, we did not observe a decrease in adiponectin expression in response to the high fat diet in the groups of mice evaluated (Figure 43b), in line with similar results obtained in this tissue in previous experiments (Figure 21b).

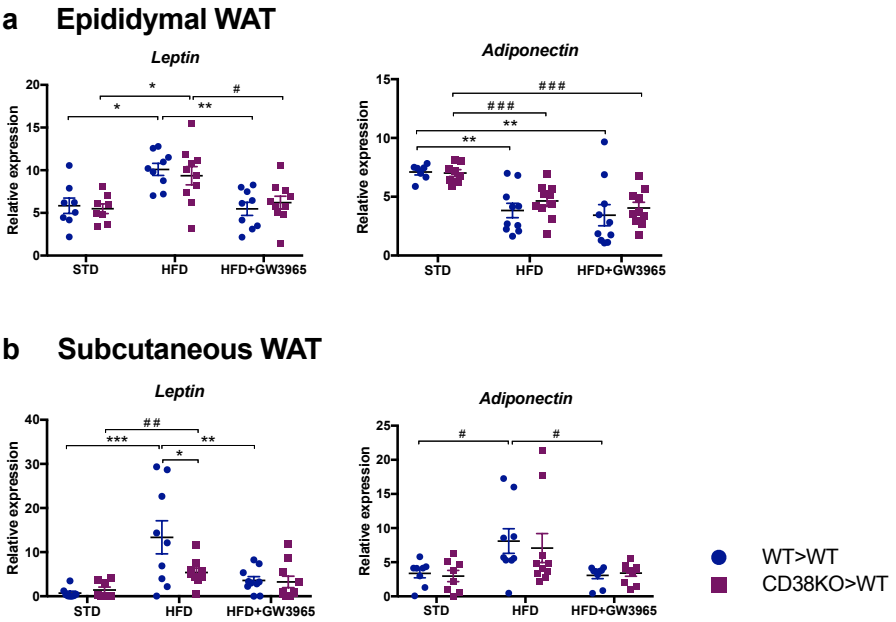


Figure 43. GW3965 administration inhibited leptin expression induced by high fat diet in epididymal white adipose tissue from mice transplanted with WT or CD38-deficient bone marrows. Eight-week-old C57BL/6 male mice were sub-lethally irradiated and then injected with 3x106 bone marrow cells from either WT (WT>WT) or CD38-deficient (CD38KO>WT) male donors. Eight weeks after the bone marrow transfer, the mice were fed either a standard chow diet (STD) or a high fat diet (HFD) for 12 weeks. The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) or GW3965 (20 mg/kg/d, HFD+GW3965) daily by oral gavage, throughout the last 14 days of the experiment. Epididymal (a) and subcutaneous (b) white adipose tissues (WAT) were isolated. The relative expression levels of selected genes were determined using qRT-PCR. Mean values of normalized relative expression ± SEM. (n=8-10 mice/group). Two-way ANOVA-Tukey posthoc, *p < 0.05, **p < 0.01, ***p < 0.001. In addition, selected conditions were compared using a T-test, # p < 0.05, ## p < 0.01, ### p < 0.001. Irrelevant comparisons have been omitted from the graphic.

Next, we evaluated the expression of canonical targets of the LXR pathway. A similar trend in *Abca1* and *Srebp1c* induction by GW3965 was observed in the white adipose tissues of mice transplanted with either WT or CD38-deficient bone marrows (Figure 44). These results suggest that the lack of expression of CD38 expression in bone marrow-derived cells does not impact the overall expression of other LXR target genes in whole white adipose tissues. In these experiments, as in those shown in Figure 25, no significant increase in *Glut4* or *Chrebp* expression was observed in response to the LXR agonist in white adipose tissues (Figure 44).

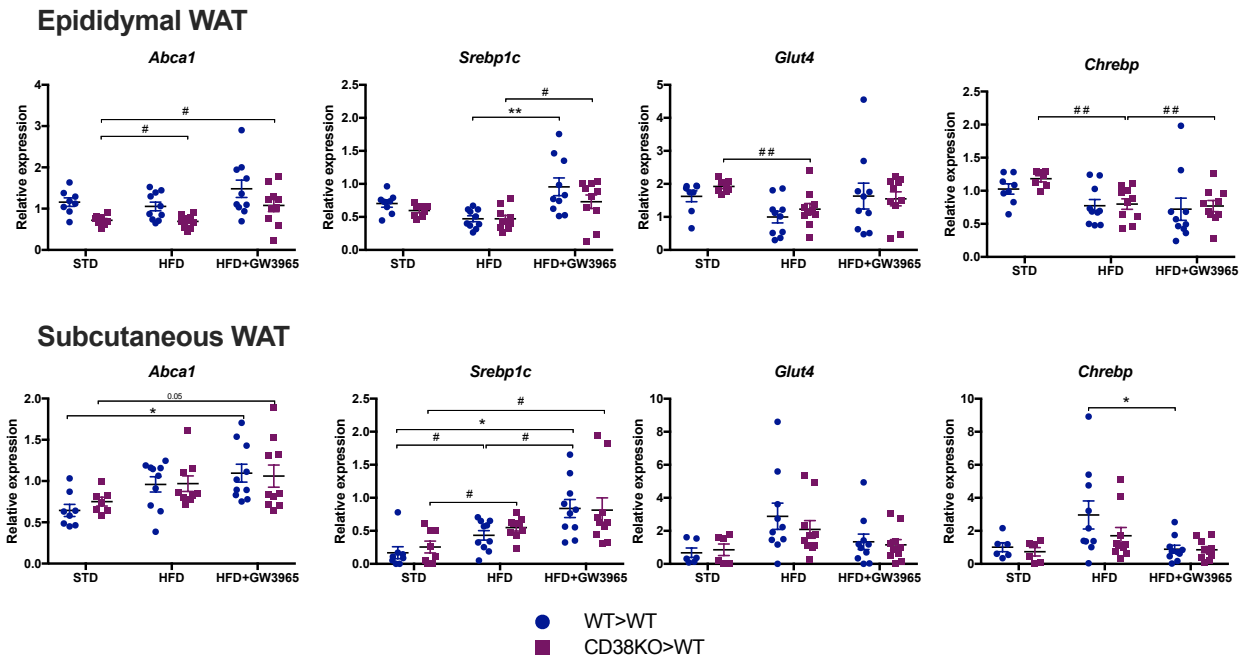


Figure 44. Effects of a high fat diet and/or GW3965 on the expression of LXR target genes in white adipose tissues of mice transplanted with WT or CD38-deficient bone marrows. Eight-week-old C57BL/6 male mice were sub-lethally irradiated and then injected with 3x106 bone marrow cells from either WT (WT>WT) or CD38-deficient (CD38KO>WT) male donors. Eight weeks after the bone marrow transfer, the mice were fed either a standard chow diet (STD) or a high fat diet (HFD) for 12 weeks. The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) or GW3965 (20 mg/kg/d, HFD+GW3965) daily by oral gavage, throughout the last 14 days of the experiment. Epididymal and subcutaneous white adipose tissues (WAT) were isolated. The relative expression levels of selected genes were determined using qRT-PCR. Mean values of normalized relative expression ± SEM. (n=8-10 mice/group). Two-way ANOVA-Tukey posthoc, *p < 0.05, **p < 0.01. In addition, selected conditions were compared using a T-test, # p < 0.05, ## p < 0.01. Irrelevant comparisons have been omitted from the graphic.

We next determined the expression levels of two important cytokines for our model, TNF-α and IL-10, in epididymal white adipose tissue. The expression of both cytokines was induced by the high fat diet in mice recipient of either WT or CD38-deficient bone marrow mice, however GW3965 did not significantly inhibit this induction (Figure 45).

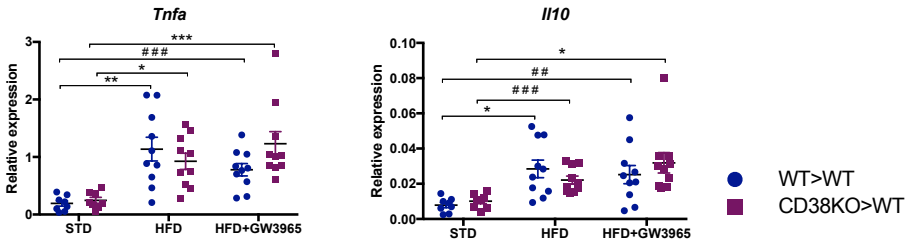


Figure 45. Effects of a high fat diet and/or GW3965 on the expression of Tnfa and Il10 in the epididymal white adipose tissue of mice transplanted with WT or CD38-deficient bone marrows.
Eight-week-old C57BL/6 male mice were sub-lethally irradiated and then injected with 3x106 bone marrow cells from either WT (WT>WT) or CD38-deficient (CD38KO>WT) male donors. Eight weeks after the bone marrow transfer, the mice were fed either a standard chow diet (STD) or a high fat diet (HFD) for 12 weeks. The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) or GW3965 (20 mg/kg/d, HFD+GW3965) daily by oral gavage, throughout the last 14 days of the experiment. Epididymal white adipose tissues were isolated. The relative expression levels of selected genes were determined using qRT-PCR. Mean values of normalized relative expression ± SEM. (n=7-10 mice/group). Two-way ANOVA-Tukey posthoc, *p < 0.05, **p < 0.01, *** p < 0.001. In addition, selected conditions were compared using a T-test, ## p < 0.01, ### p < 0.001. Irrelevant comparisons have been omitted from the graphic.

Our next step was to analyze the expression of HMOX1 and 11β-HSD1. The high fat diet caused equivalent upregulation of *Hmox1* expression in white adipose tissues from mice harbouring either WT or CD38-deficient bone marrows (Figure 46). This raises the possibility that the defect in *Hmox1* induction in response to the diet in the epididymal white adipose tissue of CD38-deficient mice (Figure 32) is not mediated by the lack of expression of CD38 in bone marrow-derived cells. In addition, LXR activation resulted in an inhibitory effect in *Hmox1* expression only in the epididymal white adipose tissue of mice carrying WT bone marrows (Figure 46). On the other hand, the genotype of the bone marrow did not influence the inhibitory effect of the high fat diet on 11β-HSD1 expression in the epididymal white adipose tissue or the restoration of its expression upon GW3965 administration (Figure 46).

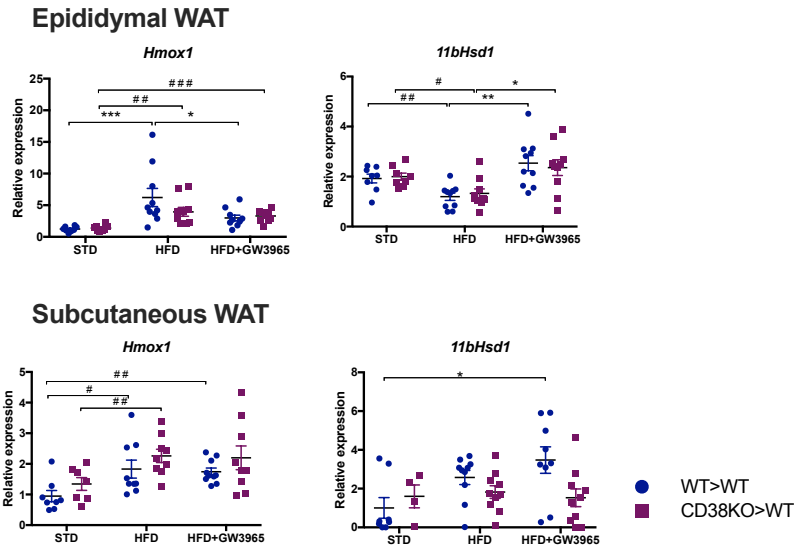


Figure 46. GW3965 administration ameliorated Hmox1 expression levels induced by high fat diet in epididymal WAT from WT-bone marrow transplanted mice. Eight-week-old C57BL/6 male mice were sub-lethally irradiated and then injected with 3x106 bone marrow cells from either WT (WT>WT) or CD38-deficient (CD38KO>WT) male donors. Eight weeks after the bone marrow transfer, the mice were fed either a standard chow diet (STD) or a high fat diet (HFD) for 12 weeks. The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) or GW3965 (20 mg/kg/d, HFD+GW3965) daily by oral gavage, throughout the last 14 days of the experiment. Epididymal and subcutaneous white adipose tissues were isolated. The relative expression levels of selected genes were determined using qRT-PCR. Mean values of normalized relative expression ± SEM. (n=8-10 mice/group). Two-way ANOVA-Tukey posthoc, *p < 0.05, **p < 0.01, *** p < 0.001. In addition, selected conditions were compared using a T-test, ## p < 0.01, ### p < 0.001. Irrelevant comparisons have been omitted from the graphic.

Finally, the expression levels of markers for infiltrated macrophages were determined. The levels of expression of *F4/80*, *Cd11c* and *Mgl1*, but not Mgl2, increased in response to the high fat diet in the epididymal white adipose tissue regardless of the genotype of the bone marrow (Figure 47), suggesting that the defect in the expression levels of these markers in this tissue in systemic CD38-deficient mice (Figure 33) are not mediated by the lack of expression of CD38 intrinsically in bone marrow-derived cells. Activation of the LXR pathway resulted in significant inhibition of the high fat diet-induced increase in *Cd11c* expression in mice recipient of WT bone marrows. A similar trend (although not reaching significance) was found in mice with CD38-deficient bone marrows (Figure 47).

The increase in *F4/80* and *Cd11c* expression induced by the high fat diet was also evidenced in the subcutaneous white adipose tissue, although a significant effect of the LXR agonist was not observed for any of these markers (Figure 47). Unexpectedly, the high fat diet induced *Mgl2* expression in this tissue in mice transplanted with WT bone marrows, an effect that was counteracted by the LXR agonist. A similar trend, although reaching lower levels in response to the high fat diet was observed in the mice carrying CD38-deficient bone marrows (Figure 47).

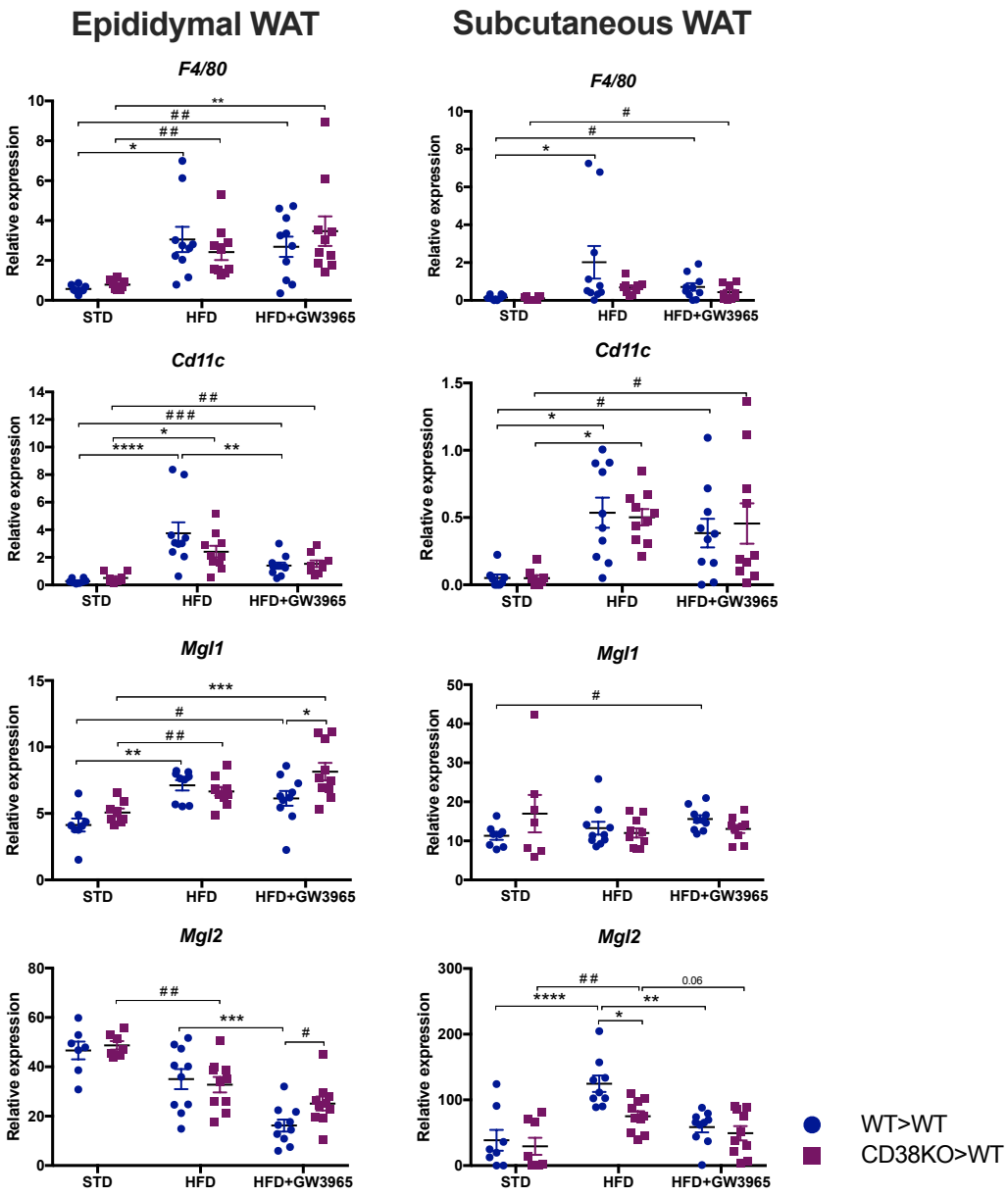
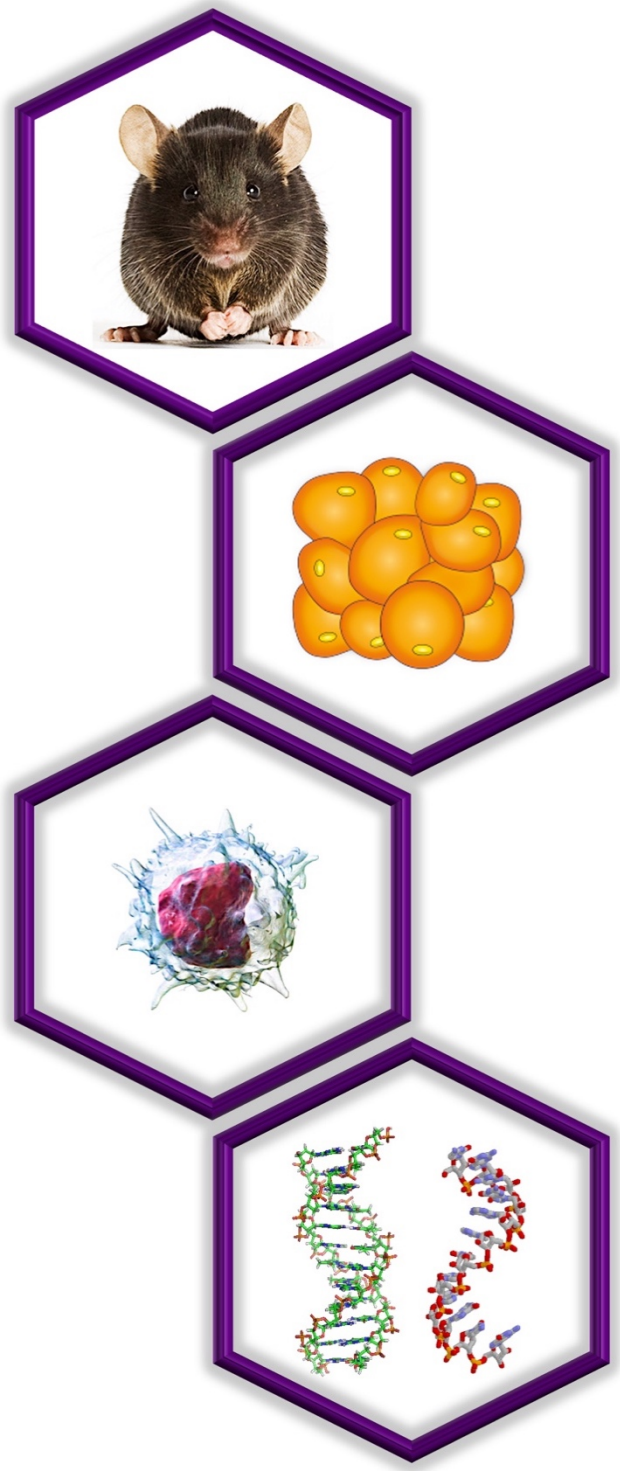


Figure 47. GW3965 administration ameliorated the CD11c expression levels induced by high fat diet in epididymal WAT from WT-bone marrow transplanted mice. Eight-week-old C57BL/6 male mice were sub-lethally irradiated and then injected with 3x106 bone marrow cells from either WT (WT>WT) or CD38-deficient (CD38KO>WT) male donors. Eight weeks after the bone marrow transfer, the mice were fed either a standard chow diet (STD) or a high fat diet (HFD) for 12 weeks. The latter group was further divided into two subgroups receiving either vehicle (carboxymethylcellulose) or GW3965 (20 mg/kg/d, HFD+GW3965) daily by oral gavage, throughout the last 14 days of the experiment. Epididymal and subcutaneous white adipose tissues were isolated. The relative expression levels of selected genes were determined using qRT-PCR. Mean values of normalized relative expression ± SEM. (n=8-10 mice/group). Two-way ANOVA-Tukey posthoc, *p < 0.05, **p < 0.01, *** p < 0.001. In addition, selected conditions were compared using a T-test, ## p < 0.01, ### p < 0.001. Irrelevant comparisons have been omitted from the graphic.

In general, the deficiency of CD38 in bone marrow-derived cells does not recapitulate the phenotype observed in full CD38 knockout mice for most of the parameters analysed. In other words, whereas the systemic CD38 deficiency resulted in increased insulin resistance associated to the high fat diet and compromised the capacity of the LXR agonist to mediate its insulin-sensitizing actions, in mice that are only deficient in CD38 in the bone marrow-derived compartment, the response to the diet or to the LXR agonist was similar than in mice recipient of WT bone marrows, with the exception of discrete events, such as the consequences of diet intervention and LXR activation on *Hmox1* expression in the epididymal white adipose tissue. Thus, these results suggest that the differences in the response to the high fat diet between WT and systemic CD38-deficient mice are not due to the lack of expression of CD38 in bone marrow-derived cells. In addition, CD38 expression in the compartment derived from the bone marrow is not a major contributor to the insulin-sensitizing actions of the LXR agonist.



DISCUSSION

Obesity is nowadays considered a pandemic disease, and its incidence correlates with the concomitant increase of a set of associated comorbidities, including T2D, cardiovascular diseases, fatty liver disease, among others, which are all grouped within the metabolic syndrome. Insulin resistance is frequently developed in obesity and seems to be a common link to these associated diseases. In fact, insulin resistance is an early feature in the development of T2D and induces a pancreatic β-cell compensatory response increasing insulin release to maintain normoglycemia. However, eventual pancreatic failure leads to hyperglycemia, the hallmark of diabetes. Therefore, many efforts have been focused to decipher the mechanisms underlying insulin resistance, being metaflammation, that is the chronic low grade inflammatory response in the adipose tissue associated to obesity, the most well-documented (Hotamisligil, 2017). In this tissue, pro-inflammatory cytokines, such as TNF-α, are initially produced by adipocytes in response to hypertrophy-induced ER stress and afterwards also by immune cells. These cytokines trigger pro-inflammatory pathways, which results in the impairment of InsR signalling and lead to insulin resistance. In this scenario, it is urgent to explore the relevance of anti-inflammatory actors as insulin-sensitizing agents.

The nuclear receptors LXRs represent interesting therapeutic targets that can be pharmacologically activated by synthetic high affinity agonists. Accumulated evidence, including work from our own group, indicates the importance of the LXR pathway in the negative control of inflammatory conditions (reviewed by Glaría, Letelier, and Valledor 2020). Putting together the pieces of evidence from different cellular models and inflammatory mediators, several mechanisms contribute to the antagonizing actions of the LXR pathway on pro-inflammatory signaling. In this work we have focused in better understanding several aspects of LXR biology. On one side, using primary murine macrophages differentiated from bone marrows, we have identified novel mechanisms that contribute to the crosstalk between LXRs and the inflammatory cytokine IFN-γ. On the other side, we have evaluated the consequences of LXR activation in a murine model of obesity-associated insulin resistance.

In this sense, the LXR pathway exerts important repressive actions on the inflammatory response to the cytokine IFN-γ (reviewed by Glaría, Letelier, and Valledor 2020). Previous work from our group showed that the LXR pathway inhibits the binding of STAT1, the main early mediator of IFN-γ signalling, to regulatory regions of several IFN-γ target genes in macrophages, without affecting STAT1 phosphorylation (Pascual-García et al., 2013). Similar effects had been also described in astrocytes (Lee et al. 2009). Another important player in the response to IFN-γ is the STAT-1 target

gene IRF1, which contributes to a second wave of transcriptional events. For this reason, the first part of this thesis evaluated whether there is functional crosstalk between LXRs and IRF1. Although we have not characterized the overall contribution of IRF1 to the macrophage response to IFN-γ, our studies indicate that IRF1 is required for the full induction of several IFN-γ-responsive genes. Despite the previously described negative actions of LXRs on STAT-1 signaling, the results from this work suggest that activation of the LXR pathway does not inhibit the induction of IRF1 expression by IFN-γ. Interestingly, however, negative crosstalk with this transcription factor is important for the repressive action of the LXR agonist on some of the genes evaluated in this work.

Reciprocally, IFN-γ signalling influences the macrophage response to LXR agonists (Pascual-García et al., 2013). In our experiments in macrophages, IRF1 deficiency impacted LXR target gene expression differentially depending on the gene analyzed. In the case of *Abca1 and Idol*, IRF1 contributed partially to the negative effects that IFN-γ signalling exerts on their expression. On the other hand, IRF1 was required for basal expression and for LXR-mediated induction of *Aim*. In contrast, LXRs and inflammatory signals cooperated for the induction of *Mertk* and *Cd38*, as previously described (Matalonga et al., 2017; Pascual-García et al., 2013). However, while functional IRF1 was necessary for these cooperative actions on *Mertk* expression, higher levels of *Cd38* induction were obtained in IRF1-deficient cells than in WT cells. The de-repression of *Cd38* was not observed in STAT1-deficient macrophages, which suggests that IRF1 plays an important fine-tuning role on the expression of *Cd38* during the macrophage response to IFN-γ. This observation contrasts with the general role of IRF1 as a transcriptional activator (Feng et al. 2021). However, several studies have shown the capacity of IRF-1 to also exert repressive actions potentially through direct binding to IRF1-binding sites (Fragale et al., 2008; Zhang et al., 2019). Despite the fact that the gene encoding CD38 contains an IRF1-binding site (Ferrero and Malavasi, 1997; Tliba et al., 2008), more work is needed in order to know if de-repression in the absence of functional IRF1 is direct or indirect in our cellular system. Of note, however, these effects might be cell type-specific, as IRF1 silencing reduced CD38 expression induced by the combination of TNF-α and IFN-γ in airway smooth muscle cells (Tliba et al., 2008).

Prior to this work we had demonstrated an important role for CD38 in anti-microbial and chemotactic responses mediated by activated LXRs (Beceiro et al., 2018; Matalonga et al., 2017). In line with the cooperation between inflammatory signals and LXRs on CD38 expression, significant induction of this gene was evidenced in several tissues in mice fed with a high fat diet and exposed to an LXR agonist. Therefore, the second objective of this thesis focused specifically on the impact

of CD38-deficiency in the insulin-sensitizing actions of the LXR pathway in a murine model of diet-induced obesity-associated insulin resistance. In these studies, we used the molecule GW3965 to activate the LXR pathway, as it is a specific LXR agonist at the doses used in our experiments. Administration of GW3965 to WT mice fed with a high fat diet resulted in a significant decrease in weight gain and in glucose tolerance, and an increase in insulin sensitivity. These changes were accompanied by a decrease in diet-induced hyperinsulinemia and in the pancreatic insulin content of obese mice. These effects on body weight, glucose tolerance and insulin resistance are in line with similar observations by other groups in diverse murine models of obesity and insulin resistance (Gao and Liu, 2013; Grefhorst et al., 2005; Laffitte et al., 2003).

When the high fat diet-induced phenotype was studied in mice with systemic CD38 deficiency, several differences were observed in comparison to WT mice. First, the CD38-deficient mice were partially protected from diet-induced obesity, hyperinsulinemia, and insulin resistance, although they showed stronger glucose intolerance in all the conditions evaluated. The phenotype in body weight and insulinemia is in line with previous work reporting the protection of CD38 null mice from diet-induced adiposity, which correlated with an increase in intracellular NAD+ levels and Sirt1 activity (Barbosa et al., 2007). In contrast to our observations, however, Barbosa et al. 2007showed an augmented glucose tolerance in aged CD38-deficient mice, in comparison to WT mice, under high fat diet conditions (Barbosa et al., 2007). It is possible that the age of the mice at the start of diet intervention is important to explain this discrepancy, especially taking into account that CD38 expression, and the concomitant NAD decline, increases with aging (Camacho-Pereira et al., 2016).

Importantly, the LXR agonist was not able to improve the performance of CD38-deficient mice in the ITT or to decrease hyperinsulinemia as efficiently as in WT mice, although it did reduce body weight and glucose intolerance. These observations suggest that the mechanisms mediating the effects of LXR agonists on insulin sensitivity and adiposity can be dissociated. In particular, the functional expression of CD38 contributes to alleviating insulin resistance, but not to reducing body weight, in response to the LXR agonist. To date, several mechanisms have been proposed to explain the improvement in glucose homeostasis by LXR agonists, including the inhibition of hepatic gluconeogenesis and the induction in GLUT4 expression and subsequent increase in glucose uptake by the adipose tissue (Cao et al., 2003; Laffitte et al., 2003). Thus, our observations on the involvement of CD38 further extends the current view of the overall mechanisms mediating the insulin-sensitizing actions of the LXR pathway.

Because CD38 plays important roles in the control of metabolism and of immune cell function, our study was expanded to analyze the expression of genes that are relevant mediators of metabolic homeostasis or of metaflammation in several tissues, with a main focus in white adipose tissues. Among the different metabolic genes analysed, leptin expression highly recapitulated the effects of the diet and of the LXR agonist on insulin sensitivity in either WT or CD38-deficient mice. These effects translated into hyperleptinemia in response to the high fat diet, which was inhibited by the LXR agonist in WT mice. CD38-deficient mice developed hyperleptinemia to a lesser extent than the WT counterparts. In general, the levels of leptin strongly associate with adiposity. The visceral white adipose tissue is highly responsible for the production of leptin in response to the high fat diet (Kumari et al., 2019), our analysis separating adipocytes from stromal cells suggest that the effects in leptin expression mostly occur in the adipocytic compartment of the white adipose tissue. The fact that CD38-deficient mice have lower leptinemia can be explained by their protection against the development of obesity and is in agreement with the data reported by Barbosa et al. 2007. Importantly, the LXR agonist did not significantly reduce leptinemia in the CD38-deficient background, despite reducing their body weight to the same extent than in WT mice. These observations further support the notion that the effects of LXR activation on leptinemia are associated with changes in insulin sensitivity, but not to changes in body weight.

Of note, while the combination of the high fat diet and the LXR agonist induced the expression of canonical LXR target genes such as *Abca1* and *Srebp1c* in the white adipose tissue, no significant induction of *Glut4* expression was detected under the same conditions, which contrasts with previous studies identifying *Glut4* as a direct LXR target gene (Dalen et al., 2003; Laffitte et al., 2003) Thus, our results suggest that a mechanism based on increased *Glut4* expression in the white adipose tissue is not a major mediator of the insulin-sensitizing actions of the LXR-CD38 axis in our model of obesity.

The importance of metaflammation in obesity-associated insulin resistance has been extensively documented. As evidenced in our own studies, obesity increases the infiltration of leukocytes and the production of several pro-inflammatory mediators in the white adipose tissue and, potentially, both adipocytes and stromal cells contribute to these effects. In murine models, adipose tissue macrophages have been defined as $F4/80^{+}CD11b^{+}$ cells (in the lean state) and as $F4/80^{+}CD11b^{+}CD11c^{+}$ cells (in the obese state) (Russo and Lumeng, 2018). Importantly, the ablation of $CD11c^{+}$ cells attenuates adipose tissue inflammation and improves glucose tolerance (Patsouris et al., 2008). Our expression analysis showed that WT mice display higher mRNA levels of the

macrophage/dendritic cells markers F4/80, CD68 and CD11c in the white adipose tissue in response to the high fat diet, and, interestingly, GW3965 administration selectively inhibited the induction of CD11c in WT but not in CD38-deficient mice. Despite robust anti-inflammatory effects of LXR agonists in different disease settings (Olaogun et al., 2020), the effects of GW3965 on *Cd11c* mRNA levels were not accompanied by broad impact on pro-inflammatory gene expression in the white adipose tissue. In this sense, the LXR agonist inhibited the expression of the cytokine TNF-α in some experiments, with several other markers of inflammation remaining unaffected.

High production of IL-6 and Th17 cell polarization have been associated with the development of obesity and insulin resistance (Ahmed and Gaffen, 2010; Lee et al., 2017), and the imbalance between Th17 and Treg populations plays a major role in obesity-dependent inflammation (Fabbrini et al., 2013; Winer et al., 2009). In *in vitro* studies, LXR activation reduced the polarization of naïve T cells to Th17 and Th1 phenotypes, and promoted the expansion of the Treg phenotype (Herold et al. 2017; Solt, Kamenecka, and Burris 2012; Yang et al. 2014). However, in our model, we did not observe effects of the LXR agonist on the mRNA expression of markers of Th1, Th17 or Treg phenotypes, namely IFN-γ, IL-17 and Foxp3, respectively.

Thus, when we take together all the experiments included in this thesis, the LXR agonist was systematically able to ameliorate insulin resistance in the WT background, however its ability to inhibit the expression of pro-inflammatory mediators was not so consistent. Therefore, we interpret that the negative effects on metaflammation are not major contributors of the insulin sensitizing actions of the LXR pathway in our model.

In agreement with the extensively documented role of CD38 in transendothelial migration and chemotaxis of immune cells to sites of infection or tissue injury (reviewed by Glaría and Valledor 2020; Piedra-Quintero et al. 2020), CD38-deficient mice showed an impairment in the frequency of leukocytes and in the mRNA expression of markers of infiltrated myeloid cells in the white adipose tissue in response to the high fat diet. Strikingly, however, these mice displayed significantly increased expression of several inflammatory mediators in different anatomic locations, such as IL-6 in the white adipose tissue, IFN-γ in the liver and spleen, and IL-17 in the liver, already under standard diet conditions, which contrast with the established pro-inflammatory roles of CD38 during the immune cell response to infection (reviewed by Glaría and Valledor 2020; Piedra-Quintero et al. 2020). However, the contribution of CD38 toward cytokine production in other conditions is controversial. In a model of LPS-induced kidney injury, CD38-deficient mice displayed increased TLR4 expression in the kidney and enhanced secretion of several inflammatory cytokines (Li et al.

2019). In addition, CD38 knockdown in the mouse macrophage cell line Raw264.7 resulted in increased secretion of several inflammatory mediators, including IL-6 (Qian et al., 2018). It is possible that CD38 expression in different cellular compartments within metabolic tissues play unknown roles in the control of cytokine expression. Therefore, the exact mechanism/s resulting in increased cytokine expression in several anatomic locations in CD38-deficient mice under standard conditions must be clarified in the future.

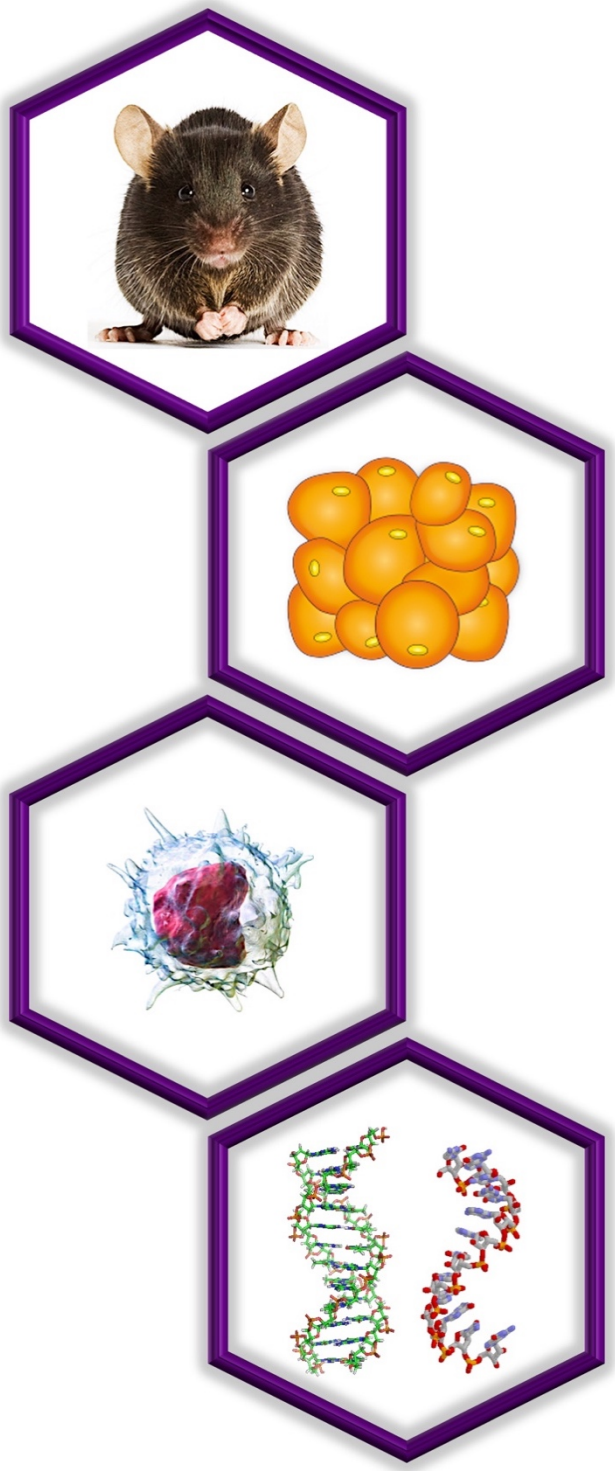
Despite the establishment of an inflammatory environment in the adipose tissue during obesity, the expression of cytokines traditionally characterized as suppressors of inflammation, namely IL-10 and TGF-β, was also increased in the white adipose tissue in response to the high-fat diet, in line with previously reported work in different obesity settings (Alessi et al., 2000; Meyerovich et al., 2016; Samad et al., 1997; Sardi et al., 2021). Initial explanations argued in favour of anti-inflammatory cytokines being produced in response to high levels of inflammatory mediators as an attempt to restore homeostasis. For instance, TNF-α can stimulate the secretion of IL-13 and IL-10 in the white adipose tissue (Juge-Aubry et al., 2005; Sauter et al., 2015). However, a promoting role for IL-10 in the development of adiposity and insulin resistance has been recently delineated. IL-10 signalling has been shown to suppress thermogenesis in adipocytes through a STAT3-dependent pathway, thus inducing obesity and the associated insulin resistance. In this line, IL-10-deficient mice are protected from diet-induced obesity and insulin resistance (Rajbhandari et al., 2018). Likewise, Tregs are the predominant IL-10–producing cells in the adipose tissue and a recent study has shown that IL-10 deletion specifically in Tregs protects against diet-induced obesity and results in an increase in insulin sensitivity (Beppu et al., 2021). Likewise, activation of TGF-β signaling has been recently shown to contribute to the development of obesity, and its blockade protects from obesity by promoting mitochondrial biogenesis and the browning of the white adipose tissue (Yadav et al., 2011). In our studies, lower expression of TGF-β, but not of IL-10, was detected in the white adipose tissue of CD38-deficient mice in both standard and high fat diet conditions. Whether impaired TGF-β production is a cause or a consequence of increased expression of several inflammatory mediators in the white adipose tissue and/or of the protection against adiposity in CD38-deficient mice remains elusive. Nevertheless, these results illustrate that the processes by which metaflammation induces insulin resistance are more complex than initially conceived, and aspects beyond the imbalance between pro- and anti-inflammatory factors require close examination.

Experiments in which the mice were transplanted with WT or CD38-deficient bone marrow cells indicate that CD38 deletion specifically in bone marrow cells is not sufficient to protect from diet-

induced obesity and insulin resistance. However, although the glucose-intolerant phenotype detected in systemic CD38-deficient mice is not reproduced in these mice, they developed higher insulin resistance in all the conditions evaluated, indicating that CD38 deficiency specifically in bone marrow cells leads to opposite consequences than the systemic CD38 deficiency regarding glucose homeostasis. Whether such differences correspond with changes in the expression of key inflammatory mediators has not been fully addressed yet.

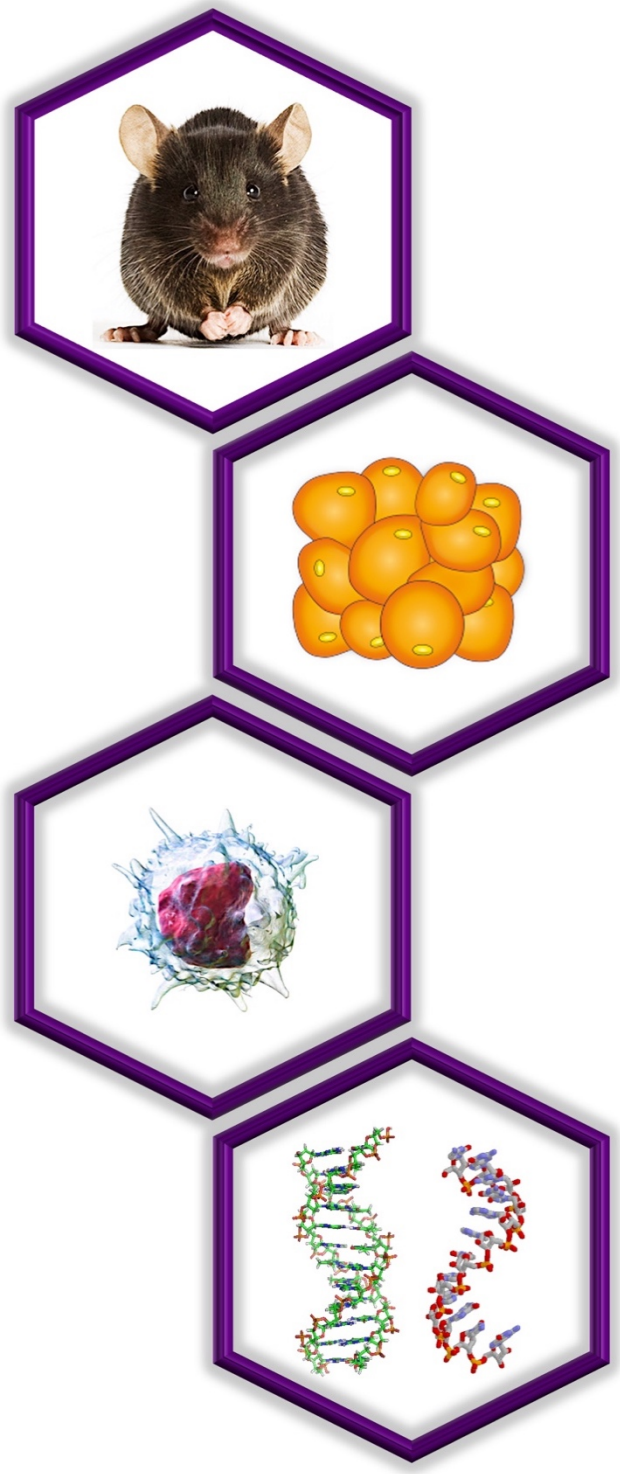
Interestingly, our studies suggest that the major contributors to the induction of CD38 expression in response to LXR activation in the epididymal white adipose tissue are bone marrow-derived cells instead of other cells, such as adipocytes or their progenitors (which may also express CD38) (Carrière et al., 2017). Attending to our initial objectives, the most important observation from the bone marrow transfer experiments is that the insulin-sensitizing effects of the LXR agonist do not require CD38 expression specifically in cells derived from the bone marrow. These observations are in line with two important results from this work. First, the LXR agonist alleviated insulin resistance without broad effects on metaflammation in the white adipose tissue. Second, the metabolic compartment, rather than the vascular stromal fraction, better recapitulated the overall effects of the diet and the LXR agonist on the expression profile of several genes, such as leptin, in the white adipose tissue.

In summary, this work unravels the importance of the multi-enzymatic protein CD38 in the insulin-sensitizing actions of the LXR pathway, and demonstrates that CD38 expression specifically in cells from the bone marrow does not contribute significantly to these effects. Based on the ubiquitous expression of CD38 in multiple cellular compartments, precise characterization of the function of the LXR-CD38 axis in different cell types will help to better understand the metabolic actions described in this work.



CONCLUSIONS

- The full induction of several macrophage pro-inflammatory genes in response to IFN-γ requires functional IRF1 expression.
- There is reciprocal crosstalk between LXRs and IRF1 on a gene-specific manner during the macrophage response to IFN-γ. In this context, IRF1 plays a fine-tuning role in the control of the expression of the LXR target CD38.
- CD38-deficient mice are partially protected from both the increase in adiposity and the development of insulin resistance in response to a high fat diet. This phenotype is not mediated by the lack of expression of CD38 in bone marrow-derived cells.
- LXR activation by GW3965 exerts insulin-sensitizing actions, which require the functional expression of CD38. These effects do not depend on CD38 expression in bone marrow-derived cells.
- In our model, obesity-associated insulin resistance is accompanied by increased mRNA levels of both pro-inflammatory (e.g. TNF-α and IL-6) and anti-inflammatory (IL-10 and TGF-β) mediators in the epididymal white adipose tissue. However, administration of GW3965 did not exert broad inhibitory actions on pro-inflammatory gene expression; only inhibition of TNF-α mRNA was observed in WT mice in some experimental settings.
- Obesity-associated insulin resistance is accompanied by an increase in the mRNA expression of several myeloid cell markers (*F4/80*, *Cd11c* and *Mgl1*) within the epididymal white adipose tissue. These effects are impaired in systemic CD38-deficient mice, but do not require intrinsic expression of CD38 in bone marrow-derived cells. In addition, LXR activation inhibits the increase in *Cd11c* mRNA expression in the epididymal white adipose tissue of obese WT mice.



REFERENCES

A-Gonzalez, N., Bensinger, S.J., Hong, C., Beceiro, S., Bradley, M.N., Zelcer, N., Deniz, J., Ramirez, C., Díaz, M., Gallardo, G., et al. (2009). Apoptotic Cells Promote Their Own Clearance and Immune Tolerance through Activation of the Nuclear Receptor LXR. Immunity *31*, 245–258.

Ahmed, M., and Gaffen, S.L. (2010). IL-17 in obesity and adipogenesis. Cytokine Growth Factor Rev. *21*, 449–453.

Aksoy, P., White, T.A., Thompson, M., and Chini, E.N. (2006a). Regulation of intracellular levels of NAD: A novel role for CD38. Biochem. Biophys. Res. Commun. *345*, 1386–1392.

Aksoy, P., Escande, C., White, T.A., Thompson, M., Soares, S., Benech, J.C., and Chini, E.N. (2006b). Regulation of SIRT 1 mediated NAD dependent deacetylation: A novel role for the multifunctional enzyme CD38. Biochem. Biophys. Res. Commun. *349*, 353–359.

Alessi, M.C., Bastelica, D., Morange, P., Berthet, B., Leduc, I., Verdier, M., Geel, O., and Juhan-Vague, I. (2000). Plasminogen activator inhibitor 1, transforming growth factor-β1, and BMI are closely associated in human adipose tissue during morbid obesity. Diabetes *49*, 1374–1380.

Amici, S.A., Young, N.A., Narvaez-Miranda, J., Jablonski, K.A., Arcos, J., Rosas, L., Papenfuss, T.L., Torrelles, J.B., Jarjour, W.N., and Guerau-de-Arellano, M. (2018). CD38 is robustly induced in human macrophages and monocytes in inflammatory conditions. Front. Immunol. *9*, 1593.

Archer, A., Stolarczyk, É., Doria, M.L., Helguero, L., Domingues, R., Howard, J.K., Mode, A., Korach-André, M., and Gustafsson, J.-Å.A. (2013). LXR activation by GW3965 alters fat tissue distribution and adipose tissue inflammation in ob/ob female mice. J. Lipid Res. *54*, 1300–1311.

Ausiello, C.M., Urbani, F., la Sala, A., Funaro, A., and Malavasi, F. (1995). CD38 ligation induces discrete cytokine mRNA expression in human cultured lymphocytes. Eur. J. Immunol. *25*, 1477–1480.

Ballak, D.B., Stienstra, R., Tack, C.J., Dinarello, C.A., and van Diepen, J.A. (2015). IL-1 family members in the pathogenesis and treatment of metabolic disease: Focus on adipose tissue inflammation and insulin resistance. Cytokine *75*, 280–290.

Barbosa, M.T.P., Soares, S.M., Novak, C.M., Sinclair, D., Levine, J.A., Aksoy, P., and Chini, E.N. (2007). The enzyme CD38 (a NAD glycohydrolase, EC 3.2.2.5) is necessary for the development of diet-induced obesity. FASEB J. *21*, 3629–3639.

Becares, N., Gage, M.C., and Pineda-Torra, I. (2017). Posttranslational modifications of lipid-activated nuclear receptors: Focus on metabolism. Endocrinology *158*, 213–225.

Beceiro, S., Pap, A., Czimmerer, Z., Sallam, T., Guillén, J.A., Gallardo, G., Hong, C., A-Gonzalez, N., Tabraue, C., Diaz, M., et al. (2018). Liver X Receptor Nuclear Receptors Are Transcriptional Regulators of Dendritic Cell Chemotaxis. Mol. Cell. Biol. *38*.

Bedinger, D.H., and Adams, S.H. (2015). Metabolic, anabolic, and mitogenic insulin responses: A tissue-specific perspective for insulin receptor activators. Mol. Cell. Endocrinol. *415*, 143–156.

Benninger, R.K.P., Dorrell, C., Hodson, D.J., and Rutter, G.A. (2018). The Impact of Pancreatic Beta Cell Heterogeneity on Type 1 Diabetes Pathogenesis. Curr. Diab. Rep. *18*, 1–9.

Bensinger, S.J., Bradley, M.N., Joseph, S.B., Zelcer, N., Janssen, E.M., Hausner, M.A., Shih, R., Parks, J.S., Edwards, P.A., Jamieson, B.D., et al. (2008). LXR Signaling Couples Sterol Metabolism to Proliferation in the Acquired Immune Response. Cell *134*, 97–111.

Beppu, L.Y., Mooli, R.G.R., Qu, X., Marrero, G.J., Finley, C.A., Fooks, A.N., Mullen, Z.P., Frias, A.B., Sipula, I., Xie, B., et al. (2021). Tregs facilitate obesity and insulin resistance via a Blimp-1/IL-10 axis. JCI Insight *6*.

Boettcher, E., Csako, G., Pucino, F., Wesley, R., and Loomba, R. (2012). Meta-analysis: Pioglitazone improves liver histology and fibrosis in patients with non-alcoholic steatohepatitis. Aliment. Pharmacol. Ther. *35*, 66–75.

Böni-Schnetzler, M., and Meier, D.T. (2019). Islet inflammation in type 2 diabetes. Semin. Immunopathol. *41*, 501–513.

Böni-Schnetzler, M., Boller, S., Debray, S., Bouzakri, K., Meier, D.T., Prazak, R., Kerr-Conte, J., Pattou, F., Ehses, J.A., Schuit, F.C., et al. (2009). Free fatty acids induce a proinflammatory response in islets via the abundantly expressed interleukin-1 receptor I. Endocrinology *150*, 5218–5229.

Böni-Schnetzler, M., Häuselmann, S.P., Dalmas, E., Meier, D.T., Thienel, C., Traub, S., Schulze, F., Steiger, L., Dror, E., Martin, P., et al. (2018). β Cell-Specific Deletion of the IL-1 Receptor Antagonist Impairs β Cell Proliferation and Insulin Secretion. Cell Rep. *22*, 1774–1786.

Bonnet, F., and Scheen, A.J. (2018). Effects of SGLT2 inhibitors on systemic and tissue low-grade inflammation: The potential contribution to diabetes complications and cardiovascular disease. Diabetes Metab. *44*, 457–464.

Bonora, B.M., Avogaro, A., and Fadini, G.P. (2020). Extraglycemic effects of SGLT2 inhibitors: A review of the evidence. Diabetes, Metab. Syndr. Obes. Targets Ther. *13*, 161–174.

Burhans, M.S., Hagman, D.K., Kuzma, J.N., Schmidt, K.A., and Kratz, M. (2019). Contribution of adipose tissue inflammation to the development of type 2 diabetes mellitus. Compr. Physiol. *9*, 1–58.

Calkin, A.C., and Tontonoz, P. (2012). Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR. Nat. Rev. Mol. Cell Biol. *13*, 213–224.

Camacho-Pereira, J., Tarragó, M.G., Chini, C.C.S., Nin, V., Escande, C., Warner, G.M., Puranik, A.S., Schoon, R.A., Reid, J.M., Galina, A., et al. (2016). CD38 Dictates Age-Related NAD Decline and Mitochondrial Dysfunction through an SIRT3-Dependent Mechanism. Cell Metab. *23*, 1127–1139.

Canesin, G., Hejazi, S.M., Swanson, K.D., and Wegiel, B. (2020). Heme-Derived Metabolic Signals Dictate Immune Responses. Front. Immunol. *11*, 66.

Cao, G., Liang, Y., Broderick, C.L., Oldham, B.A., Beyer, T.P., Schmidt, R.J., Zhang, Y., Stayrook, K.R., Suen, C., Otto, K.A., et al. (2003). Antidiabetic action of a liver X receptor agonist mediated by inhibition of hepatic gluconeogenesis. J. Biol. Chem. *278*, 1131–1136.

Carey, A.L., Steinberg, G.R., Macaulay, S.L., Thomas, W.G., Holmes, A.G., Ramm, G., Prelovsek, O., Hohnen-Behrens, C., Watt, M.J., James, D.E., et al. (2006). Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. Diabetes *55*, 2688–2697.

Carrière, A., Jeanson, Y., Côté, J.A., Dromard, C., Galinier, A., Menzel, S., Barreau, C., Dupuis-Coronas, S., Arnaud, E., Girousse, A., et al. (2017). Identification of the ectoenzyme CD38 as a marker of committed preadipocytes. Int. J. Obes. *41*, 1539–1546.

Cha, J.Y., and Repa, J.J. (2007). The Liver X Receptor (LXR) and hepatic lipogenesis: The carbohydrate-response element-binding protein is a target gene of LXR. J. Biol. Chem. *282*, 743–751.

Chai, W., Liu, J., Jahn, L.A., Fowler, D.E., Barrett, E.J., and Liu, Z. (2011). Salsalate attenuates free fatty acid-induced microvascular and metabolic insulin resistance in humans. Diabetes Care *34*, 1634–1638.

Chawla, A., Repa, J.J., Evans, R.M., and Mangelsdorf, D.J. (2001). Nuclear receptors and lipid physiology: Opening the x-files. Science (80-.). *294*, 1866–1870.

Chen, J.D., and Evans, R.M. (1995). A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature *377*, 454–457.

Chen, R., Yan, J., Liu, P., and Wang, Z. (2015). Effects of thiazolidinedione therapy on inflammatory markers of type 2 diabetes: A meta-analysis of randomized controlled trials. PLoS One *10*.

Chini, E. (2009). CD38 as a Regulator of Cellular NAD: A Novel Potential Pharmacological Target for Metabolic Conditions. Curr. Pharm. Des. *15*, 57–63.

Cho, K.W., Morris, D.L., and Lumeng, C.N. (2014). Flow cytometry analyses of adipose tissue macrophages. In Methods in Enzymology, (Academic Press Inc.), pp. 297–314.

Cho, K.W., Zamarron, B.F., Muir, L.A., Singer, K., Porsche, C.E., DelProposto, J.B., Geletka, L., Meyer, K.A.,

O'Rourke, R.W., and Lumeng, C.N. (2016). Adipose Tissue Dendritic Cells Are Independent Contributors to Obesity-Induced Inflammation and Insulin Resistance. J. Immunol. *197*, 3650–3661.

Chung, K.J., Nati, M., Chavakis, T., and Chatzigeorgiou, A. (2018). Innate immune cells in the adipose tissue. Rev. Endocr. Metab. Disord. *19*, 283–292.

Cipolletta, D., Feuerer, M., Li, A., Kamei, N., Lee, J., Shoelson, S.E., Benoist, C., and Mathis, D. (2012). PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue T reg cells. Nature *486*, 549–553.

Cockayne, D.A., Muchamuel, T., Grimaldi, J.C., Muller-Steffner, H., Randall, T.D., Lund, F.E., Murray, R., Schuber, F., and Howard, M.C. (1998). Mice Deficient for the Ecto-Nicotinamide Adenine Dinucleotide Glycohydrolase CD38 Exhibit Altered Humoral Immune Responses. Blood *92*, 1324–1333.

Costanzo, A.E., Taylor, K.R., Dutt, S., Han, P.P., Fujioka, K., and Jameson, J.M. (2015). Obesity impairs γδ T cell homeostasis and antiviral function in humans. PLoS One *10*.

Cui, G., Qin, X., Wu, L., Zhang, Y., Sheng, X., Yu, Q., Sheng, H., Xi, B., Zhang, J.Z., and Zang, Y.Q. (2011). Liver X receptor (LXR) mediates negative regulation of mouse and human Th17 differentiation. J. Clin. Invest. *121*, 658–670.

Dalen, K.T., Ulven, S.M., Bamberg, K., Gustafsson, J.Å., and Nebb, H.I. (2003). Expression of the Insulin-responsive Glucose Transporter GLUT4 in Adipocytes Is Dependent on Liver X Receptor α. J. Biol. Chem. *278*, 48283–48291.

Damle, R.N., Wasil, T., Fais, F., Ghiotto, F., Valetto, A., Allen, S.L., Buchbinder, A., Budman, D., Dittmar, K., Kolitz, J., et al. (1999). Ig V Gene Mutation Status and CD38 Expression As Novel Prognostic Indicators in Chronic Lymphocytic Leukemia. Blood *94*, 1840–1847.

Dandona, P., Aljada, A., Mohanty, P., Ghanim, H., Hamouda, W., Assian, E., and Ahmad, S. (2001). Insulin Inhibits Intranuclear Nuclear Factor κB and Stimulates IκB in Mononuclear Cells in Obese Subjects: Evidence for an Anti-inflammatory Effect? J. Clin. Endocrinol. Metab. *86*, 3257–3265.

Deaglio, S., Zubiaur, M., Gregorini, A., Bottarel, F., Ausiello, C.M., Dianzani, U., Sancho, J., and Malavasi, F. (2002). Human CD38 and CD16 are functionally dependent and physically associated in natural killer cells. Blood *99*, 2490–2498.

Deckert, J., Wetzel, M.C., Bartle, L.M., Skaletskaya, A., Goldmacher, V.S., Vallée, F., Zhou-Liu, Q., Ferrari, P., Pouzieux, S., Lahoute, C., et al. (2014). SAR650984, a novel humanized CD38-targeting antibody, demonstrates potent antitumor activity in models of multiple myeloma and other CD38+ hematologic malignancies. Clin. Cancer Res. *20*, 4574–4583.

DeFuria, J., Belkina, A.C., Jagannathan-Bogdan, M., Snyder-Cappione, J., Carr, J.D., Nersesova, Y.R., Markham, D., Strissel, K.J., Watkins, A.A., Zhu, M., et al. (2013). B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. Proc. Natl. Acad. Sci. U. S. A. *110*, 5133–5138.

Deng, T., Liu, J., Deng, Y., Minze, L., Xiao, X., Wright, V., Yu, R., Li, X.C., Blaszczak, A., Bergin, S., et al. (2017). Adipocyte adaptive immunity mediates diet-induced adipose inflammation and insulin resistance by decreasing adipose Treg cells. Nat. Commun. *8*, 1–11.

Deshpande, D.A., Guedes, A.G.P., Lund, F.E., Subramanian, S., Walseth, T.F., and Kannan, M.S. (2017). CD38 in the pathogenesis of allergic airway disease: Potential therapeutic targets. Pharmacol. Ther. *172*, 116–126.

Dobrian, A.D., Ma, Q., Lindsay, J.W., Leone, K.A., Ma, K., Coben, J., Galkina, E. V, and Nadler, J.L. (2011). Dipeptidyl peptidase IV inhibitor sitagliptin reduces local inflammation in adipose tissue and in pancreatic islets of obese mice. Am. J. Physiol. - Endocrinol. Metab. *300*, 410–421.

Donath, M.Y., and Shoelson, S.E. (2011). Type 2 diabetes as an inflammatory disease. Nat. Rev. Immunol. *11*, 98–107.

Donath, M.Y., Dinarello, C.A., and Mandrup-Poulsen, T. (2019). Targeting innate immune mediators in type 1 and type 2 diabetes. Nat. Rev. Immunol. *19*, 734–746.

Van De Donk, N.W.C.J., Richardson, P.G., and Malavasi, F. (2018). CD38 antibodies in multiple myeloma: Back to the future. Blood *131*, 13–29.

Dorrell, C., Schug, J., Canaday, P.S., Russ, H.A., Tarlow, B.D., Grompe, M.T.M.M.T.M., Horton, T., Hebrok, M., Streeter, P.R., Kaestner, K.H., et al. (2016). Human islets contain four distinct subtypes of β cells. Nat. Commun. *7*, 1–9.

Drent, E., Themeli, M., Poels, R., de Jong-Korlaar, R., Yuan, H., de Bruijn, J., Martens, A.C.M., Zweegman, S., van de Donk, N.W.C.J., Groen, R.W.J., et al. (2017). A Rational Strategy for Reducing On-Target Off-Tumor Effects of CD38-Chimeric Antigen Receptors by Affinity Optimization. Mol. Ther. *25*, 1946–1958.

Drew, J.E., Farquharson, A.J., Horgan, G.W., and Williams, L.M. (2016). Tissue-specific regulation of sirtuin and nicotinamide adenine dinucleotide biosynthetic pathways identified in C57Bl/6 mice in response to high-fat feeding. J. Nutr. Biochem. *37*, 20–29.

Dror, E., Dalmas, E., Meier, D.T., Wueest, S., Thévenet, J., Thienel, C., Timper, K., Nordmann, T.M., Traub, S., Schulze, F., et al. (2017). Postprandial macrophage-derived IL-1β stimulates insulin, and both synergistically promote glucose disposal and inflammation. Nat. Immunol. *18*, 283–292.

Du, Y., Dai, Q., Zhang, H., Li, Q., Song, K., Fu, Y., Min, W., Liu, Z., and Li, R. (2019). CD38 deficiency downregulates the onset and pathogenesis of collagen-induced arthritis through the NF-κB pathway. J. Immunol. Res. *2019*.

Ellingsgaard, H., Ehses, J.A., Hammar, E.B., Van Lommel, L., Quintens, R., Martens, G., Kerr-Conte, J., Pattou, F., Berney, T., Pipeleers, D., et al. (2008). Interleukin-6 regulates pancreatic α-cell mass expansion. Proc. Natl. Acad. Sci. U. S. A. *105*, 13163–13168.

Evans, R.M., and Mangelsdorf, D.J. (2014). Nuclear receptors, RXR, and the big bang. Cell *157*, 255–266.

Fabbrini, E., Cella, M., McCartney, S.A., Fuchs, A., Abumrad, N.A., Pietka, T.A., Chen, Z., Finck, B.N., Han, D.H., Magkos, F., et al. (2013). Association between specific adipose tissue CD4+ T-cell populations and insulin resistance in obese individuals. Gastroenterology *145*, 366-374.e3.

Fang, F., Lu, Y., Ma, D.L., Du, T.T., Shao, S.Y., and Yu, X.F. (2013). A meta-analysis of salicylates for type 2 diabetes mellitus. J. Huazhong Univ. Sci. Technol. - Med. Sci. *33*, 1–14.

Farack, L., Golan, M., Egozi, A., Dezorella, N., Bahar Halpern, K., Ben-Moshe, S., Garzilli, I., Tóth, B., Roitman, L., Krizhanovsky, V., et al. (2019). Transcriptional Heterogeneity of Beta Cells in the Intact Pancreas. Dev. Cell *48*, 115-125.e4.

Faulds, M.H., Zhao, C., and Dahlman-Wright, K. (2010). Molecular biology and functional genomics of liver X receptors (LXR) in relationship to metabolic diseases. Curr. Opin. Pharmacol. *10*, 692–697.

Fedele, G., Frasca, L., Palazzo, R., Ferrero, E., Malavasi, F., and Ausiello, C.M. (2004). CD38 is expressed on human mature monocyte-derived dendritic cells and is functionally involved in CD83 expressioin and IL-12 induction. Eur. J. Immunol. *34*, 1342–1350.

Feng, H., Zhang, Y.B., Gui, J.F., Lemon, S.M., and Yamane, D. (2021). Interferon regulatory factor 1 (IRF1) and anti-pathogen innate immune responses. PLoS Pathog. *17*.

Feng, X., Zhang, L., Acharya, C., An, G., Wen, K., Qiu, L., Munshi, N.C., Tai, Y.T., and Anderson, K.C. (2017). Targeting CD38 suppresses induction and function of T regulatory cells to mitigate immunosuppression in multiple myeloma. Clin. Cancer Res. *23*, 4290–4300.

Fernø, J., Strand, K., Mellgren, G., Stiglund, N., and Björkström, N.K. (2020). Natural Killer Cells as Sensors of Adipose Tissue Stress. Trends Endocrinol. Metab. *31*, 3–12.

Ferrero, E., and Malavasi, F. (1997). Human CD38, a leukocyte receptor and ectoenzyme, is a member of a novel eukaryotic gene family of nicotinamide adenine dinucleotide+-converting enzymes: extensive structural homology with the genes for murine bone marrow stromal cell antigen 1 and aplysi…. J. Immunol. *159*.

Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., Lee, J., Goldfine, A.B., Benoist, C.,

Shoelson, S., et al. (2009). Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat. Med. *15*, 930–939.

Fève, B., and Bastard, J.P. (2009). The role of interleukins in insulin resistance and type 2 diabetes mellitus. Nat. Rev. Endocrinol. *5*, 305–311.

Fiévet, C., and Staels, B. (2009). Liver X receptor modulators: Effects on lipid metabolism and potential use in the treatment of atherosclerosis. Biochem. Pharmacol. *77*, 1316–1327.

Fragale, A., Gabriele, L., Stellacci, E., Borghi, P., Perrotti, E., Ilari, R., Lanciotti, A., Remoli, A.L., Venditti, M., Belardelli, F., et al. (2008). IFN Regulatory Factor-1 Negatively Regulates CD4 + CD25 + Regulatory T Cell Differentiation by Repressing Foxp3 Expression. J. Immunol. *181*, 1673–1682.

Francisco, V., Pino, J., Campos-Cabaleiro, V., Ruiz-Fernández, C., Mera, A., Gonzalez-Gay, M.A., Gómez, R., and Gualillo, O. (2018). Obesity, fat mass and immune system: Role for leptin. Front. Physiol. *9*.

Frayling, T.M., Timpson, N.J., Weedon, M.N., Zeggini, E., Freathy, R.M., Lindgren, C.M., Perry, J.R.B., Elliott, K.S., Lango, H., Rayner, N.W., et al. (2007). A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *316*, 889–894.

Funaro, A., Morra, M., Calosso, L., Zini, M.G., Ausiello, C.M., and Malavasi, F. (1997). Role of the human CD38 molecule in B cell activation and proliferation. Tissue Antigens *49*, 7–15.

Galione, A. (2019). NAADP receptors. Cold Spring Harb. Perspect. Biol. *11*, a035071.

Gao, M., and Liu, D. (2013). The Liver X Receptor Agonist T0901317 Protects Mice from High Fat Diet-Induced Obesity and Insulin Resistance. AAPS J. *15*, 258–266.

Garvey, W.T., Van Gaal, L., Leiter, L.A., Vijapurkar, U., List, J., Cuddihy, R., Ren, J., and Davies, M.J. (2018). Effects of canagliflozin versus glimepiride on adipokines and inflammatory biomarkers in type 2 diabetes. Metabolism. *85*, 32–37.

George, A.M., Jacob, A.G., and Fogelfeld, L. (2015). Lean diabetes mellitus: An emerging entity in the era of obesity. World J. Diabetes *6*, 613.

Ghadge, A.A., and Khaire, A.A. (2019). Leptin as a predictive marker for metabolic syndrome. Cytokine *121*.

Glaría, E., and Valledor, A.F. (2020). Roles of CD38 in the Immune Response to Infection. Cells *9*.

Glaría, E., Letelier, N.A., and Valledor, A.F. (2020). Integrating the roles of liver X receptors in inflammation and infection: mechanisms and outcomes. Curr. Opin. Pharmacol. *53*, 55–65.

Gonzalez-Franquesa, A., and Patti, M.E. (2017). Insulin resistance and mitochondrial dysfunction. In Advances in Experimental Medicine and Biology, (Springer, Cham), pp. 465–520.

Greenberg, A.S., Egan, J.J., Wek, S.A., Garty, N.B., Blanchette-Mackie, E.J., and Londos, C. (1991). Perilipin, a major hormonally regulated adipocyte-specific phosphoprotein associated with the periphery of lipid storage droplets. J. Biol. Chem. *266*, 11341–11346.

Grefhorst, A., Van Dijk, T.H., Hammer, A., Van Der Sluijs, F.H., Havinga, R., Havekes, L.M., Romijn, J.A., Groot, P.H., Reijngoud, D.J., and Kuipers, F. (2005). Differential effects of pharmacological liver X receptor activation on hepatic and peripheral insulin sensitivity in lean and ob/ob mice. Am. J. Physiol. - Endocrinol. Metab. *289*, E829–E838.

Gregor, M.F., and Hotamisligil, G.S. (2011). Inflammatory mechanisms in obesity. Annu. Rev. Immunol. *29*, 415–445.

Grozio, A., Sociali, G., Sturla, L., Caffa, I., Soncini, D., Salis, A., Raffaelli, N., De Flora, A., Nencioni, A., and Bruzzone, S. (2013). CD73 Protein as a Source of Extracellular Precursors for Sustained NAD+ Biosynthesis in FK866-treated Tumor Cells. J. Biol. Chem. *288*, 25938–25949.

Grundy, S.M. (2012). Pre-Diabetes, Metabolic Syndrome, and Cardiovascular Risk. J. Am. Coll. Cardiol. *59*, 635 LP – 643.

Gual, P., Le Marchand-Brustel, Y., and Tanti, J.F. (2005). Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. In Biochimie, pp. 99–109.

Guerreiro, S., Privat, A.L., Bressac, L., and Toulorge, D. (2020). CD38 in Neurodegeneration and Neuroinflammation. Cells *9*, 471.

Guillemot-Legris, O., Mutemberezi, V., and Muccioli, G.G. (2016). Oxysterols in Metabolic Syndrome: From Bystander Molecules to Bioactive Lipids. Trends Mol. Med. *22*, 594–614.

Gupta, D., Jetton, T.L., LaRock, K., Monga, N., Satish, B., Lausier, J., Peshavaria, M., and Leahy, J.L. (2017). Temporal characterization of β cell-adaptive and -maladaptive mechanisms during chronic high-fat feeding in C57BL/6NTac mice (American Society for Biochemistry and Molecular Biology Inc.).

Gutierrez, G.D., Gromada, J., and Sussel, L. (2017). Heterogeneity of the pancreatic beta cell. Front. Genet. *8*, 22.

Halban, P.A., Polonsky, K.S., Bowden, D.W., Hawkins, M.A., Ling, C., Mather, K.J., Powers, A.C., Rhodes, C.J., Sussel, L., and Weir, G.C. (2014). β-Cell failure in type 2 diabetes: Postulated mechanisms and prospects for prevention and treatment. Diabetes Care *37*, 1751–1758.

Halleux, C.M., Takahashi, M., Delporte, M.L., Detry, R., Funahashi, T., Matsuzawa, Y., and Brichard, S.M. (2001). Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. Biochem. Biophys. Res. Commun. *288*, 1102–1107.

Han, M.S., Jung, D.Y., Morel, C., Lakhani, S.A., Kim, J.K., Flavell, R.A., and Davis, R.J. (2013). JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation. Science (80-.). *339*, 218–222.

Harman-Boehm, I., Blüher, M., Redel, H., Sion-Vardy, N., Ovadia, S., Avinoach, E., Shai, I., Klöting, N., Stumvoll, M., Bashan, N., et al. (2007). Macrophage infiltration into omental versus subcutaneous fat across different populations: Effect of regional adiposity and the comorbidities of obesity. J. Clin. Endocrinol. Metab. *92*, 2240–2247.

Hasib, A., Ng, M.T., Gault, V.A., Khan, D., Parthsarathy, V., Flatt, P.R., and Irwin, N. (2017). An enzymatically stable GIP/xenin hybrid peptide restores GIP sensitivity, enhances beta cell function and improves glucose homeostasis in high-fat-fed mice. Diabetologia *60*, 541–552.

Heimberg, H., De Vos, A., Vandercammen, A., Van Schaftingen, E., Pipeleers, D., and Schuit, F. (1993). Heterogeneity in glucose sensitivity among pancreatic β-cells is correlated to differences in glucose phosphorylation rather than glucose transport. EMBO J. *12*, 2873–2879.

Heinonen, S., Buzkova, J., Muniandy, M., Kaksonen, R., Ollikainen, M., Ismail, K., Hakkarainen, A., Lundbom, J., Lundbom, N., Vuolteenaho, K., et al. (2015). Impaired mitochondrial biogenesis in adipose tissue in acquired obesity. Diabetes *64*, 3135–3145.

Herold, M., Breuer, J., Hucke, S., Knolle, P., Schwab, N., Wiendl, H., and Klotz, L. (2017). Liver X receptor activation promotes differentiation of regulatory T cells. PLoS One *12*, 1–13.

Herzog, B., Hallberg, M., Seth, A., Woods, A., White, R., and Parker, M.G. (2007). The nuclear receptor cofactor, receptor-interacting protein 140, is required for the regulation of hepatic lipid and glucose metabolism by liver X receptor. Mol. Endocrinol. *21*, 2687–2697.

Hirai, S., Ohyane, C., Kim, Y. Il, Lin, S., Goto, T., Takahashi, N., Kim, C.S., Kang, J., Yu, R., and Kawada, T. (2014). Involvement of mast cells in adipose tissue fibrosis. Am. J. Physiol. - Endocrinol. Metab. *306*, E247–E255.

Hogan, A.E., Gaoatswe, G., Lynch, L., Corrigan, M.A., Woods, C., O'Connell, J., and O'Shea, D. (2014). Glucagon-like peptide 1 analogue therapy directly modulates innate immune-mediated inflammation in individuals with type 2 diabetes mellitus. Diabetologia *57*, 781–784.

Horenstein, A.L., Stockinger, H., Imhof, B.A., and Malavasi, F. (1998). CD38 binding to human myeloid cells is mediated by mouse and human CD31. Biochem. J. *330*, 1129–1135.

Hörlein, A.J., Näär, A.M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Söderström, M., Glass, C.K., et al. (1995). Ligand-independent repression by the thyroid hormone receptor mediated by a

nuclear receptor co-repressor. Nature *377*, 397–404.

Hotamisligil, G.S. (2017). Inflammation, metaflammation and immunometabolic disorders. Nature *542*, 177–185.

Hotamisligil, G.S., Shargill, N.S., and Spiegelman, B.M. (1993). Adipose expression of tumor necrosis factor-α: Direct role in obesity-linked insulin resistance. Science (80-.). *259*, 87–91.

Hotamisligil, G.S., Arner, P., Caro, J.F., Atkinson, R.L., and Spiegelman, B.M. (1995). Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. J. Clin. Invest. *95*, 2409–2415.

Huang, W., and Glass, C.K. (2010). Nuclear receptors and inflammation control: Molecular mechanisms and pathophysiological relevance. Arterioscler. Thromb. Vasc. Biol. *30*, 1542–1549.

Huang, C., Yuan, L., and Cao, S. (2015a). Endogenous GLP-1 as a key self-defense molecule against lipotoxicity in pancreatic islets. Int. J. Mol. Med. *36*, 173–185.

Huang, Y., Fu, X., Lyu, X., Xu, Z., He, Z., Zhang, Y., Zeng, Y., He, F., and Huang, G. (2015b). Activation of LXR attenuates collagen-induced arthritis via suppressing BLyS production. Clin. Immunol. *161*, 339–347.

Huber, J., Kiefer, F.W., Zeyda, M., Ludvik, B., Silberhumer, G.R., Prager, G., Zlabinger, G.J., and Stulnig, T.M. (2008). CC chemokine and CC chemokine receptor profiles in visceral and subcutaneous adipose tissue are altered in human obesity. J. Clin. Endocrinol. Metab. *93*, 3215–3221.

Hudish, L.I., Reusch, J.E.B., and Sussel, L. (2019). β Cell dysfunction during progression of metabolic syndrome to type 2 diabetes. J. Clin. Invest. *129*, 4001–4008.

Huh, J.Y., Park, J., Kim, J.I., Park, Y.J., Lee, Y.K., and Kim, J.B. (2017). Deletion of CD1d in adipocytes aggravates adipose tissue inflammation and insulin resistance in obesity. Diabetes *66*, 835–847.

Ikehata, F., Satoh, J., Nata, K., Tohgo, A., Nakazawa, T., Kato, I., Kobayashi, S., Akiyama, T., Takasawa, S., Toyota, T., et al. (1998). Autoantibodies against CD38 (ADP-ribosyl cyclase/cyclic adp-ribose hydrolase) that impair glucose-induced insulin secretion in noninsulin- dependent diabetes patients. J. Clin. Invest. *102*, 395–401.

Ito, A., Hong, C., Rong, X., Zhu, X., Tarling, E.J., Hedde, P.N., Gratton, E., Parks, J., and Tontonoz, P. (2015). LXRs link metabolism to inflammation through Abca1-dependent regulation of membrane composition and TLR signaling. Elife *4*.

Jaganathan, R., Ravindran, R., and Dhanasekaran, S. (2018). Emerging Role of Adipocytokines in Type 2 Diabetes as Mediators of Insulin Resistance and Cardiovascular Disease. Can. J. Diabetes *42*, 446-456.e1.

Jais, A., Einwallner, E., Sharif, O., Gossens, K., Lu, T.T.H., Soyal, S.M., Medgyesi, D., Neureiter, D., Paier-Pourani, J., Dalgaard, K., et al. (2014). Heme oxygenase-1 drives metaflammation and insulin resistance in mouse and man. Cell *158*, 25–40.

Jeon, J.Y., Nam, J.Y., Kim, H.A., Park, Y.B., Bae, S.C., and Suh, C.H. (2014). Liver X receptors alpha gene (NR1H3) promoter polymorphisms are associated with systemic lupus erythematosus in Koreans. Arthritis Res. Ther. *16*, R112.

Jia, G., DeMarco, V.G., and Sowers, J.R. (2016). Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. Nat. Rev. Endocrinol. *12*, 144–153.

Jiao, P., Ma, J., Feng, B., Zhang, H., Alan Diehl, J., Eugene Chin, Y., Yan, W., and Xu, H. (2011). FFA-induced adipocyte inflammation and insulin resistance: Involvement of ER stress and IKKβ pathways. Obesity *19*, 483–491.

Johnson, J.D., Ford, E.L., Bernal-Mizrachi, E., Kusser, K.L., Luciani, D.S., Han, Z., Tran, H., Randall, T.D., Lund, F.E., and Polonsky, K.S. (2006). Suppressed insulin signaling and increased apoptosis in Cd38-null islets. Diabetes *55*, 2737–2746.

Johnston, N.R., Mitchell, R.K., Haythorne, E., Pessoa, M.P., Semplici, F., Ferrer, J., Piemonti, L., Marchetti, P., Bugliani, M., Bosco, D., et al. (2016). Beta Cell Hubs Dictate Pancreatic Islet Responses to Glucose. Cell Metab.

24, 389–401.

Jonas, M.I., Kurylowicz, A., Bartoszewicz, Z., Lisik, W., Jonas, M., Wierzbicki, Z., Chmura, A., Pruszczyk, P., and Puzianowska-Kuznicka, M. (2015). Interleukins 6 and 15 levels are higher in subcutaneous adipose tissue, but obesity is associated with their increased content in visceral fat depots. Int. J. Mol. Sci. *16*, 25817–25830.

Jonas, M.I., Kurylowicz, A., Bartoszewicz, Z., Lisik, W., Jonas, M., Domienik-Karlowicz, J., and Puzianowska-Kuznicka, M. (2017). Adiponectin/resistin interplay in serum and in adipose tissue of obese and normal-weight individuals. Diabetol. Metab. Syndr. *9*, 457–464.

Juge-Aubry, C.E., Somm, E., Pernin, A., Alizadeh, N., Giusti, V., Dayer, J.M., and Meier, C.A. (2005). Adipose tissue is a regulated source of interleukin-10. Cytokine *29*, 270–274.

Jurgens, C.A., Toukatly, M.N., Fligner, C.L., Udayasankar, J., Subramanian, S.L., Zraika, S., Aston-Mourney, K., Carr, D.B., Westermark, P., Westermark, G.T., et al. (2011). β-Cell loss and β-cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. Am. J. Pathol. *178*, 2632–2640.

Kadowaki, T., Yamauchi, T., Kubota, N., Hara, K., Ueki, K., and Tobe, K. (2006). Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J. Clin. Invest. *116*, 1784–1792.

Kahn, S.E., Hull, R.L., and Utzschneider, K.M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature *444*, 840–846.

Kameswaran, V., Bramswig, N.C., McKenna, L.B., Penn, M., Schug, J., Hand, N.J., Chen, Y., Choi, I., Vourekas, A., Won, K.J., et al. (2014). Epigenetic regulation of the DLK1-MEG3 MicroRNA cluster in human type 2 diabetic islets. Cell Metab. *19*, 135–145.

Kato, I., Takasawa, S., Akabane, A., Tanaka, O., Abe, H., Takamura, T., Suzuki, Y., Nata, K., Yonekura, H., Yoshimoto, T., et al. (1995). Regulatory role of CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase) in insulin secretion by glucose in pancreatic β cells: Enhanced insulin secretion in CD38-expressing transgenic mice. J. Biol. Chem. *270*, 30045–30050.

Kato, I., Yamamoto, Y., Fujimura, M., Noguchi, N., Takasawa, S., and Okamoto, H. (1999). CD38 disruption impairs glucose-induced increases in cyclic ADP-ribose, [Ca2+](i), and insulin secretion. J. Biol. Chem. *274*, 1869–1872.

Katsiki, N., Mikhailidis, D.P., and Banach, M. (2018). Leptin, cardiovascular diseases and type 2 diabetes mellitus review-article. Acta Pharmacol. Sin. *39*, 1176–1188.

Kim, E.K., Lee, S.H., Jhun, J.Y., Byun, J.K., Jeong, J.H., Lee, S.-Y., Kim, J.K., Choi, J.Y., and Cho, M.-L. (2016). Metformin Prevents Fatty Liver and Improves Balance of White/Brown Adipose in an Obesity Mouse Model by Inducing FGF21. Mediators Inflamm. *2016*, 5813030.

Kohlgruber, A.C., Gal-Oz, S.T., Lamarche, N.M., Shimazaki, M., Duquette, D., Nguyen, H.N., Mina, A.I., Paras, T., Tavakkoli, A., Von Andrian, U., et al. (2018). γδ T cells producing interleukin-17A regulate adipose regulatory T cell homeostasis and thermogenesis /631/250/256 /631/250/2504 article. Nat. Immunol. *19*, 464–474.

Kolodin, D., Van Panhuys, N., Li, C., Magnuson, A.M., Cipolletta, D., Miller, C.M., Wagers, A., Germain, R.N., Benoist, C., and Mathis, D. (2015). Antigen- and cytokine-driven accumulation of regulatory t cells in visceral adipose tissue of lean mice. Cell Metab. *21*, 543–557.

Kothari, V., Galdo, J.A., and Mathews, S.T. (2016). Hypoglycemic agents and potential anti-inflammatory activity. J. Inflamm. Res. *9*, 27–38.

Krejcik, J., Casneuf, T., Nijhof, I.S., Verbist, B., Bald, J., Plesner, T., Syed, K., Liu, K., Van De Donk, N.W.C.J., Weiss, B.M., et al. (2016). Daratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. Blood *128*, 384–394.

Kumari, R., Kumar, S., and Kant, R. (2019). An update on metabolic syndrome: Metabolic risk markers and adipokines in the development of metabolic syndrome. Diabetes Metab. Syndr. Clin. Res. Rev. *13*, 2409–2417.

Kuryłowicz, A., and Kózniewski, K. (2020). Anti-inflammatory strategies targeting metaflammation in type 2 diabetes. Molecules *25*.

Lackey, D.E., and Olefsky, J.M. (2016). Regulation of metabolism by the innate immune system. Nat. Rev. Endocrinol. *12*, 15–20.

Laffitte, B.A., Joseph, S.B., Walczak, R., Pei, L., Wilpitz, D.C., Collins, J.L., and Tontonoz, P. (2001). Autoregulation of the Human Liver X Receptor α Promoter. Mol. Cell. Biol. *21*, 7558–7568.

Laffitte, B.A., Chao, L.C., Li, J., Walczak, R., Hummasti, S., Joseph, S.B., Castrillo, A., Wilpitz, D.C., Mangelsdorf, D.J., Collins, J.L., et al. (2003). Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. Proc. Natl. Acad. Sci. U. S. A. *100*, 5419–5424.

Lagathu, C., Yvan-Charvet, L., Bastard, J.P., Maachi, M., Quignard-Boulangé, A., Capeau, J., and Caron, M. (2006). Long-term treatment with interleukin-1β induces insulin resistance in murine and human adipocytes. Diabetologia *49*, 2162–2173.

Lair, B., Laurens, C., Van Den Bosch, B., and Moro, C. (2020). Novel insights and mechanisms of lipotoxicity-driven insulin resistance. Int. J. Mol. Sci. *21*, 1–27.

Lamkanfi, M., Mueller, J.L., Vitari, A.C., Misaghi, S., Fedorova, A., Deshayes, K., Lee, W.P., Hoffman, H.M., and Dixit, V.M. (2009). Glyburide inhibits the Cryopyrin/Nalp3 inflammasome. J. Cell Biol. *187*, 61–70.

Lande, R., Urbani, F., Di Carlo, B., Sconocchia, G., Deaglio, S., Funaro, A., Malavasi, F., and Ausiello, C.M. (2002). CD38 ligation plays a direct role in the induction of IL-1β, IL-6, and IL-10 secretion in resting human monocytes. Cell. Immunol. *220*, 30–38.

Larsen, C.M., Faulenbach, M., Vaag, A., Vølund, A., Ehses, J.A., Seifert, B., Mandrup-Poulsen, T., and Donath, M.Y. (2007). Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N. Engl. J. Med. *356*, 1517–1526.

Lee, Y.S., and Jun, H.S. (2016). Anti-Inflammatory Effects of GLP-1-Based Therapies beyond Glucose Control. Mediators Inflamm. *2016*.

Lee, J.H., Park, S.M., Kim, O.S., Lee, C.S., Woo, J.H., Park, S.J., Joe, E. hye, and Jou, I. (2009). Differential SUMOylation of LXRα and LXRβ Mediates Transrepression of STAT1 Inflammatory Signaling in IFN-γ-Stimulated Brain Astrocytes. Mol. Cell *35*, 806–817.

Lee, S.H., Jhun, J., Byun, J.-K., Kim, E.-K., Jung, K., Lee, J.E., Choi, J.Y., Park, S.-H., and Cho, M.-L. (2017). IL-17 axis accelerates the inflammatory progression of obese in mice via TBK1 and IKBKE pathway. Immunol. Lett. *184*, 67–75.

Lee, Y.S., Wollam, J., and Olefsky, J.M. (2018). An Integrated View of Immunometabolism. Cell *172*, 22–40.

Levy, D.E., and Darnell, J.E. (2002). STATs: transcriptional control and biological impact. Nat. Rev. Mol. Cell Biol. *3*, 651–662.

Li, C., DiSpirito, J.R., Zemmour, D., Spallanzani, R.G., Kuswanto, W., Benoist, C., and Mathis, D. (2018). TCR Transgenic Mice Reveal Stepwise, Multi-site Acquisition of the Distinctive Fat-Treg Phenotype. Cell *174*, 285-299.e12.

Li, Q., Wu, C., Liu, Z., Zhang, H., Du, Y., Liu, Y., Song, K., Shi, Q., and Li, R. (2019). Increased TLR4 Expression Aggravates Sepsis by Promoting IFN- γ Expression in CD38 -/- Mice. J. Immunol. Res. *2019*.

Liu, J., Divoux, A., Sun, J., Zhang, J., Clément, K., Glickman, J.N., Sukhova, G.K., Wolters, P.J., Du, J., Gorgun, C.Z., et al. (2009). Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. Nat. Med. *15*, 940–945.

Lo, J., Bernstein, L.E., Canavan, B., Torriani, M., Jackson, M.B., Ahima, R.S., and Grinspoon, S.K. (2007). Effects of TNF-α neutralization on adipocytokines and skeletal muscle adiposity in the metabolic syndrome. Am. J. Physiol. - Endocrinol. Metab. *293*, 102–109.

Lund, F.E., Yu, N., Kim, K.M., Reth, M., and Howard, M.C. (1996). Signaling through CD38 augments B cell antigen receptor (BCR) responses and is dependent on BCR expression. J. Immunol. *157*.

Lynch, L., Nowak, M., Varghese, B., Clark, J., Hogan, A.E., Toxavidis, V., Balk, S.P., O'Shea, D., O'Farrelly, C., and Exley, M.A. (2012). Adipose Tissue Invariant NKT Cells Protect against Diet-Induced Obesity and Metabolic Disorder through Regulatory Cytokine Production. Immunity *37*, 574–587.

Malavasi, F., Deaglio, S., Funaro, A., Ferrero, E., Horenstein, A.L., Ortolan, E., Vaisitti, T., and Aydin, S. (2008). Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. Physiol. Rev. *88*, 841–886.

Malavasi, F., Deaglio, S., Damle, R., Cutrona, G., Ferrarini, M., and Chiorazzi, N. (2011). CD38 and chronic lymphocytic leukemia: A decade later. Blood *118*, 3470–3478.

Mallone, R., Funaro, A., Zubiar, M., Baj, G., Ausiello, C.M., Tacchetti, C., Sancho, J., Grossi, C., and Malavasi, F. (2001). Signaling through CD38 induces NK cell activation. Int. Immunol. *13*, 397–409.

Marinho, T. de S., Ornellas, F., Aguila, M.B., and Mandarim-de-Lacerda, C.A. (2020). Browning of the subcutaneous adipocytes in diet-induced obese mouse submitted to intermittent fasting. Mol. Cell. Endocrinol. *513*.

Matalonga, J., Glaria, E., Bresque, M., Escande, C., Carbó, J.M., Kiefer, K., Vicente, R., León, T.E., Beceiro, S., Pascual-García, M., et al. (2017). The Nuclear Receptor LXR Limits Bacterial Infection of Host Macrophages through a Mechanism that Impacts Cellular NAD Metabolism. Cell Rep. *18*, 1241–1255.

Matrai, Z. (2005). CD38 as a prognostic marker in CLL. Hematology *10*, 39–46.

Mclaughlin, T., Ackerman, S.E., Shen, L., and Engleman, E. (2017). Role of innate and adaptive immunity in obesity-associated metabolic disease. J. Clin. Invest. *127*, 5–13.

Mehta, P., Nuotio-Antar, A.M., and Smith, C.W. (2015). γδ T cells promote inflammation and insulin resistance during high fat diet-induced obesity in mice. J. Leukoc. Biol. *97*, 121–134.

Menn, G. Le, Sibille, B., Murdaca, J., Rousseau, A.S., Squillace, R., Vergoni, B., Cormont, M., Niot, I., Grimaldi, P.A., Mothe-Satney, I., et al. (2019). Decrease in αβ/γδ T-cell ratio is accompanied by a reduction in high-fat diet-induced weight gain, insulin resistance, and inflammation. FASEB J. *33*, 2553–2562.

van der Meulen, T., Mawla, A.M., DiGruccio, M.R., Adams, M.W., Nies, V., Dólleman, S., Liu, S., Ackermann, A.M., Cáceres, E., Hunter, A.E., et al. (2017). Virgin Beta Cells Persist throughout Life at a Neogenic Niche within Pancreatic Islets. Cell Metab. *25*, 911-926.e6.

Meyerovich, K., Fukaya, M., Terra, L.F., Ortis, F., Eizirik, D.L., and Cardozo, A.K. (2016). The non-canonical NF-κB pathway is induced by cytokines in pancreatic beta cells and contributes to cell death and proinflammatory responses in vitro. Diabetologia *59*, 512–521.

Miyazawa, M., Subbaramaiah, K., Bhardwaj, P., Zhou, X.K., Wang, H., Falcone, D.J., Giri, D.D., and Dannenberg, A.J. (2018). Pioglitazone inhibits periprostatic white adipose tissue inflammation in obese mice. Cancer Prev. Res. *11*, 215–226.

Morandi, F., Horenstein, A.L., Costa, F., Giuliani, N., Pistoia, V., and Malavasi, F. (2018). CD38: A target for immunotherapeutic approaches in multiple myeloma. Front. Immunol. *9*.

Moreno, L., Perez, C., Zabaleta, A., Manrique, I., Alignani, D., Ajona, D., Blanco, L., Lasa, M., Maiso, P., Rodriguez, I., et al. (2019). The mechanism of action of the anti-CD38 monoclonal antibody isatuximab in multiple myeloma. Clin. Cancer Res. *25*, 3176–3187.

Morris, D.L., Cho, K.W., DelProposto, J.L., Oatmen, K.E., Geletka, L.M., Martinez-Santibanez, G., Singer, K., and Lumeng, C.N. (2013). Adipose tissue macrophages function as antigen-presenting cells and regulate adipose tissue cd4+ t cells in mice. Diabetes *62*, 2762–2772.

Muñoz, P., Navarro, M.D.C., Pavón, E.J., Salmerón, J., Malavasi, F., Sancho, J., and Zubiaur, M. (2003). CD38 Signaling in T Cells Is Initiated within a Subset of Membrane Rafts Containing Lck and the CD3-ζ Subunit of the T Cell Antigen Receptor. J. Biol. Chem. *278*, 50791–50802.

Nauck, M.A., Heimesaat, M.M., Orskov, C., Holst, J.J., Ebert, R., and Creutzfeldt, W. (1993). Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in

patients with type- 2 diabetes mellitus. J. Clin. Invest. *91*, 301–307.

Nieto-Vazquez, I., Fernandez-Veledo, S., De Alvaro, C., and Lorenzo, M. (2008). Dual role of interleukin-6 in regulating insulin sensitivity in murine skeletal muscle. Diabetes *57*, 3211–3221.

Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., Otsu, M., Hara, K., Ueki, K., Sugiura, S., et al. (2009). CD8 + effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat. Publ. Gr.

Nishimura, S., Manabe, I., Takaki, S., Nagasaki, M., Otsu, M., Yamashita, H., Sugita, J., Yoshimura, K., Eto, K., Komuro, I., et al. (2013). Adipose natural regulatory B cells negatively control adipose tissue inflammation. Cell Metab. *18*, 759–766.

Odegaard, J.I., Ricardo-Gonzalez, R.R., Goforth, M.H., Morel, C.R., Subramanian, V., Mukundan, L., Eagle, A.R., Vats, D., Brombacher, F., Ferrante, A.W., et al. (2007). Macrophage-specific PPARγ controls alternative activation and improves insulin resistance. Nature *447*, 1116–1120.

Okamoto, Y., Higashiyama, H., Rong, J.X., McVey, M.J., Kinoshita, M., Asano, S., and Hansen, M.K. (2007). Comparison of mitochondrial and macrophage content between subcutaneous and visceral fat in db/db mice. Exp. Mol. Pathol. *83*, 73–83.

Olaogun, I., Farag, M., and Hamid, P. (2020). The Pathophysiology of Type 2 Diabetes Mellitus in Non-obese Individuals: An Overview of the Current Understanding. Cureus *53*, 55–65.

Olefsky, J.M., and Glass, C.K. (2009). Macrophages, inflammation, and insulin resistance. Annu. Rev. Physiol. *72*, 219–246.

Ormazabal, V., Nair, S., Elfeky, O., Aguayo, C., Salomon, C., and Zuñiga, F.A. (2018). Association between insulin resistance and the development of cardiovascular disease. Cardiovasc. Diabetol. *17*, 122.

Park, H.K., Kwak, M.K., Kim, H.J., and Ahima, R.S. (2017). Linking resistin, inflammation, and cardiometabolic diseases. Korean J. Intern. Med. *32*, 239–247.

Park, M.C., Kwon, Y.J., Chung, S.J., Park, Y.B., and Lee, S.K. (2010). Liver X receptor agonist prevents the evolution of collagen-induced arthritis in mice. Rheumatology (Oxford). *49*, 882–890.

Partida-Sánchez, S., Goodrich, S., Kusser, K., Oppenheimer, N., Randall, T.D., and Lund, F.E. (2004). Regulation of dendritic cell trafficking by the ADP-ribosyl cyclase CD38: Impact on the development of humoral immunity. Immunity *20*, 279–291.

Pascual-García, M., Rué, L., León, T., Julve, J., Carbó, J.M., Matalonga, J., Auer, H., Celada, A., Escolà-Gil, J.C., Steffensen, K.R., et al. (2013). Reciprocal Negative Cross-Talk between Liver X Receptors (LXRs) and STAT1: Effects on IFN-γ–Induced Inflammatory Responses and LXR-Dependent Gene Expression. J. Immunol. *190*, 6520–6532.

Patsouris, D., Li, P.P., Thapar, D., Chapman, J., Olefsky, J.M., and Neels, J.G. (2008). Ablation of CD11c-Positive Cells Normalizes Insulin Sensitivity in Obese Insulin Resistant Animals. Cell Metab. *8*, 301–309.

Pepper, C., Lin, T.T., Pratt, G., Hewamana, S., Brennan, P., Hiller, L., Hills, R., Ward, R., Starczynski, J., Austen, B., et al. (2008). Mcl-1 expression has in vitro and in vivo significance in chronic lymphocytic leukemia and is associated with other poor prognostic markers. Blood *112*, 3807–3817.

Piedra-Quintero, Z.L., Wilson, Z., Nava, P., and Guerau-de-Arellano, M. (2020). CD38: An Immunomodulatory Molecule in Inflammation and Autoimmunity. Front. Immunol. *11*.

Poitout, V., Amyot, J., Semache, M., Zarrouki, B., Hagman, D., and Fontés, G. (2010). Glucolipotoxicity of the pancreatic beta cell. Biochim. Biophys. Acta - Mol. Cell Biol. Lipids *1801*, 289–298.

Postigo, J., Iglesias, M., Cerezo-Wallis, D., Rosal-Vela, A., García-Rodríguez, S., Zubiaur, M., Sancho, J., Merino, R., and Merino, J. (2012). Mice deficient in CD38 develop an attenuated form of collagen type II-induced arthritis. PLoS One *7*.

Poy, M.N., Hausser, J., Trajkovski, M., Braun, M., Collins, S., Rorsman, P., Zavolan, M., and Stoffel, M. (2009).

miR-375 maintains normal pancreatic α- and β-cell mass. Proc. Natl. Acad. Sci. U. S. A. *106*, 5813–5818.

Prasad, M., Chen, E.W., Toh, S.A., and Gascoigne, N.R.J. (2020). Autoimmune responses and inflammation in type 2 diabetes (John Wiley and Sons Inc.).

Prieur, X., Roszer, T., and Ricote, M. (2010). Lipotoxicity in macrophages: evidence from diseases associated with the metabolic syndrome. Biochim. Biophys. Acta *1801*, 327—337.

Pugazhenthi, U., Velmurugan, K., Tran, A., Mahaffey, G., and Pugazhenthi, S. (2010). Anti-inflammatory action of exendin-4 in human islets is enhanced by phosphodiesterase inhibitors: Potential therapeutic benefits in diabetic patients. Diabetologia *53*, 2357–2368.

Pupilli, C., Giannini, S., Marchetti, P., Lupi, R., Antonelli, A., Malavasi, F., Takasawa, S., Okamoto, H., and Ferrannini, E. (1999). Autoantibodies to CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase) in Caucasian patients with diabetes: Effects on insulin release from human islets. Diabetes *48*, 2309–2315.

Qatanani, M., Tan, Y., Dobrin, R., Greenawalt, D.M., Hu, G., Zhao, W., Olefsky, J.M., Sears, D.D., Kaplan, L.M., and Kemp, D.M. (2013). Inverse regulation of inflammation and mitochondrial function in adipose tissue defines extreme insulin sensitivity in morbidly obese patients. Diabetes *62*, 855–863.

Qian, Y., Chen, C., Ma, L., Wang, Z., Wang, L.F., Zuo, L., Yang, Y., Huang, X., Jiang, M., Wang, X., et al. (2018). CD38 deficiency promotes inflammatory response through activating sirt1/NF-κB-mediated inhibition of TLR2 expression in macrophages. Mediators Inflamm. *2018*.

Rajbhandari, P., Thomas, B.J., Feng, A.C., Hong, C., Wang, J., Vergnes, L., Sallam, T., Wang, B., Sandhu, J., Seldin, M.M., et al. (2018). IL-10 Signaling Remodels Adipose Chromatin Architecture to Limit Thermogenesis and Energy Expenditure. Cell *172*, 218-233.e17.

Reilly, S.M., and Saltiel, A.R. (2017). Adapting to obesity with adipose tissue inflammation. Nat. Rev. Endocrinol. *13*, 633–643.

Reinherz, E.L., Kung, P.C., Goldstein, G., Levey, R.H., and Schlossman, S.F. (1980). Discrete stages of human intrathymic differentiation: Analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc. Natl. Acad. Sci. U. S. A. *77*, 1588–1592.

Reiniš, M., Morra, M., Funaro, A., Di Primio, R., and Malavasi, F. (1997). Functional associations of CD38 with CD3 on the T-cell membrane. J. Biol. Regul. Homeost. Agents *11*, 137–142.

Rena, G., Hardie, D.G., and Pearson, E.R. (2017). The mechanisms of action of metformin. Diabetologia *60*, 1577–1585.

Repa, J.J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J.M.A., Shimomura, I., Shan, B., Brown, M.S., Goldstein, J.L., and Mangelsdorf, D.J. (2000). Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRα and LXRβ. Genes Dev. *14*, 2819–2830.

Rosen, E.D., Sarraf, P., Troy, A.E., Bradwin, G., Moore, K., Milstone, D.S., Spiegelman, B.M., and Mortensen, R.M. (1999). PPARγ Is Required for the Differentiation of Adipose Tissue In Vivo and In Vitro. Mol. Cell *4*, 611–617.

Russo, L., and Lumeng, C.N. (2018). Properties and functions of adipose tissue macrophages in obesity (Blackwell Publishing Ltd).

Ryysy, L., Häkkinen, A.M., Goto, T., Vehkavaara, S., Westerbacka, J., Halavaara, J., and Yki-Järvinen, H. (2000). Hepatic fat content and insulin action on free fatty acids and glucose metabolism rather than insulin absorption are associated with insulin requirements during insulin therapy in type 2 diabetic patients. Diabetes *49*, 749–758.

Saltiel, A.R., and Olefsky, J.M. (2017). Inflammatory mechanisms linking obesity and metabolic disease. J. Clin. Invest. *127*, 1–4.

Samad, F., Yamamoto, K., Pandey, M., and Loskutoff, D.J. (1997). Elevated expression of transforming growth factor-β in adipose tissue from obese mice. Mol. Med. *3*, 37–48.

Sardi, C., Martini, E., Mello, T., Camelliti, S., Sfondrini, L., Marcucci, F., Kallikourdis, M., Sommariva, M., and Rumio, C. (2021). Effect of acetylsalicylic acid on inflamed adipose tissue. Insulin resistance and hepatic steatosis in a mouse model of diet-induced obesity. Life Sci. *264*, 118618.

Sauter, N.S., Thienel, C., Plutino, Y., Kampe, K., Dror, E., Traub, S., Timper, K., Bédat, B., Pattou, F., Kerr-Conte, J., et al. (2015). Angiotensin II induces interleukin-1b-mediated islet inflammation and β-cell dysfunction independently of vasoconstrictive effects. Diabetes *64*, 1273–1283.

Schultz, J.R., Tu, H., Luk, A., Repa, J.J., Medina, J.C., Li, L., Schwendner, S., Wang, S., Thoolen, M., Mangelsdorf, D.J., et al. (2000). Role of LXRs in control of lipogenesis. Genes Dev. *14*, 2831–2838.

Sconocchia, G., Titus, J.A., Mazzoni, A., Visintin, A., Pericle, F., Hicks, S.W., Malavasi, F., and Segal, D.M. (1999). CD38 triggers cytotoxic responses in activated human natural killer cells. Blood *94*, 3864–3871.

Secor McVoy, J.R., Oughli, A.A., and Oh, U. (2015). Liver X receptor-dependent inhibition of microglial nitric oxide synthase 2. J. Neuroinflammation *12*, 1–14.

Shaw, J.E., Sicree, R.A., and Zimmet, P.Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res. Clin. Pract. *87*, 4–14.

Shoelson, S.E., Lee, J., and Goldfine, A.B. (2006). Inflammation and insulin resistance. J. Clin. Invest. *116*, 1793–1801.

Sims, J.E., and Smith, D.E. (2010). The IL-1 family: Regulators of immunity. Nat. Rev. Immunol. *10*, 89–102.

Solinas, G., and Becattini, B. (2017). JNK at the crossroad of obesity, insulin resistance, and cell stress response. Mol. Metab. *6*, 174–184.

Solt, L.A., Kamenecka, T.M., and Burris, T.P. (2012). LXR-Mediated Inhibition of CD4+ T Helper Cells. PLoS One *7*.

Song, E.K., Lee, Y.R., Kim, Y.R., Yeom, J.H., Yoo, C.H., Kim, H.K., Park, H.M., Kang, H.S., Kim, J.S., Kim, U.H., et al. (2012). NAADP Mediates Insulin-Stimulated Glucose Uptake and Insulin Sensitization by PPARγ in Adipocytes. Cell Rep. *2*, 1607–1619.

Song, N.Y., Lee, Y.H., Na, H.K., Baek, J.H., and Surh, Y.J. (2018). Leptin induces SIRT1 expression through activation of NF-E2-related factor 2: Implications for obesity-associated colon carcinogenesis. Biochem. Pharmacol. *153*, 282–291.

Spann, N.J., Garmire, L.X., McDonald, J.G., Myers, D.S., Milne, S.B., Shibata, N., Reichart, D., Fox, J.N., Shaked, I., Heudobler, D., et al. (2012). Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses. Cell *151*, 138–152.

Spiegelman, B.M., and Hotamisligil, G.S. (1993). Through thick and thin: Wasting, obesity, and TNFα. Cell *73*, 625–627.

Stefanovic-Racic, M., Yang, X., Turner, M.S., Mantell, B.S., Stolz, D.B., Sumpter, T.L., Sipula, I.J., Dedousis, N., Scott, D.K., Morel, P.A., et al. (2012). Dendritic Cells Promote Macrophage Infiltration and Comprise a Substantial Proportion of Obesity-Associated Increases in CD11c + Cells in Adipose Tissue and Liver. 2330 DIABETES *61*.

Steinman, R.M. (2008). Dendritic Cells In Vivo: A Key Target for a New Vaccine Science. Immunity *29*, 319–324.

Stulnig, T.M., Steffensen, K.R., Gao, H., Reimers, M., Dahlman-Wright, K., Schuster, G.U., and Gustafsson, J.A. (2002a). Novel roles of Liver X receptors exposed by gene expression profiling in liver and adipose tissue. Mol. Pharmacol. *62*, 1299–1305.

Stulnig, T.M., Oppermann, U., Steffensen, K.R., Schuster, G.U., and Gustafsson, J.Å. (2002b). Liver X receptors downregulate 11β-hydroxysteroid dehydrogenase type 1 expression and activity. Diabetes *51*, 2426–2433.

Sun, J., Ni, Q., Xie, J., Xu, M., Zhang, J., Kuang, J., Wang, Y., Ning, G., and Wang, Q. (2020). β-Cell Dedifferentiation in Patients with T2D with Adequate Glucose Control and Nondiabetic Chronic Pancreatitis.

J. Clin. Endocrinol. Metab. *104*, 83–94.

Sun, Q., Li, J., and Gao, F. (2014). New insights into insulin: The anti-inflammatory effect and its clinical relevance. World J. Diabetes *5*, 89.

Talukdar, S., Oh, D.Y., Bandyopadhyay, G., Li, D., Xu, J., McNelis, J., Lu, M., Li, P., Yan, Q., Zhu, Y., et al. (2012). Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat. Med. *18*, 1407–1412.

Tliba, O., Damera, G., Banerjee, A., Gu, S., Baidouri, H., Keslacy, S., and Amrani, Y. (2008). Cytokines induce an early steroid resistance in airway smooth muscle cells: Novel role of interferon regulatory factor-1. Am. J. Respir. Cell Mol. Biol. *38*, 463–472.

Unamuno, X., Gómez-Ambrosi, J., Rodríguez, A., Becerril, S., Frühbeck, G., and Catalán, V. (2018). Adipokine dysregulation and adipose tissue inflammation in human obesity (Blackwell Publishing Ltd).

Vaisitti, T., Audrito, V., Serra, S., Bologna, C., Arruga, F., Brusa, D., Buonincontri, R., Gizdic, B., and Deaglio, S. (2015). Multiple Metamorphoses of CD38 from Prognostic Marker to Disease Modifier to Therapeutic Target in Chronic Lymphocytic Leukemia. Curr. Top. Med. Chem. *13*, 2955–2964.

Valledor, A.F., and Ricote, M. (2004). Nuclear receptor signaling in macrophages. Biochem. Pharmacol. *67*, 201–212.

Valledor, A.F., Comalada, M., Xaus, J., and Celadal, A. (2000). The differential time-course of extracellular-regulated kinase activity correlates with the macrophage response toward proliferation or activation. J. Biol. Chem. *275*, 7403–7409.

van der Veen, J.N., van Dijk, T.H., Vrins, C.L.J., van Meer, H., Havinga, R., Bijsterveld, K., Tietge, U.J.F., Groen, A.K., and Kuipers, F. (2009). Activation of the liver X receptor stimulates trans-intestinal excretion of plasma cholesterol. J. Biol. Chem. *284*, 19211–19219.

Venkateswaran, A., Laffitte, B.A., Joseph, S.B., Mak, P.A., Wilpitz, D.C., Edwards, P.A., and Tontonoz, P. (2000). Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXRα. Proc. Natl. Acad. Sci. U. S. A. *97*, 12097–12102.

Viardot, A., Grey, S.T., Mackay, F., and Chisholm, D. (2007). Potential antiinflammatory role of insulin via the preferential polarization of effector T cells toward a T helper 2 phenotype. Endocrinology *148*, 346–353.

Villarreal, D., Pradhan, G., Wu, C.S., Allred, C.D., Guo, S., and Sun, Y. (2019). A simple high efficiency protocol for pancreatic islet isolation from mice. J. Vis. Exp. *2019*, e57048.

Vilsbøll, T., Krarup, T., Madsbad, S., and Holst, J. (2002). Defective amplification of the late phase insulin response to glucose by gip in obese type ii diabetic patients. Diabetologia *45*, 1111–1119.

Wagner, B.L., Valledor, A.F., Shao, G., Daige, C.L., Bischoff, E.D., Petrowski, M., Jepsen, K., Baek, S.H., Heyman, R.A., Rosenfeld, M.G., et al. (2003). Promoter-Specific Roles for Liver X Receptor/Corepressor Complexes in the Regulation of ABCA1 and SREBP1 Gene Expression. Mol. Cell. Biol. *23*, 5780–5789.

Wang, H., Shen, L., Sun, X., Liu, F., Feng, W., Jiang, C.C.C.C.C., Chu, X., Ye, X., Jiang, C.C.C.C.C., Wang, Y., et al. (2019). Adipose group 1 innate lymphoid cells promote adipose tissue fibrosis and diabetes in obesity (Nature Publishing Group).

Wang, L.F., Miao, L.J., Wang, X.N., Huang, C.C., Qian, Y.S., Huang, X., Wang, X.L., Jin, W.Z., Ji, G.J., Fu, M., et al. (2018a). CD38 deficiency suppresses adipogenesis and lipogenesis in adipose tissues through activating Sirt1/PPARγ signaling pathway. J. Cell. Mol. Med. *22*, 101–110.

Wang, N., Lan, D., Chen, W., Matsuura, F., and Tall, A.R. (2004). ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. Proc. Natl. Acad. Sci. U. S. A. *101*, 9774–9779.

Wang, Y., Zhang, Y., Hughes, T., Zhang, J., Caligiuri, M.A., Benson, D.M., and Yu, J. (2018b). Fratricide of NK cells in daratumumab therapy for multiple myeloma overcome by ex vivo–expanded autologous NK cells. Clin. Cancer Res. *24*, 4006–4017.

de Weers, M., Tai, Y.-T., van der Veer, M.S., Bakker, J.M., Vink, T., Jacobs, D.C.H., Oomen, L.A., Peipp, M., Valerius, T., Slootstra, J.W., et al. (2011). Daratumumab, a Novel Therapeutic Human CD38 Monoclonal Antibody, Induces Killing of Multiple Myeloma and Other Hematological Tumors. J. Immunol. *186*, 1840–1848.

Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., and Ferrante, A.W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. *112*, 1796–1808.

Wensveen, F.M., Valentić, S., Šestan, M., Wensveen, T.T., and Polić, B. (2015a). The "Big Bang" in obese fat: Events initiating obesity-induced adipose tissue inflammation. Eur. J. Immunol. *45*, 2446–2456.

Wensveen, F.M., Jelenčić, V., Valentić, S., Šestan, M., Wensveen, T.T., Theurich, S., Glasner, A., Mendrila, D., Štimac, D., Wunderlich, F.T., et al. (2015b). NK cells link obesity-induced adipose stress to inflammation and insulin resistance. Nat. Immunol. *16*, 376–385.

Widenmaier, S.B., Kim, S.J., Yang, G.K., De Los Reyes, T., Nian, C., Asadi, A., Seino, Y., Kieffer, T.J., Kwok, Y.N., and McIntosh, C.H.S. (2010). A GIP receptor agonist exhibits β-cell anti-apoptotic actions in rat models of diabetes resulting in improved β-cell function and glycemic control. PLoS One *5*.

Winer, D.A., Winer, S., Shen, L., Wadia, P.P., Yantha, J., Paltser, G., Tsui, H., Wu, P., Davidson, M.G., Alonso, M.N., et al. (2011). B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat. Med. *17*, 610–617.

Winer, S., Paltser, G., Chan, Y., Tsui, H., Engleman, E., Winer, D., and Dosch, H.M. (2009). Obesity predisposes to Th17 bias. Eur. J. Immunol. *39*, 2629–2635.

Woo, C.Y., Jang, J.E., Lee, S.E., Koh, E.H., and Lee, K.U. (2019). Mitochondrial dysfunction in adipocytes as a primary cause of adipose tissue inflammation. Diabetes Metab. J. *43*, 247–256.

Wu, L., Wen, H., Zhou, Y., Yu, H., Liu, Y., Bai, L., Kijlstra, A., and Yang, P. (2014). Activation of the Liver X Receptor Inhibits Th17 and Th1 Responses in Behcet's Disease and Vogt-Koyanagi-Harada Disease. Curr. Mol. Med. *14*, 712–722.

Xourgia, E., Tzouganatou, E.-M., Papazafiropoulou, A., and Melidonis, A. (2019). Anti-inflammatory properties of antidiabetic agents. World J. Meta-Analysis *7*, 129–141.

Xu, H., Teoman Uysal, K., David Becherer, J., Arner, P., and Hotamisligil, G.S. (2002). Altered tumor necrosis factor-α (TNF-α) processing in adipocytes and increased expression of transmembrane TNF-α in obesity. Diabetes *51*, 1876–1883.

Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., Sole, J., Nichols, A., Ross, J.S., Tartaglia, L.A., et al. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J. Clin. Invest. *112*, 1821–1830.

Yabe, D., Seino, Y., Fukushima, M., and Seino, S. (2015). β Cell Dysfunction Versus Insulin Resistance in the Pathogenesis of Type 2 Diabetes in East Asians. Curr. Diab. Rep. *15*, 1–9.

Yadav, H., Quijano, C., Kamaraju, A.K., Gavrilova, O., Malek, R., Chen, W., Zerfas, P., Zhigang, D., Wright, E.C., Stuelten, C., et al. (2011). Protection from obesity and diabetes by blockade of TGF-β/Smad3 signaling. Cell Metab. *14*, 67–79.

Yanai, H., and Yoshida, H. (2019). Beneficial effects of adiponectin on glucose and lipid metabolism and atherosclerotic progression: Mechanisms and perspectives. Int. J. Mol. Sci. *20*.

Yang, H., Zheng, S., Qiu, Y., Yang, Y., Wang, C., Yang, P., Li, Q., and Lei, B. (2014a). Activation of liver X receptor alleviates ocular inflammation in experimental autoimmune uveitis. Investig. Ophthalmol. Vis. Sci. *55*, 2795–2804.

Yang, L., Yuan, J., and Zhou, Z. (2014b). Emerging Roles of Dipeptidyl Peptidase 4 Inhibitors: Anti-Inflammatory and Immunomodulatory Effect and Its Application in Diabetes Mellitus. Can. J. Diabetes *38*, 473–479.

Yu, P., Liu, Z., Yu, X., Ye, P., Liu, H., Xue, X., Yang, L., Li, Z., Wu, Y., Fang, C., et al. (2019). Direct Gating of the

TRPM2 Channel by cADPR via Specific Interactions with the ADPR Binding Pocket. Cell Rep. *27*, 3684-3695.e4.

Zatterale, F., Longo, M., Naderi, J., Raciti, G.A., Desiderio, A., Miele, C., and Beguinot, F. (2020). Chronic Adipose Tissue Inflammation Linking Obesity to Insulin Resistance and Type 2 Diabetes (Frontiers Media S.A.).

Żelechowska, P., Agier, J., Kozłowska, E., and Brzezińska-Błaszczyk, E. (2018). Mast cells participate in chronic low-grade inflammation within adipose tissue. Obes. Rev. *19*, 686–697.

Zhang, X., Du, K., Lou, Z., Ding, K., Zhang, F., Zhu, J., and Chang, Z. (2019). The CtBP1-HDAC1/2-IRF1 transcriptional complex represses the expression of the long noncoding RNA GAS5 in human osteosarcoma cells. Int. J. Biol. Sci. *15*, 1460–1471.

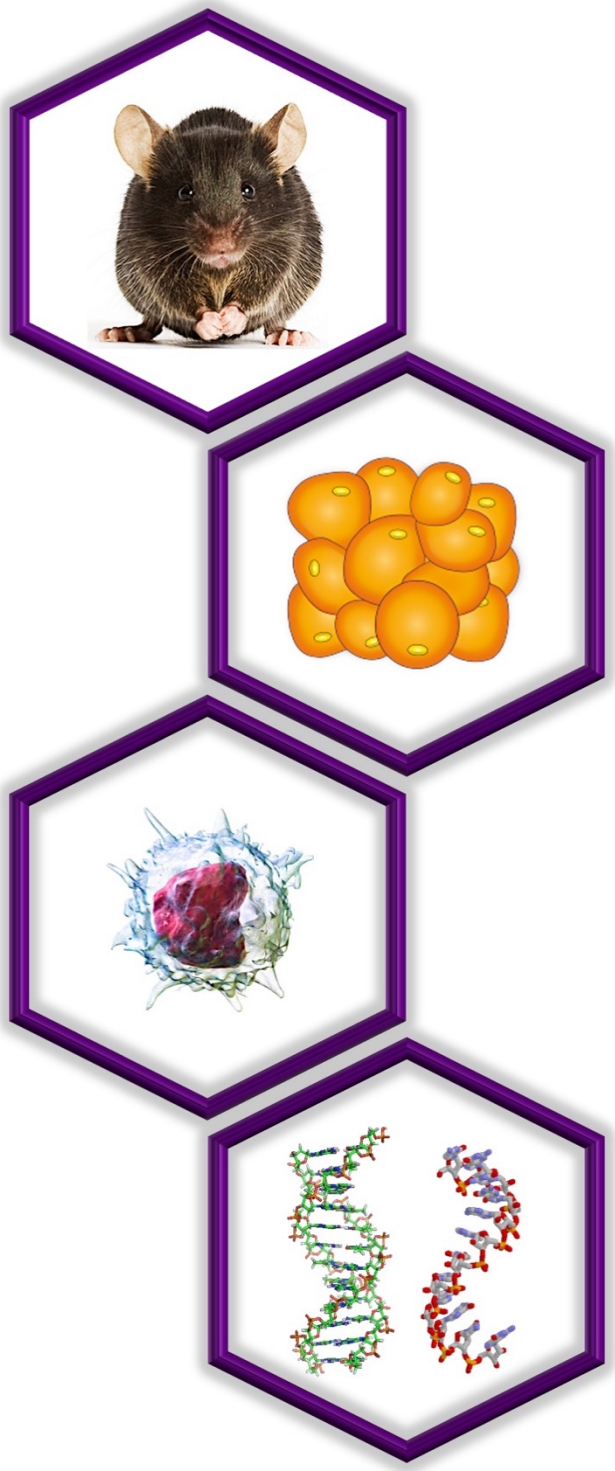
Zhou, S., Tang, X., and Chen, H.Z. (2018). Sirtuins and Insulin Resistance. Front. Endocrinol. (Lausanne). *9*.

Zhu, Y., Tchkonia, T., Stout, M.B., Giorgadze, N., Wang, L., Li, P.W., Heppelmann, C.J., Bouloumié, A., Jensen, M.D., Robert Bergen, H., et al. (2015). Inflammation and the depot-specific secretome of human preadipocytes. Obesity *23*, 989–999.

Zubiaur, M., Izquierdo, M., Terhorst, C., Malavasi, F., and Sancho, J. (1997). CD38 ligation results in activation of the Raf-1/mitogen-activated protein kinase and the CD3-zeta/zeta-associated protein-70 signaling pathways in Jurkat T lymphocytes. J. Immunol. *159*, 193–205.

Zubiaur, M., Fernández, O., Ferrero, E., Salmerón, J., Malissen, B., Malavasi, F., and Sancho, J. (2002). CD38 is associated with lipid rafts and upon receptor stimulation leads to Akt/protein kinase B and Erk activation in the absence of the CD3-ζ immune receptor tyrosine-based activation motifs. J. Biol. Chem. *277*, 13–22.

Zuo, W., Liu, N., Zeng, Y., Liu, Y., Li, B., Wu, K., Xiao, Y., and Liu, Q. (2020). CD38: A Potential Therapeutic Target in Cardiovascular Disease. Cardiovasc. Drugs Ther. 2020 354 *35*, 815–828.



ANNEXES

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Current Opinion in Pharmacology

Integrating the roles of liver X receptors in inflammation and infection: mechanisms and outcomes

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Liver X receptors (LXRs) are transcription factors from the nuclear receptor family that can be pharmacologically activated by high-affinity agonists. LXR activation exerts a combination of metabolic and anti-inflammatory actions that result in the modulation of immune responses and in the amelioration of inflammatory disorders. In addition, LXR agonists modulate the metabolism of infected cells and limit the infectivity and/or growth of several pathogens. This review gives an overview of the recent advances in understanding the complexity of the mechanisms through which the LXR pathway controls inflammation and host–cell pathogen interaction.

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Current Opinion in Pharmacology 2020, **53**:55–65

This review comes from a themed issue on **Immunomodulation**

Edited by **Tamás Röszer**

For a complete overview see the Issue and the Editorial

Available online 28th June 2020

https://doi.org/10.1016/j.coph.2020.05.001

Introduction

Liver X receptors (LXRs), namely, NR1H3 (LXRα) and NR1H2 (LXRβ), are transcription factors from the nuclear receptor family (reviewed in Ref. [1]). LXRβ is ubiquitously expressed, whereas LXRα expression is more predominant in tissues that are highly involved in lipid metabolism. Within the immune system, macrophages, dendritic cells, and neutrophils express both isoforms, B lymphocytes express mainly LXRβ, and T cell populations have been reported to express either LXRβ or both isoforms [2–5,6•]. LXRs can be activated by endogenous agonists, including specific oxysterols and intermediates of cholesterol biosynthesis, and by specific high-affinity agonists that are frequently used *in vivo* to explore the consequences of pharmacological LXR activation.

LXRs form heterodimers with retinoid X receptors (RXRs) on LXR response elements and, once activated by agonists, they positively regulate the expression of target genes. Recent studies have proposed three possible modes of action for LXRα-mediated transcriptional activation [7•]. Two mechanisms are based on the canonical induction of target gene expression by RXR-LXR heterodimers in a pharmacologically responsive-manner. In the absence of agonistic activation, the target genes are repressed by LXR/RXR heterodimers, which may lead to de-repression in the absence of functional LXRs [8]. A third mechanism was proposed, by which the expression of a number of transcripts depends on the presence of LXRs, but these transcripts are not upregulated upon pharmacological LXR activation [7•].

Most of the targets that are positively induced in response to LXR agonists play key roles in lipid and glucose metabolism (reviewed in Ref. [1]). These include (but are not restricted to) several sterol transporters from the ATP binding cassette (ABC) family, for example, ABCA1 and ABCG1; transcription factors sterol regulatory element-binding protein 1c (SREBP1c) and carbohydrate regulatory element-binding protein with important lipogenic roles; the E3 ubiquitin ligase inducible degrader of the low-density lipoprotein receptor (IDOL); and several apolipoproteins involved in lipid transport.

The use of immortalized murine macrophages that express equivalent levels of FLAG-tagged LXRα or LXRβ in an LXR-deficient background has contributed in defining the specific roles of LXR isoforms in gene regulation. In addition to a signature simultaneously regulated by both isoforms, LXRα selectively regulates the expression of genes linked to the control of apoptosis and leukocyte migration, whereas LXRβ-specific functions are associated with lymphocyte differentiation and selection [7•].

In addition to their positive effects on gene transcription, LXRs can negatively affect the expression of inflammatory mediators through a plethora of mechanisms, which will be further revised in the following section. Agonist-bound LXRs undergo conjugation to small ubiquitin-related modifier (SUMO), a process known as SUMOylation, which is required for some of the repressive actions of these proteins [9]. Moreover, a study in astrocytes proposed different SUMOylation pathways for agonist-bound LXRα and LXRβ, mediated by separate members of the SUMO E3 ligase family [10].

In a complex scenario combining metabolic and anti-inflammatory actions, LXRs are able to modulate immune responses. These actions are particularly relevant in the management of an infection, as a number of pathogens are able to hijack host metabolic pathways for their own benefit. This review integrates the recent conceptual advances in understanding the complexity of mechanisms used by the LXR pathway to control inflammation and the response of the host to infection.

LXRs as attenuators of inflammatory disorders

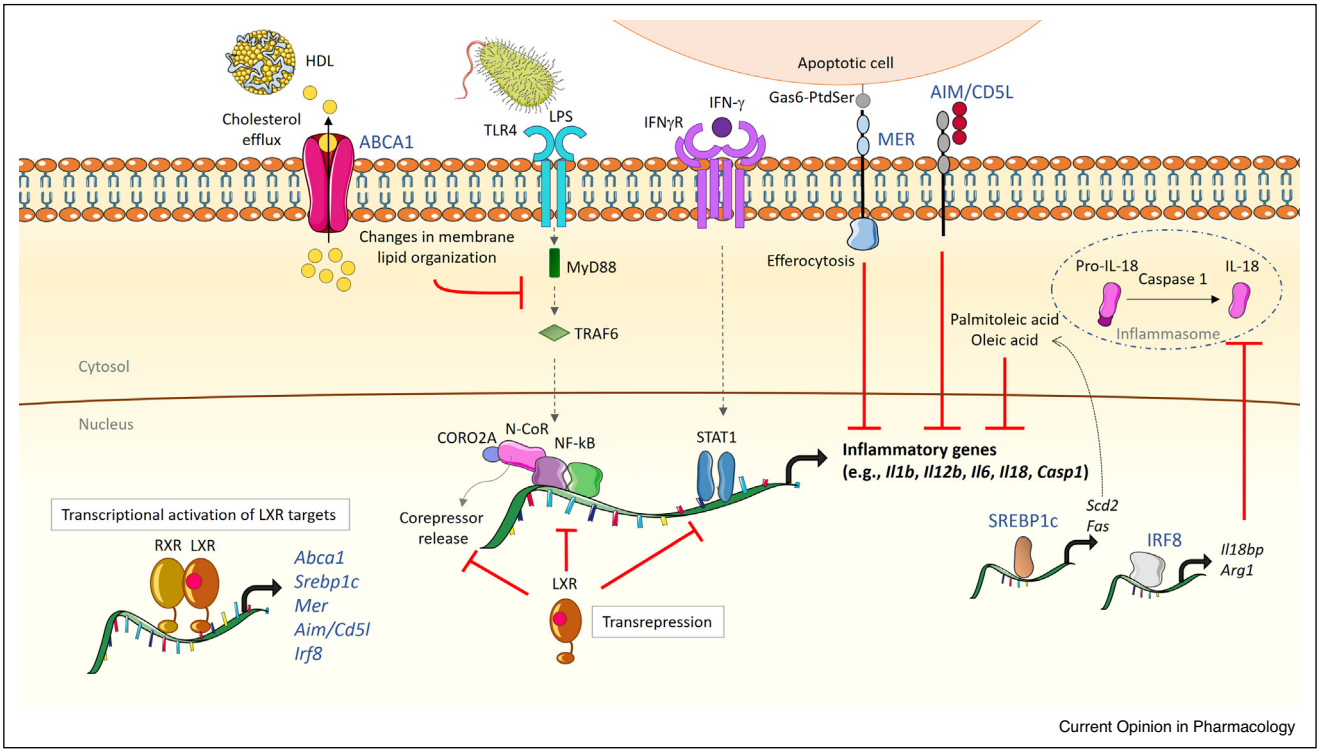
Accumulated evidence indicates the importance of the LXR pathway in the negative control of inflammatory conditions. For example, pharmacological activation of LXRs reduced the extent of the inflammatory response in murine models of dermatitis [11,12], neuroinflammation [13,14], lupus [15], arthritis [16], and atherosclerosis [12], consistent with the fact that LXR-deficient mice develop an age-related lupus-like autoimmune disease [17]. Furthermore, several polymorphisms affecting the promoter region of the gene encoding LXRα were associated with susceptibility to systemic lupus erythematosus in a Korean cohort [18].

To explain the anti-inflammatory actions of pharmacologically activated LXRs, many studies have focused on the capability of high-affinity agonists to repress pro-inflammatory gene expression in macrophages and other cell types activated by the engagement of toll-like receptors (TLRs) or by endogenous inflammatory cytokines [9,12,14,19,20]. The LXR pathway impairs the transcriptional activity of nuclear factor kappa B (NF-κB) [12] and the recruitment of signal transducer and activator of transcription (STAT)1 to target gene promoters [10,14]. Putting together the pieces of evidence reported by different groups, it is apparent that several mechanisms contribute to the antagonizing actions of the LXR pathway on pro-inflammatory signaling (Figure 1). First, agonist-bound LXRs underwent SUMOylation and exerted transrepression by inhibiting the removal of nuclear receptor co-repressor (NCoR) complexes from pro-inflammatory gene promoters in response to lipopolysaccharide (LPS) [9,21,22]. In macrophages, this process involves the interaction of SUMOylated LXRs with the actin-binding protein CORONIN 2A (CORO2A). This interaction prevented actin recruitment to inflammatory gene promoters [21], in line with more recent evidence on the important roles of nuclear actin in the transcriptional control of macrophage activation [23]. In the hepatic acute phase response in mice, the anti-inflammatory effects were selectively mediated by SUMOylated LXRβ and its interaction with the corepressor complex subunit G protein pathway suppressor 2 (GPS2) [22]. LXRβ also attenuated inflammatory cytokine production in murine mast cells stimulated with LPS or FcεRI crosslinking [24]. By contrast, both SUMOylated LXRα and LXRβ contributed in inhibiting the transcriptional response of murine macrophages and astrocytes to interferon (IFN)-γ through interference with STAT1 [10,14], which supports the notion that the relative contribution of each isoform depends on the cell type and the inflammatory trigger.

Direct repressive actions have also been proposed involving the binding of LXRs to specific sites within macrophage inflammatory gene enhancer elements and potential chromatin closure, although additional studies are required to better define this mechanism. Gene signatures affected by this repressive activity are associated with leukocyte cell–cell adhesion and neutrophil chemotaxis, in line with the inhibitory effects of LXR agonists on neutrophil infiltration in a model of zymosan-induced peritonitis in mice [25].

Other mechanisms contributing to the repression of inflammation imply the increased transcription of LXR targets in macrophages (Figure 1). First, the cholesterol and phospholipid transporter ABCA1, whose upregulation results in changes in membrane cholesterol homeostasis that are able to disrupt the recruitment of key adaptor molecules to lipid rafts, thereby antagonizing TLR signaling [20]. Second, several enzymes involved in the synthesis of fatty acids (fatty acid synthase) and in their conversion to derivatives with anti-inflammatory properties (predominantly mediated by stearoyl-CoA desaturase-2 (SCD2) and its products 9Z palmitoleic acid and oleic acid). The induction of these enzymes is exerted directly by LXRs or indirectly through the upregulation of the transcription factor SREBP1c, depending on the type of agonist mediating LXR activation [26]. Third, MER, a receptor tyrosine kinase that recognizes the plasma protein growth arrest-specific 6 (GAS6) bound to phosphatidylserine (PtdSer) on the surface of apoptotic bodies and contributes to apoptotic cell clearance. The upregulation of MER has been proposed as a mechanism coupling the engulfment of apoptotic cells (efferocytosis) with the suppression of inflammatory pathways. Indeed, LXR deficiency resulted in an aberrant pro-inflammatory response of macrophages to apoptotic cells and in the development of autoimmune disease in mice [17]. Fourth, interferon regulatory factor (IRF)8, a transcription factor with multiple roles in myeloid cells. Through the upregulation of IRF8, the LXR pathway indirectly induced the expression of interleukin (IL)-18 binding protein (IL18BP) in the murine and human systems. IL18BP is a potent endogenous inhibitor of the pro-inflammatory cytokine IL-18 [27]. In parallel, LXR agonists also repressed IL18 transcription and blocked the processing of pro-IL-18 to its bioactive form by interfering with pro-caspase 1 expression and activation, indicating that the LXR pathway uses a combination of mechanisms to inhibit IL-18 production [27]. In addition, increased expression of IRF8 in murine macrophages overexpressing LXRα resulted in the upregulation of the anti-inflammatory enzyme arginase 1 [28].

Figure 1



LXRs inhibit the inflammatory response in macrophages through multiple mechanisms.
TLR signaling or IFN-γ stimulation induce inflammatory gene expression. Agonist-bound LXRs mediate mechanisms of transrepression, which interfere with the release of corepressors or with the activity/recruitment of transcription factors (NF-κB, STAT1) required for inflammatory gene expression. In addition, LXRs inhibit inflammation indirectly through the transcriptional activation of LXR targets (in blue) involved in the modulation of metabolic and/or immune responses. The cholesterol efflux mediated by ABCA1 results in changes in the lipid composition of the membrane, which interferes with TLR signaling. SREBP1c induces the expresion of enzymes involved in the generation of lipids with anti-inflammatory properties. MER couples efferocytosis with the suppression of the inflammatory response. AIM/CD5L enhances the expression of molecules involved in the resolution of inflammation and promotes an anti-inflammatory profile. IRF8 induces the expression of IL18BP, which binds to secreted IL-18 and inhibits its biological actions. Some elements in this image have been downloaded from SMART – Servier Medical ART. *Arg1*, arginase 1; *Casp1*, caspase 1; *Fas*, fatty acid synthase; HDL, high-density lipoprotein; IFNγR, IFN-γ receptor; *Il1b*, interleukin 1β; *Il12b*, interleukin 12?subunit b; *Il6*, interleukin 6; MyD88, myeloid differentiation primary response 88; TRAF6, tumor necrosis factor receptor associated factor 6.

Aside from the mechanisms described above, LXR agonists also induce the expression of apoptosis inhibitory factor secreted by macrophages (AIM)/CD5L [29,30]. In the murine system, this effect is mediated specifically by LXRα [7•,30]. AIM/CD5L is a soluble scavenger receptor that can also act as a pattern-recognition receptor [31]. The endogenous production of human AIM/CD5L enhanced the expression of molecules involved in the resolution of inflammation, namely, MER and CD163, increased autophagy, and promoted an anti-inflammatory profile in human monocytes, resembling the actions of IL-10 [32•], which suggests the possibility that AIM might also be involved in facilitating the resolution of inflammation in response to LXR agonists.

In contrast to predominant anti-inflammatory activities of LXR agonists in macrophages, both pro-inflammatory and anti-inflammatory actions have been reported in dendritic cells. In this regard, LXR activation downregulated the expression of the actin-bundling protein fascin in human myeloid dendritic cells, suppressing T cell stimulation due to inefficient immunological synapse formation [33]. However, prolonged NF-κB activation was detected in a different study, which translated into increased pro-inflammatory and T cell stimulatory activities [34]. Moreover, LXR agonism increased the chemotaxis of murine dendritic cells to signals generated in inflammatory settings, such as chemokine (C–C motif) ligand (CCL)19 and CCL21. This action was mediated through transcriptional activation of the ectoenzyme CD38, which is capable of converting nicotinamide adenine dinucleotide (NAD) into cyclic adenosine diphosphoribose (cADPR), an important second messenger in leukocyte trafficking [6•]. These contrasting observations raise the question as to whether the effects of the LXR pathway are influenced by additional factors involved in dendritic cell maturation, which requires further exploration.

In addition to the actions in myeloid cells, LXR agonists inhibited the differentiation of murine and human helper T (Th)17 cells [35], which are a subset of $CD4^+$ T cells that secrete IL-17 and contribute to the pathogeny of inflammatory diseases [36]. An indirect mechanisms was proposed, by which LXR-induced SREBP1 negatively interfered with the activity of the transcription factor aryl hydrocarbon receptor on the *Il17* promoter. The differentiation of other $CD4^+$ T cell populations was also inhibited by LXR agonists [37], consistent with the anti-proliferative actions of LXRβ in murine T cells mediated by the upregulation of ABCG1 and subsequent changes in sterol homeostasis [3]. Moreover, LXR activation induced regulatory T cell (Treg) expansion. Although a molecular mechanism was not defined, the oral administration of an LXR agonist in mice increased the abundance of gut-associated Treg with high suppressive capacity [38], which may provide additional explanation to the protective effects of the LXR pathway against the development of autoimmune diseases.

The interplay between the metabolic actions of LXRs and their role in the modulation of adaptive immune responses was further illustrated by the observation that excessive lipid accumulation in LXRβ-deficient antigen presenting cells induced the expression of B cell activating factor (BAFF) and a proliferation inducing ligand (APRIL) that support B cell survival and differentiation [39]. This scenario triggered the expansion of auto-reactive B cells and contributed to the development of autoimmune disease. In addition, despite the fact that B cells mostly express the LXRβ isoform, the activation of LXRα repressed BAFF production in human B cell lines through interference with NF-κB, STAT1 and mothers against decapentaplegic homolog 3 (SMAD3) signaling [40].

Beyond the anti-inflammatory actions in immune cells, transcriptional activation by LXRs impairs inflammatory responses in the liver in the context of metabolic disease. In particular, lysophosphatidylcholine acyltransferase 3 (LPCAT3) is highly induced by LXR agonists in hepatic cells, where it drives the incorporation of unsaturated fatty acids into phospholipids [41]. The activity of LPCAT3 resulted in reduced membrane lipid saturation, thus inhibiting pro-inflammatory c-Src kinase activation, and in decreased availability of saturated lipids for the synthesis of inflammatory mediators.

Impact of metabolic and anti-inflammatory actions of LXRs on host cell–pathogen interaction

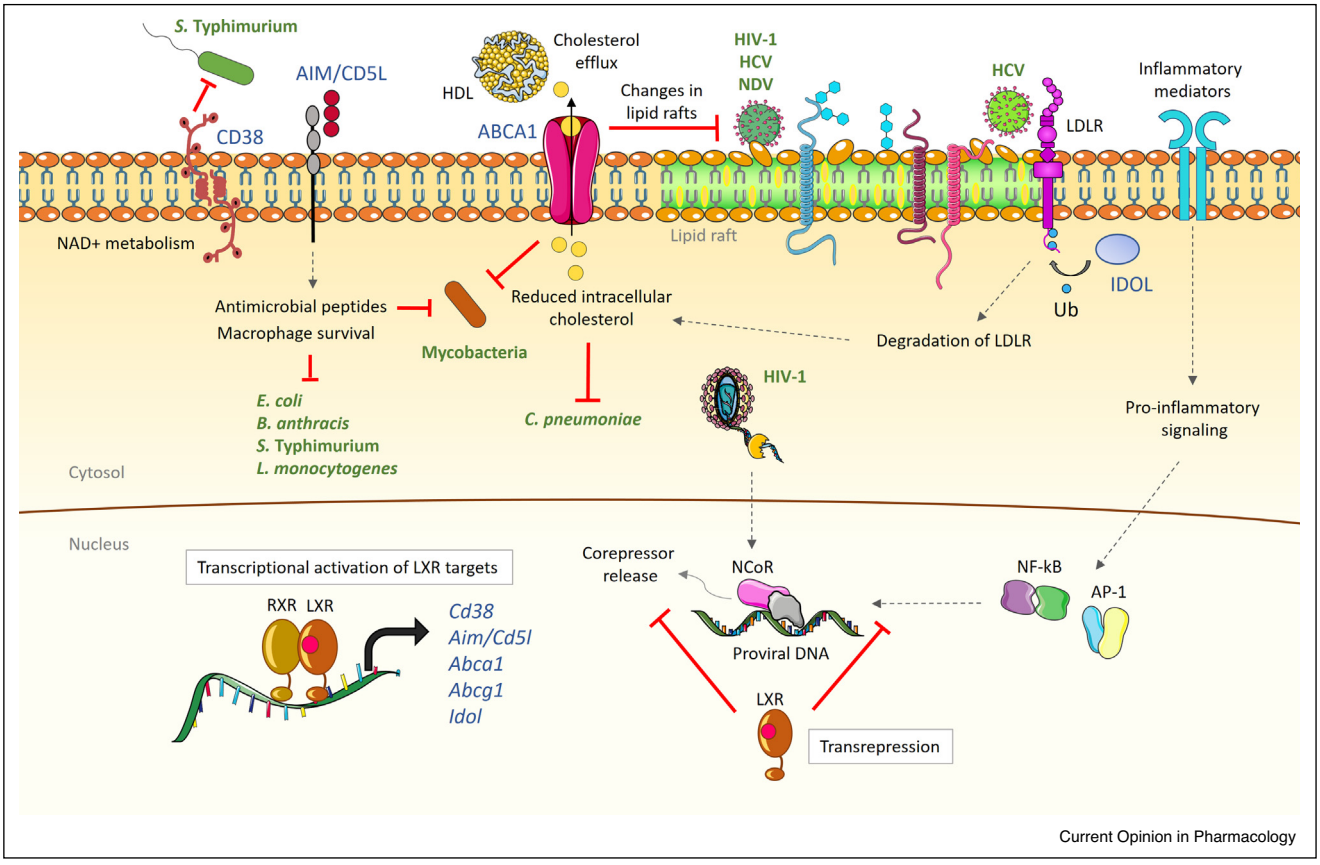
Despite contributing to immunopathology, inflammatory responses are crucial for the establishment of an effective immune response against infection. Based on the anti-inflammatory actions of the LXR pathway, one could expect that LXR agonism would lead to deficient immune responses against infection. However, as will be discussed in this section, several studies have shown otherwise. Notably, a number of pathogens have developed mechanisms to hijack the host immune response and establish intracellular infection, particularly in phagocytic cells, even under adverse inflammatory conditions. Metabolic reprogramming of host cells or adaptation to their metabolic status are indeed common strategies used by intracellular pathogens for survival and replication [42].

Interestingly, many studies have shown increased expression and/or activity of LXR isoforms in leukocytes infected by intracellular pathogens [30,43•,44–46]. Although the signaling pathway/s leading to increased LXR expression during infection have not been fully characterized, muramyl dipeptide, a ligand of nucleotide-binding oligomerization domain-containing protein 2 (NOD2) that is present in many bacteria, was able to induce LXRα expression in murine macrophages [30]. In addition, type I and II IFNs and IL-36, which are produced during the immune response to infection, as well as LPS from Gram-negative bacteria, upregulated the expression of enzymes that transform free cholesterol into endogenous LXR agonists, such as 25-hydroxycholesterol (25-HC) [14,43•,47,48]. However, the involvement of LXRs in the physiological actions of 25-HC is still unclear [49]. Sterile acute inflammation also increased LXR expression and activity through a mechanism requiring functional MER signaling [50], in line with the observation that efferocytosis via MER activates the LXR pathway [17].

By contrast, LXRα expression was inhibited in experimental models of sepsis [51,52] and the transcriptional control of LXR target genes was compromised in several infection/inflammatory settings [14,15,53]. In this regard, TLR3/4 ligands and IFN-γ interfered with the LXR-mediated control of cholesterol metabolism through activation of IRF3 and STAT1, respectively [14,53]. Competition for the coactivator p300/CREB-binding protein (CBP) was proposed as a mechanism for IRF3 and STAT1 to inhibit the transcriptional activity of LXRs on specific target genes.

Such divergent consequences of infection/inflammation on LXR signaling have fueled the need to explore the roles of this pathway in host–pathogen interaction (Figure 2). Initial studies in mice defined the general role for LXRs in promoting macrophage survival after infection by different bacteria, namely *Listeria monocytogenes*, *Bacillus anthracis*, *Escherichia coli*, and *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), which correlated with the upregulated expression of the anti-apoptotic molecule AIM/CD5L, a specific target of LXRα, and with the downregulation of pro-apoptotic factors [29,30]. Deficient LXR expression, particularly in bone marrow-derived cells, resulted in a higher susceptibility to

Figure 2



LXR activation induces protective mechanisms that limit viral and bacterial infection.
LXR agonists upregulate the expression of LXR targets (in blue) that contribute in reducing the infection by several pathogens (names of pathogens in green). AIM/CD5L confers resistance to apoptosis and induces the synthesis of antimicrobial peptides. CD38 reduces intracellular NAD + levels and the infection by *S.* Typhimurium, ABCA1 promotes cholesterol efflux. As a consequence, reduced intracellular cholesterol limits the growth of mycobacteria and, potentially, of other bacterial strains that depend on intracellular cholesterol. In addition, changes in the cholesterol levels within lipid rafts may interfere with the entry of several viruses into host cells. IDOL, by virtue of its role in controling the turnover of the LDLR, inhibits the capability of HCV to infect host cells. LXRs can also affect the intracellular replication of HIV-1 through mechanisms of transrepression, which affect corepressor release or transcription factor recruitment to the proviral DNA. Ub, ubiquitin. Some elements in this image have been downloaded from SMART – Servier Medical ART.

infection by *L. monocytogenes*, with increased bacterial burden and neutrophilic abscesses in the liver and a lower survival rate [30]. In studies comparing the relative contribution of LXR isoforms, the lack of expression of LXRα was responsible for the increased susceptibility to *L. monocytogenes*.

Later on, a solid amount of evidence supported the involvement of LXRs in the control of the infection by *Mycobacterium tuberculosis*. In human macrophages and in a murine model of mycobacterial infection, LXR agonists reduced the intracellular bacterial burden [43•,44,45]. In line with these observations, LXR-deficient mice had higher bacterial burdens and increased granulomatous lesions in the lungs and underwent more rapid progression to systemic infection than their wild-type counterparts [45]. The increased susceptibility of LXR-deficient mice was associated with the impaired activities of the innate and adaptive immune systems, including the infiltration of neutrophils to the lungs and the establishment of local Th1 and Th17 responses. These observations are in contrast with the general anti-inflammatory roles of the LXR pathway in non-infectious inflammatory diseases described in the previous section. Interestingly, whereas both LXRα and LXRβ participated in limiting mycobacterial infection in human macrophages *in vitro* [43•], LXRα was specifically required to control the course of infection in mice [45], mirroring the selective contribution of this isoform in the protection against *L. monocytogenes* [30].

In addition, LXR agonists increased the production of antimicrobial peptides in *M. tuberculosis*-infected macrophages [43•], consistent with the capability of the LXRα

target AIM/CD5L to enhance this mechanism of defense and to contribute to mycobacterial clearance [54]. Therefore, it is plausible that activities regulated by AIM/CD5L beyond the control of apoptotic cell death also contribute to the protective effects of LXR agonists against bacterial infection. On the other hand, in contrast to the pro-survival actions described above, LXR agonists promoted apoptosis in human macrophages infected with *M. tuberculosis*, which may represent a mycobactericidal strategy [44]. Although the mechanisms leading to increased cell death were not determined, further investigation is required to better understand the implications of the LXR-AIM axis in different types of infection and how this pathway integrates with the other transcriptional effects of LXR agonists.

In this regard, the upregulation of the LXR targets ABCA1 and ABCG1, which mediate intracellular cholesterol efflux, may also represent an important host mechanism for inhibiting mycobacterial growth [44]. Indeed, interference with ABCA1 expression facilitated the growth of the vaccine strain Bacille Calmette–Guérin in human macrophages [55], probably because mycobacteria have a preference for intracellular fatty acids and cholesterol as carbon sources (reviewed in [56]). The obligate intracellular bacterium *Chlamydia pneumoniae* also relies heavily on intracellular cholesterol and uses the TIR domain-containing adapter inducing IFN-β (TRIF)-IRF3 signaling pathway to promote the conversion of infected macrophages into cholesterol-loaded foam cells [57]. Although this study did not evaluate the effects on cholesterol transporters, the results are consistent with the capability of IRF3 to inhibit ABCA1 expression [53]. Interestingly, LXR activation interfered with IRF3 activity and inhibited foam cell formation during *C. pneumoniae* infection [57]. Therefore, it is possible that LXR agonists use cooperative mechanisms based on the induction of ABCA1/G1 and the repressive actions on IRF3 to limit the accumulation of cholesterol and control the infection by bacterial species that benefit from intracellular lipid storages.

Accumulated data support that alterations in the membrane cholesterol as a consequence of increased ABCA1 expression may also affect other critical steps in the infection cycle of several pathogens. Lipid rafts are membrane microdomains enriched in cholesterol and glycosphingolipids that concentrate molecules specifically targeted by a number of microorganisms for host cell binding, invasion, or dissemination, as well as receptors that initiate signaling pathways in host cells in response to environmental stimuli [58]. Indeed, a number of pathogens disrupt cellular cholesterol homeostasis either to promote lipid raft formation and gain entry into host cells or to hijack host cell signaling pathways that facilitate intracellular survival/replication [59]. For example, human immunodeficiency virus (HIV)-1, via its protein Nef, diminished cholesterol efflux from macrophages by modulating the post-transcriptional expression of ABCA1 and its redistribution, thus facilitating viral infectivity [60]. Reciprocally, the activation of the LXR-ABCA1 axis resulted in antiviral effects against HIV-1, including inhibitory effects on viral entry into human $CD4^+$ T cells [61], on virus production and the fusion activity of the virions [62], and on the capability of human dendritic cells to capture HIV-1 and trans-infect T cells [63]. Furthermore, pharmacological treatment with an LXR agonist reduced the viral load in humanized models of HIV infection in mice [62,64]. The antiviral effects were not exclusive for HIV infection, as the control of cholesterol homeostasis by the LXR-ABCA1 pathway also impacted the capability of hepatitis C virus (HCV) to establish virus-host cell fusions and consequently enter the liver cells [65], as well as both the entry and replication capacity of Newcastle disease virus (NDV) [66].

In addition to mechanisms for cholesterol efflux, LXRs control cholesterol uptake through the transcriptional upregulation of IDOL, an E3 ubiquitin ligase that triggers the ubiquitination and degradation of several members of the low-density lipoprotein receptor (LDLR) family [67]. Therefore, the role of IDOL in lowering intracellular cholesterol could help, in combination with the activity of ABCA1/G1, reduce the infectivity and/or growth of some pathogens. Moreover, HCV associates with lipoproteins and benefits from the surface expression of the LDLR to infect hepatocytes (reviewed in [68]). As the overexpression of IDOL inhibited the infection of human hepatocytes with HCV [69], it is plausible that a reduction in the LDLR levels represents an additional mechanism mediating the inhibitory actions of LXR agonists on HCV entry into host cells.

Most studies exploring the role of synthetic LXR agonists in viral infection have not addressed the exact contribution of LXR isoforms. However, the expression of at least LXRα (in the absence of pharmacological treatment) was required to restrict the reactivation of gammaherpesvirus in chronically infected mice [70]. LXRα-deficiency resulted in viral reactivation in peritoneal cells, but not in splenocytes, despite intact virus-specific T cell responses.

The recent discovery of the multifunctional protein CD38 as an additional LXR transcriptional target provided new insights to the way LXR agonists control bacterial infection [6•,71•]. Indeed, CD38 exerts multiple roles in the regulation of the immune response to pathogens [72]. Its expression in cells originating at the bone marrow was required for LXR agonists to ameliorate the clinical severity of *S.* Typhimurium infection in mice [71•]. These effects were consistent with the reduced internalization of *S.* Typhimurium by macrophages [71•] and may be influenced by an enhanced migratory potential of

dendritic cells [6•] upon activation of the LXR-CD38 axis. CD38 displays strong NADase activity, being able to modulate cellular NAD^+ homeostasis while generating calcium-mobilizing second messengers [73]. It also exerts important receptorial and accessory functions in immune cells. Interestingly, the effects of LXR agonists on bacterial cell internalization were overcome with exogenous supplementation of NAD^+ [71•], highlighting the potential significance of intracellular NAD^+ levels in host cell–pathogen interaction. Whether the effects in NAD^+ metabolism cooperate with other LXR-mediated metabolic changes in the control of infection has not been determined. In addition, the contribution of the LXR-CD38 axis in controlling the progression of other types of infection requires investigation.

The LXR pathway can also impact the course of infection through mechanisms based on transcriptional repression. As an example, LXR agonists repressed the basal transcription of HIV-1 in infected macrophages and counteracted HIV-1 replication in response to TLR signaling. These effects were mediated by preventing the release of the corepressor NCoR and inhibiting the recruitment of NF-κB, AP1 components, and CBP to the proviral DNA [74]. Additionally, the repression of pro-inflammatory genes was also proposed as a potential mechanism to downregulate the activation of HIV-1 expression in infected cells.

In line with anti-inflammatory effects in the context of endotoxemia [75], LXR agonists reduced organ dysfunction and mortality associated with sepsis in rodent models [51,52]. The functional expression of silent mating type information regulation 2 homolog (SIRT)-1 was required for the protective effects of LXR agonists on myocardial function in septic mice, which coincided with a reduction in NF-κB activity, oxidative stress, and myocardial cell apoptosis, although the mechanism leading to increased SIRT-1 transcription/activation was not defined [51]. In addition, evidence was provided for a selective role of LXRα, but not of LXRβ, in the protection against liver injury during sepsis [52], which contrasts with the role of LXRβ in ameliorating the hepatic acute response [22]. In general, these observations argue that the LXR pathway plays a role in limiting exacerbated tissue damage due to infection. However, in a different study, LXR agonism increased sepsis-induced mortality in mice due to an impairment of neutrophil infiltration to the infection site [5], raising the possibility that the outcome of LXR activation in sepsis depends on additional factors, which warrants further investigation.

In contrast to the predominant protective effects of the LXR pathway on bacterial and viral infections, the anti-inflammatory environment potentiated by LXR agonists may be a favorable scenario for certain pathogens. In this regard, LXR deficiency conferred resistance to the parasite *Leishmania chagasi/infantum* [76], despite the fact that *Leishmania* spp. are NAD^+ auxotrophs and highly sensitive to the host cell membrane cholesterol for infection [77]. Resistance to infection was associated with increased production of nitric oxide and IL-1β and augmented parasite killing by LXR-deficient macrophages [76]. Similarly, LXR agonists enhanced mortality during *Klebsiella pneumoniae* infection in mice, which correlated with the changes in the course of infiltration of neutrophils to the infected lungs [78]. The inhibition of chemokine-induced RhoA activation was proposed as a potential underlying mechanism.

Putting together all of the pieces of evidence obtained from the different models of infection, the modulation of inflammatory and metabolic responses by LXRs has different consequences depending on the pathogen. Therefore, targeting the LXR pathway as a strategy against infection must take into account the multiple mechanisms contributing to the effects of LXRs in host cell–pathogen interaction.

Conclusions and future perspectives

Because of the emergence of antimicrobial resistances and the absence of effective vaccines for a large number of pathogens, one of the major necessities in public health is the development of innovative host-directed therapies (HDTs) against infection. LXRs, by virtue of their condition as druggable targets and their multiple roles at the intersection between metabolism and inflammation, are promising candidates for HDT.

As summarized in this review, LXR activation exerts a protective role in many pre-clinical models of viral and bacterial infection. Different studies have focused on at least one molecular mechanism to explain these protective effects, but it is likely that several mechanisms cooperate simultaneously to reduce the capacity of infection of pathogens and the inflammatory response. As discussed here, some commonalities exist in relation to the metabolic resources hijacked by different pathogens. Accumulated evidence points toward the LXR pathway as part of the host response to modulate the metabolism of the infected cell and limit the infectivity and/or growth of intracellular pathogens, a role that can be boosted upon pharmacological LXR activation. In this regard, cholesterol metabolism is targeted by LXR agonists in a manner that is beneficial to limiting the infection, at least in animal and *in vitro* studies. Reciprocally, pathogens that are able to interfere with the capacity of LXRs to alter the host cell metabolism may benefit from a more favorable environment. In fact, there is significant evidence of the LXR pathway itself being modulated at the level of both expression and activity by signals derived from pathogen recognition or from cytokines produced at the infection site.

Table 1

Specific contributions of LXR isoforms to the control of inflammation and infection

Disease/cellular model	Trigger	Species	LXR isoform	Effects	Ref.
Macrophages (*in vitro*)	LPS; IFN-γ	Mouse	LXRα/LXRβ	Repression of inflammatory genes	[12,14]
Astrocytes (*in vitro*)	IFN-γ	Mouse	LXRα/LXRβ	Repression of inflammatory genes	[10]
Lupus-like autoimmunity (*in vivo*)	Aging	Mouse	LXRα/LXRβ	Protection from autoimmunity	[17]
Hepatic acute phase response (*in vivo*)	LPS	Mouse	LXRβ	Repression of acute phase response	[22]
Ear inflammation (*in vivo*)	TPA	Mouse	LXRβ	Inhibition of inflammation	[11]
Mast cells (*in vitro*)	LPS; FcεRI crosslinking	Mouse	LXRβ	Repression of inflammatory cytokine production	[24]
T cells (*in vivo*; *in vitro*)	Aging; mitogens	Mouse	LXRβ	Inhibition of proliferation	[3]
Antigen presenting cells (*in vivo*)	Cholesterol accumulation	Mouse	LXRβ	Limitation of B cell expansion	[39]
B cell lines (*in vitro*)	Basal conditions	Human	LXRα	Repression of BAFF production	[40]
Macrophages (*in vitro*)	*M. tuberculosis*	Human	LXRα/LXRβ	Limitation of mycobacterial infection	[43•]
M. tuberculosis infection *in vivo*	*M. tuberculosis*	Mouse	LXRα	Increased resistance to infection	[45]
L. monocytogenes infection *in vivo*	*L. monocytogenes*	Mouse	LXRα	Increased resistance to infection	[30]
GHV infection *in vivo*	GHV	Mouse	LXRα	Restriction of viral reactivation in peritoneal cells	[70]
Cecal ligation and puncture (*in vivo*)	Sepsis	Mouse	LXRα	Protection against liver injury	[52]

Ref., reference. TPA, phorbol 12-myristate-13-acetate.

In addition, excessive tissue damage due to an exacerbated immune response is a common feature in infection and in inflammatory disorders. Beyond its role in limiting the extent of infection, activated LXRs trigger mechanisms to keep the inflammatory response under control and to avoid excessive organ injury in pre-clinical studies.

Given their role at the intersection of lipid metabolism and immune responses, the effects of LXR activation in the context of infection have been studied in depth in macrophages. Indeed, despite their relevance in microbial killing and in the recruitment of immune cells to the site of infection, macrophages are commonly targeted by intracellular pathogens for their replication and dissemination [79]. Therefore, LXRs limit the extent of infection and restrict excessive inflammatory responses in a cell type that represents a selective niche for intracellular infection and, at the same time, is crucial for the preservation of tissue integrity. Despite the importance of LXRs in macrophage biology, this review also integrates data showing the beneficial effects of LXR agonists in other host cells that are targets of the infection, especially in the context of viral infection.

A major limitation in the use of LXR agonists is their adverse effects in pre-clinical models of disease due to the activation of a lipogenic program [80]. Based on hepatic LXRα as the main isoform involved in agonist-induced lipogenesis, attempts have been made to develop LXRβ-specific ligands to circumvent this problem (reviewed in Ref. [1]). However, this kind of approach would probably have limitations as a HDT against infection. Whereas the anti-inflammatory effects of LXR agonists depend on LXRβ in a number of disease models in mice, LXRα activity is essential for the development of protective immune responses against several types of infection (Table 1). Therefore, the development of more sophisticated agonists that are capable of promoting selective LXR functions while inhibiting specific targets [81••] and/or new routes of administration targeting specific immune compartments [82•] deserves further attention in the context of infection.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Estibaliz Glaría: Conceptualization, Writing - original draft, Writing - review & editing. **Nicole A Letelier:** Conceptualization, Writing - original draft, Writing - review & editing. **Annabel F Valledor:** Conceptualization, Writing - original draft, Supervision, Funding acquisition, Writing - review & editing.

Acknowledgements

This work was supported by grants from the Spanish Ministry of Economy and Competitivity (MINECO) to AFV (SAF2017-89510-R) and to the NuRCaMeIn network (SAF2017-90604REDT), and from Fundació La Marató de TV3 to AFV (201605-31). EG received a fellowship from the University of Barcelona (APIF). NAL received a fellowship from CONICYT (Comisión Nacional de Investigación Científica y Tecnología) (PFCHA/DOCTORADO BECAS CHILE/2016-72170639), Chilean Ministry of Education.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Schulman IG: **Liver X receptors link lipid metabolism and inflammation**. *FEBS Lett* 2017, **591**:2978-2991.

2. Walcher D, Kümmel A, Kehrle B, Bach H, Grüb M, Durst R, Hombach V, Marx N: **LXR activation reduces proinflammatory cytokine expression in human CD4-positive lymphocytes**. *Arterioscler Thromb Vasc Biol* 2006, **26**:1022-1028.

3. Bensinger SJ, Bradley MN, Joseph SB, Zelcer N, Janssen EM, Hausner MA, Shih R, Parks JS, Edwards PA, Jamieson BD *et al.*: **LXR signaling couples sterol metabolism to proliferation in the acquired immune response**. *Cell* 2008, **134**:97-111.

4. Diehl CJ, Barish GD, Downes M, Chou MY, Heinz S, Glass CK, Evans RM, Witztum JL: **Research resource: comparative nuclear receptor atlas: basal and activated peritoneal B-1 and B-2 cells**. *Mol Endocrinol* 2011, **25**:529-545.

5. Souto FO, Castanheira FVS, Trevelin SC, Lima BHF, Cebinelli GCM, Turato WM, Auxiliadora-Martins M, Basile-Filho A, Alves-Filho JC, Cunha FQ: **Liver X receptor activation impairs neutrophil functions and aggravates sepsis**. *J Infect Dis* 2020, **221**:1542-1553.

6. • Beceiro S, Pap A, Czimmerer Z, Sallam T, Guillén JA, Gallardo G, Hong C, A-Gonzalez N, Tabraue C, Diaz M *et al.*: **Liver X receptor nuclear receptors are transcriptional regulators of dendritic cell chemotaxis**. *Mol Cell Biol* 2018, **38** e00534-17.

Using gain- and loss-of-function models, this work shows that dendritic cells depend on functional LXRs to migrate *in vivo*. The study also defines CD38 as the molecular mechanism by which LXR agonists enhance dendritic cell chemotaxis.

7. • Ramón-Vázquez A, de la Rosa JV, Tabraue C, Lopez F, Díaz-Chico BN, Bosca L, Tontonoz P, Alemany S, Castrillo A: **Common and differential transcriptional actions of nuclear receptors liver X receptors α and β in macrophages**. *Mol Cell Biol* 2019, **39** e00376-18.

Through the design of immortalized macrophages expressing equivalent amounts of tagged LXRα and LXRβ in an LXR-deficient background, the authors characterize the genomic distribution and transcriptional capacity of each isoform. The study shows that LXRα and LXRβ control the transcription of receptor-exclusive sets of genes.

8. Wagner BL, Valledor AF, Shao G, Daige CL, Bischoff ED, Petrowski M, Jepsen K, Baek SH, Heyman RA, Rosenfeld MG *et al.*: **Promoter-specific roles for liver X receptor/corepressor complexes in the regulation of ABCA1 and SREBP1 gene expression**. *Mol Cell Biol* 2003, **23**:5780-5789.

9. Ghisletti S, Huang W, Ogawa S, Pascual G, Lin M-E, Willson TM, Rosenfeld MG, Glass CK: **Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma**. *Mol Cell* 2007, **25**:57-70.

10. Lee JH, Park SM, Kim OS, Lee CS, Woo JH, Park SJ, Joe E, Jou I: **Differential SUMOylation of LXRalpha and LXRbeta mediates transrepression of STAT1 inflammatory signaling in IFN-gamma-stimulated brain astrocytes**. *Mol Cell* 2009, **35**:806-817.

11. Fowler AJ, Sheu MY, Schmuth M, Kao J, Fluhr JW, Rhein L, Collins JL, Willson TM, Mangelsdorf DJ, Elias PM *et al.*: **Liver X receptor activators display anti-inflammatory activity in irritant and allergic contact dermatitis models: liver-X-receptor-specific inhibition of inflammation and primary cytokine production**. *J Invest Dermatol* 2003, **120**:246-255.

12. Joseph SB, Castrillo A, Laffitte BA, Mangelsdorf DJ, Tontonoz P: **Reciprocal regulation of inflammation and lipid metabolism by liver X receptors**. *Nat Med* 2003, **9**:213-219.

13. Hindinger C, Hinton DR, Kirwin SJ, Atkinson RD, Burnett ME, Bergmann CC, Stohlman SA: **Liver X receptor activation decreases the severity of experimental autoimmune encephalomyelitis**. *J Neurosci Res* 2006, **1234**:1225-1234.

14. Pascual-García M, Rué L, León T, Julve J, Carbó JM, Matalonga J, Auer H, Celada A, Escolà-Gil JC, Steffensen KR *et al.*: **Reciprocal negative cross-talk between liver X receptors (LXRs) and STAT1: effects on IFN-γ–induced inflammatory responses and LXR-dependent gene expression**. *J Immunol* 2013, **190**:6520-6532.

15. Han S, Zhuang H, Shumyak S, Wu J, Xie C, Li H, Yang LJ, Reeves WH: **Liver X receptor agonist therapy prevents diffuse alveolar hemorrhage in murine lupus by repolarizing macrophages**. *Front Immunol* 2018, **9**:135.

16. Park M-C, Kwon Y-J, Chung S-J, Park Y-B, Lee S-K: **Liver X receptor agonist prevents the evolution of collagen-induced arthritis in mice**. *Rheumatology (Oxford)* 2010, **49**:882-890.

17. A-Gonzalez N, Bensinger SJ, Hong C, Beceiro S, Bradley MN, Zelcer N, Deniz J, Ramirez C, Díaz M, Gallardo G *et al.*: **Apoptotic cells promote their own clearance and immune tolerance through activation of the nuclear receptor LXR**. *Immunity* 2009, **31**:245-258.

18. Jeon JY, Nam JY, Kim HA, Park YB, Bae SC, Suh CH: **Liver X receptors alpha gene (NR1H3) promoter polymorphisms are associated with systemic lupus erythematosus in Koreans**. *Arthritis Res Ther* 2014, **16**:R112.

19. Endo-Umeda K, Nakashima H, Komine-Aizawa S, Umeda N, Seki S, Makishima M: **Liver X receptors regulate hepatic F4/80 + CD11b+ kupffer cells/macrophages and innate immune responses in mice**. *Sci Rep* 2018, **8**:9281.

20. Ito A, Hong C, Rong X, Zhu X, Tarling EJ, Hedde PN, Gratton E, Parks J, Tontonoz P: **LXRs link metabolism to inflammation through Abca1-dependent regulation of membrane composition and TLR signaling**. *eLife* 2015, **4**:e08009.

21. Huang W, Ghisletti S, Saijo K, Gandhi M, Aouadi M, Tesz GJ, Zhang DX, Yao J, Czech MP, Goode BL *et al.*: **Coronin 2A mediates actin-dependent de-repression of inflammatory response genes**. *Nature* 2011, **470**:414-418.

22. Venteclef N, Jakobsson T, Ehrlund A, Damdimopoulos A, Mikkonen L, Ellis E, Nilsson L-M, Parini P, Jänne OA, Gustafsson J-A *et al.*: **GPS2-dependent corepressor/SUMO pathways govern anti-inflammatory actions of LRH-1 and LXRbeta in the hepatic acute phase response**. *Genes Dev* 2010, **24**:381-395.

23. Misu S, Takebayashi M, Miyamoto K: **Nuclear actin in development and transcriptional reprogramming**. *Front Genet* 2017, **8**:27.

24. Nunomura S, Okayama Y, Matsumoto K, Hashimoto N, Endo-Umeda K, Terui T, Makishima M, Ra C: **Activation of LXRs using the synthetic agonist GW3965 represses the production of pro-inflammatory cytokines by murine mast cells**. *Allergol Int* 2015, **64(Suppl)**:S11-S17.

25. Thomas DG, Doran AC, Fotakis P, Westerterp M, Antonson P, Jiang H, Jiang XC, Gustafsson JÅ, Tabas I, Tall AR: **LXR suppresses inflammatory gene expression and neutrophil migration through cis-repression and cholesterol efflux**. *Cell Rep* 2018, **25**:3774-3785.e4.

26. Spann NJ, Garmire LX, McDonald JG, Myers DS, Milne SB, Shibata N, Reichart D, Fox JN, Shaked I, Heudobler D *et al.*: **Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses**. *Cell* 2012, **151**:138-152.

27. Pourcet B, Gage MC, Leon TE, Waddington KE, Pello OM, Steffensen KR, Castrillo A, Valledor AF, Pineda-Torra I: **The nuclear receptor LXR modulates interleukin-18 levels in macrophages through multiple mechanisms**. *Sci Rep* 2016, **6**:25481.

28. Pourcet B, Feig JE, Vengrenyuk Y, Hobbs A, Kepka-Lenhart D, Garabedian M, Morris SM, Fisher EA, Pineda-Torra I: **LXRα regulates macrophage arginase 1 through PU.1 and interferon regulatory factor 8**. *Circ Res* 2011, **109**:492-501.

29. Valledor AF, Hsu LC, Ogawa S, Sawka-Verhelle D, Karin M, Glass CK: **Activation of liver X receptors and retinoid X receptors prevents bacterial-induced macrophage apoptosis**. *Proc Natl Acad Sci U S A* 2004, **101**:17813-17818.

30. Joseph SB, Bradley MN, Castrillo A, Bruhn KW, Mak PA, Pei L, Hogenesch J, O'connell RM, Cheng G, Saez E *et al.*: **LXR-dependent gene expression is important for macrophage survival and the innate immune response**. *Cell* 2004, **119**:299-309.

31. Sarrias MR, Roselló S, Sánchez-Barbero F, Sierra JM, Vila J, Yélamos J, Vives J, Casals C, Lozano F: **A role for human SPα as a pattern recognition receptor**. *J Biol Chem* 2005, **280**:35391-35398.

32. • Sanjurjo L, Aran G, Téllez É, Amézaga N, Armengol C, López D, Prats C, Sarrias MR: **CD5L promotes M2 macrophage polarization through autophagy-mediated upregulation of ID3**. *Front Immunol* 2018, **9**:480.

This paper establishes the relevance of CD5L as a potential target in therapeutic strategies aiming at modulating human macrophage polarization.

33. Geyeregger R, Zeyda M, Bauer W, Kriehuber E, Säemann MD, Zlabinger GJ, Maurer D, Stulnig TM: **Liver X receptors regulate dendritic cell phenotype and function through blocked induction of the actin-bundling protein fascin**. *Blood* 2007, **109**:4288-4295.

34. Töröcsik D, Baráth M, Benko S, Széles L, Dezso B, Póliska S, Hegyi Z, Homolya L, Szatmári I, Lányi A *et al.*: **Activation of liver X receptor sensitizes human dendritic cells to inflammatory stimuli**. *J Immunol* 2010, **184**:5456-5465.

35. Cui G, Qin X, Wu L, Zhang Y, Sheng X, Yu Q, Sheng H, Xi B, Zhang JZ, Zang YQ: **Liver X receptor (LXR) mediates negative regulation of mouse and human Th17 differentiation**. *J Clin Invest* 2011, **121**:658-670.

36. Yasuda K, Takeuchi Y, Hirota K: **The pathogenicity of Th17 cells in autoimmune diseases**. *Semin Immunopathol* 2019, **41**:283-297.

37. Solt LA, Kamenecka TM, Burris TP: **LXR-mediated inhibition of CD4+ T helper cells**. *PLoS One* 2012, **7**:e46615.

38. Herold M, Breuer J, Hucke S, Knolle P, Schwab N, Wiendl H, Klotz L: **Liver X receptor activation promotes differentiation of regulatory T cells**. *PLoS One* 2017, **12**:e0184985.

39. Ito A, Hong C, Oka K, Salazar JV, Diehl C, Witztum JL, Diaz M, Castrillo A, Bensinger SJ, Chan L *et al.*: **Cholesterol accumulation in CD11c+ immune cells is a causal and targetable factor in autoimmune disease**. *Immunity* 2016, **45**:1311-1326.

40. Huang Y, Fu X, Lyu X, Xu Z, He Z, Zhang Y, Zeng Y, He F, Huang G: **Activation of LXR attenuates collagen-induced arthritis via suppressing BLyS production**. *Clin Immunol* 2015, **161**:339-347.

41. Rong X, Albert CJ, Hong C, Duerr MA, Chamberlain BT, Tarling EJ, Ito A, Gao J, Wang B, Edwards PA *et al.*: **LXRs regulate ER stress and inflammation through dynamic modulation of membrane phospholipid composition**. *Cell Metab* 2013, **18**:685-697.

42. Eisenreich W, Rudel T, Heesemann J, Goebel W: **How viral and intracellular bacterial pathogens reprogram the metabolism of host cells to allow their intracellular replication**. *Front Cell Infect Microbiol* 2019, **9**:42.

43. • Ahsan F, Maertzdorf J, Guhlich-Bornhof U, Kaufmann SHE, Moura-Alves P: **IL-36/LXR axis modulates cholesterol metabolism and immune defense to *Mycobacterium tuberculosis***. *Sci Rep* 2018, **8**:1520.

This work describes a novel mechanism of crosstalk by which coordinated IL-36 and LXR signaling limit mycobacterial growth in human macrophages. The conclusions from this study may also have implications in other infection settings.

44. Bouttier M, Laperriere D, Memari B, Mangiapane J, Fiore A, Mitchell E, Verway M, Behr MA, Sladek R, Barreiro LB *et al.*: **Alu repeats as transcriptional regulatory platforms in macrophage responses to *M. tuberculosis* infection**. *Nucleic Acids Res* 2016, **44**:10571-10587.

45. Korf H, Vander Beken S, Romano M, Steffensen KR, Stijlemans B, Gustafsson J-Å, Grooten J, Huygen K: **Liver X receptors contribute to the protective immune response against *Mycobacterium tuberculosis* in mice**. *J Clin Invest* 2009, **119**:1626-1637.

46. Lange PT, Schorl C, Sahoo D, Tarakanova VL: **Liver X receptors suppress activity of cholesterol and fatty acid synthesis pathways to oppose gammaherpesvirus replication**. *mBio* 2018, **9**:e01115-18.

47. Dang EV, McDonald JG, Russell DW, Cyster JG: **Oxysterol restraint of cholesterol synthesis prevents AIM2 inflammasome activation**. *Cell* 2017, **171**:1057-1071.e11.

48. Park K, Scott AL: **Cholesterol 25-hydroxylase production by dendritic cells and macrophages is regulated by type I interferons**. *J Leukoc Biol* 2010, **88**:1081-1087.

49. Cyster JG, Dang EV, Reboldi A, Yi T: **25-Hydroxycholesterols in innate and adaptive immunity**. *Nat Rev Immunol* 2014, **14**:731-743.

50. Choi JY, Seo JY, Yoon YS, Lee YJ, Kim HS, Kang JL: **Mer signaling increases the abundance of the transcription factor LXR to promote the resolution of acute sterile inflammation**. *Sci Signal* 2015, **8**:ra21.

51. Han D, Li X, Li S, Su T, Fan L, Fan W-S, Qiao H-Y, Chen J-W, Fan M-M, Li X-J *et al.*: **Reduced silent information regulator 1 signaling exacerbates sepsis-induced myocardial injury and mitigates the protective effect of a liver X receptor agonist**. *Free Radic Biol Med* 2017, **113**:291-303.

52. Wang YY, Ryg U, Dahle MK, Steffensen KR, Thiemermann C, Chaudry IH, Reinholt FP, Collins JL, Nebb HI, Aasen AO *et al.*: **Liver X receptor protects against liver injury in sepsis caused by rodent cecal ligation and puncture**. *Surg Infect (Larchmt)* 2011, **12**:283-289.

53. Castrillo A, Joseph SB, Vaidya SA, Haberland M, Fogelman AM, Cheng G, Tontonoz P: **Crosstalk between LXR and toll-like receptor signaling mediates bacterial and viral antagonism of cholesterol metabolism**. *Mol Cell* 2003, **12**:805-816.

54. Sanjurjo L, Amézaga N, Vilaplana C, Cáceres N, Marzo E, Valeri M, Cardona PJ, Sarrias MR: **The scavenger protein apoptosis inhibitor of macrophages (AIM) potentiates the antimicrobial response against *Mycobacterium tuberculosis* by enhancing autophagy**. *PLoS One* 2013, **8**:e79670.

55. Long J, Roy RB, Zhang YJ, Antrobus R, Du Y, Smith DL, Weekes MP, Javid B: **Plasma membrane profiling reveals upregulation of ABCA1 by infected macrophages leading to restriction of mycobacterial growth**. *Front Microbiol* 2016, **7**:1086.

56. Huang L, Nazarova EV, Russell DG: ***Mycobacterium tuberculosis*: bacterial fitness within the host macrophage**. *Microbiol Spectr* 2019, **7**.

57. Chen S, Sorrentino R, Shimada K, Bulut Y, Doherty TM, Crother TR, Arditi M: ***Chlamydia pneumoniae* -induced foam cell formation requires MyD88-dependent and -independent signaling and is reciprocally modulated by liver X receptor activation**. *J Immunol* 2008, **181**:7186-7193.

58. Bukrinsky MI, Mukhamedova N, Sviridov D: **Lipid rafts and pathogens: the art of deception and exploitation**. *J Lipid Res* 2019, **61**:601-610.

59. Samanta D, Mulye M, Clemente TM, Justis AV, Gilk SD: **Manipulation of host cholesterol by obligate intracellular bacteria**. *Front Cell Infect Microbiol* 2017, **7**:165.

60. Mujawar Z, Rose H, Morrow MP, Pushkarsky T, Dubrovsky L, Mukhamedova N, Fu Y, Dart A, Orenstein JM, Bobryshev YV *et al.*: **Human immunodeficiency virus impairs reverse cholesterol transport from macrophages**. *PLoS Biol* 2006, **4**:e365.

61. Jiang H, Badralmaa Y, Yang J, Lempicki R, Hazen A, Natarajan V: **Retinoic acid and liver X receptor agonist synergistically inhibit HIV infection in CD4+ T cells by up-regulating ABCA1-mediated cholesterol efflux**. *Lipids Health Dis* 2012, **11**:69.

62. Morrow MP, Grant A, Mujawar Z, Dubrovsky L, Pushkarsky T, Kiselyeva Y, Jennelle L, Mukhamedova N, Remaley AT, Kashanchi F *et al.*: **Stimulation of the liver X receptor pathway inhibits HIV-1 replication via induction of ATP-binding cassette transporter A1**. *Mol Pharmacol* 2010, **78**:215-225.

63. Hanley TM, Blay Puryear W, Gummuluru S, Viglianti GA: **PPARgamma and LXR signaling inhibit dendritic cell-**

mediated HIV-1 capture and trans-infection. *PLoS Pathog* 2010, **6**:e1000981.

64. Ramezani A, Dubrovsky L, Pushkarsky T, Sviridov D, Karandish S, Raj DS, Fitzgerald ML, Bukrinsky M: **Stimulation of liver X receptor has potent anti-HIV effects in a humanized mouse model of HIV infection**. *J Pharmacol Exp Ther* 2015, **354**:376-383.

65. Bocchetta S, Maillard P, Yamamoto M, Gondeau C, Douam F, Lebreton S, Lagaye S, Pol S, Helle F, Plengpanich W *et al.*: **Up-regulation of the ATP-binding cassette transporter A1 inhibits hepatitis C virus infection**. *PLoS One* 2014, **9**:e92140.

66. Sheng Xiang-Xiang, Sun Ying-Jie, Zhan Yuan, Qu Yu-Rong, Wang Hua-Xia, Luo Miao, Liao Ying, Qiu Xu-Sheng, Ding Chan, Fan Hong-Jie *et al.*: **The LXR ligand GW3965 inhibits Newcastle disease virus infection by affecting cholesterol homeostasis**. *Arch Virol* 2016, **161**:2491-2501.

67. Zelcer N, Hong C, Boyadjian R, Tontonoz P: **LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor**. *Science* 2009, **325**:100-104.

68. Grassi G, Di Caprio G, Fimia GM, Ippolito G, Tripodi M, Alonzi T: **Hepatitis C virus relies on lipoproteins for its life cycle**. *World J Gastroenterol* 2016, **22**:1953-1965.

69. Zeng J, Wu Y, Liao Q, Li L, Chen X, Chen X: **Liver X receptors agonists impede hepatitis C virus infection in an Idol-dependent manner**. *Antiviral Res* 2012, **95**:245-256.

70. Lange PT, Jondle CN, Darrah EJ, Johnson KE, Tarakanova VL: **LXR alpha restricts gammaherpesvirus reactivation from latently infected peritoneal cells**. *J Virol* 2019, **93** e02071-18.

71. • Matalonga J, Glaria E, Bresque M, Escande C, Carbó JM, Kiefer K, Vicente R, León TE, Beceiro S, Pascual-García M *et al.*: **The nuclear receptor LXR limits bacterial infection of host macrophages through a mechanism that impacts cellular NAD metabolism**. *Cell Rep* 2017, **18**:1241-1255.

This report described for the first time that the multifunctional enzyme CD38 is a transcriptional target of LXR. Through upregulation of this enzyme, LXR agonists modulated the levels of intracellular NAD+, which might have physiological implications beyond the management of infection.

72. Glaría E, Valledor AF: **Roles of CD38 in the immune response to infection**. *Cells* 2020, **9**:228.

73. Chini EN, Chini CCS, Espindola Netto JM, de Oliveira GC, van Schooten W: **The pharmacology of CD38/NADase: an emerging target in cancer and diseases of aging**. *Trends Pharmacol Sci* 2018, **39**:424-436.

74. Hanley TM, Viglianti GA: **Nuclear receptor signaling inhibits HIV-1 replication in macrophages through multiple trans-repression mechanisms**. *J Virol* 2011, **85**:10834-10850.

75. Wang YY, Dahle MK, Steffensen KR, Reinholt FP, Collins JL, Thiemermann C, Aasen AO, Gustafsson JÅ, Wang JE: **Liver x receptor agonist GW3965 dose-dependently regulates lps-mediated liver injury and modulates posttranscriptional TNF-α production and p38 mitogen-activated protein kinase activation in liver macrophages**. *Shock* 2009, **32**:548-553.

76. Bruhn KW, Marathe C, Maretti-Mira AC, Nguyen H, Haskell J, Tran TA, Vanchinathan V, Gaur U, Wilson ME, Tontonoz P *et al.*: **LXR deficiency confers increased protection against visceral Leishmania infection in mice**. *PLoS Negl Trop Dis* 2010, **4**:e886.

77. Kumar GA, Jafurulla M, Chattopadhyay A: **The membrane as the gatekeeper of infection: cholesterol in host–pathogen interaction**. *Chem Phys Lipids* 2016, **199**:179-185.

78. Smoak K, Madenspacher J, Jeyaseelan S, Williams B, Dixon D, Poch KR, Nick JA, Worthen GS, Fessler MB: **Effects of liver X receptor agonist treatment on pulmonary inflammation and host defense**. *J Immunol* 2008, **180**:3305-3312.

79. Price JV, Vance RE: **The macrophage paradox**. *Immunity* 2014, **41**:685-693.

80. Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ *et al.*: **Role of LXRs in control of lipogenesis**. *Genes Dev* 2000, **14**:2831-2838.

81. •• Muse ED, Yu S, Edillor CR, Tao J, Spann NJ, Troutman TD, Seidman JS, Henke A, Roland JT, Ozeki KA *et al.*: **Cell-specific discrimination of desmosterol and desmosterol mimetics confers selective regulation of LXR and SREBP in macrophages**. *Proc Natl Acad Sci U S A* 2018, **115**:E4680-E4689.

This study reveals the capacity of certain endogenous LXR ligands to dissociate LXR functions in a cell-specific manner, which is important for the development of new agonists with diminished adverse effects.

82. • Cao E, Lindgren A, Martinsson S, Hu L, Lindfors L, Sigfridsson K, Skantze U, Michaëlsson E, Trevaskis NL, Porter CJH: **Promoting intestinal lymphatic transport targets a liver-X receptor (LXR) agonist (WAY-252,623) to lymphocytes and enhances immunomodulation**. *J Control Release* 2019, **296**:29-39.

This paper reports a new delivery route for an LXR agonist, in combination with a lymph-directing long chain lipid-based formulation, which targets the agonist specifically to lymphocytes. This type of approach may facilitate the modulation of immune responses while avoiding the adverse lipogenic effects of conventional LXR agonist administration.

antioxidants

MDPI

Article

Nicotinamide Prevents Apolipoprotein B-Containing Lipoprotein Oxidation, Inflammation and Atherosclerosis in Apolipoprotein E-Deficient Mice

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Received: 30 October 2020; Accepted: 18 November 2020; Published: 21 November 2020

Abstract: The potential of nicotinamide (NAM) to prevent atherosclerosis has not yet been examined. This study investigated the effect of NAM supplementation on the development of atherosclerosis in a mouse model of the disease. The development of aortic atherosclerosis was significantly reduced (NAM low dose: 45%; NAM high dose: 55%) in NAM-treated, apolipoprotein (Apo)E-deficient mice challenged with a Western diet for 4 weeks. NAM administration significantly increased (1.8-fold) the plasma concentration of proatherogenic ApoB-containing lipoproteins in NAM high-dose (HD)-treated mice compared with untreated mice. However, isolated ApoB-containing lipoproteins from NAM HD mice were less prone to oxidation than those of untreated mice. This result was consistent with the decreased (1.5-fold) concentration of oxidized low-density lipoproteins in this group. Immunohistochemical staining of aortas from NAM-treated mice showed significantly increased levels of IL-10 (NAM low-dose (LD): 1.3-fold; NAM HD: 1.2-fold), concomitant with a significant decrease in the relative expression of TNFα (NAM LD: −44%; NAM HD: −57%). An improved anti-inflammatory pattern was reproduced in macrophages cultured in the presence of NAM. Thus, dietary NAM supplementation in ApoE-deficient mice prevented the development of atherosclerosis and improved protection against ApoB-containing lipoprotein oxidation and aortic inflammation.

Keywords: macrophage; cytokine; ATP-binding cassette (ABC) transporters; niacinamide; vitamin B3; cardiovascular disease

1. Introduction

Coronary artery disease (CAD) represents one of the main outcomes of cardiovascular disease [1]. Atherosclerosis is an inflammatory process that is characterized by the infiltration of macrophages and other inflammatory cell subsets in the arterial wall and subsequently contributes to cardiovascular disease [2–4]. Statins have been proven to be effective at reducing cardiovascular disease-related mortality and morbidity [5]. However, a substantial risk of adverse cardiovascular outcomes persists [6,7]. Therefore, over the last few decades, researchers have expressed increasing interest in investigating other pharmacological agents to further decrease the residual cardiovascular disease risk [8].

Nicotinic acid (NA) administration produces favorable antiatherogenic effects in vivo [9–18] and on humans [19]. NA significantly reduced CAD progression or clinical cardiovascular events in several studies [20]. However, the results from these earlier clinical trials have not been replicated in more recent trials that also included statins [20]. The occurrence of adverse side effects of NA therapy has also limited its use in clinical practice.

Dietary supplementation with other vitamin B_3 derivatives, such as nicotinamide mononucleotide (NMN) and nicotinamide riboside (NR), confer favorable age-related vasoprotective effects by reversing endothelial dysfunction, attenuating oxidative stress, or rescuing age-related changes in gene expression [21–25]. However, these favorable effects have not been linked to changes in the inflammatory status of target tissues in these studies. Moreover, atherosclerosis was not directly assessed in these studies.

Nicotinamide (NAM), the amide form of vitamin B_3, has also been shown to exert potent anti-inflammatory effects on chronic inflammatory diseases, including intestinal [26] or dermatological diseases [27–30]. Consistent with these findings, a dietary deficiency of vitamin B_3 forms has been associated with different inflammatory processes, including dermatitis, irritation, inflammation of mucous membranes and tubular inflammation [31–35]. The anti-inflammatory effects of NAM have also been explored in vitro in immune cell types [36–45], which are involved in chronic inflammatory processes such as atherosclerosis. Notably, in one of these studies [35], the authors proposed that NAM may promote monocyte differentiation into macrophages with restricted inflammatory traits.

Increased oxidation often underlies inflammation and triggers cardiovascular disease [46]. Importantly, NAM also exerts a beneficial effect on oxidative stress [47]. Indeed, this vitamin B_3 form prevents both protein and lipid oxidation [48], at least in part through its ability to scavenge reactive oxidative species [47]. Antioxidant effects of other vitamin B_3 derivatives, such as NR [49] and NMN [18], on vascular cells have also been reported.

Based on experimental evidence, NAM might also protect against atherogenesis in vitro [36–45]; however, the effect of NAM has not yet been assessed in vivo. We therefore tested the hypothesis that the administration of NAM in vivo prevents the development of atherosclerosis in a murine model of substantial hypercholesterolemia and atherosclerosis.

2. Materials and Methods

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau (Procedure ID 10434). The methods were conducted in accordance with the approved guidelines. The effect of NAM supplementation on male apolipoprotein (Apo)E-deficient mice on a C57BL/6J genetic background was examined. Two doses of NAM (high dose: 1%; low dose: 0.25%) (cat#N0636, Merck KGaA, Darmstadt, Germany) were administered to mice in the drinking water, starting at the same time as the high-fat diet was initiated. The design of the intervention is shown in Figure S1.

The biochemical parameters were analyzed using commercial kits and adapted to a COBAS 6000/c501 autoanalyzer (Roche Diagnostics, Basel, Switzerland), as described in a previous study [50]. NAM and me-NAM levels were analyzed using high-performance liquid chromatography (HPLC) with mass spectrometry (MS). Plasma concentrations of cytokines (IL-10, IL-6, IL-4 and TNFα) were analyzed using Luminex xMAP® technology (Millipore Corporation, Billerica, MA, USA). The susceptibility of mouse lipoproteins to copper-induced lipid oxidation and the capacity of NAM to inhibit the oxidative modification of human low-density lipoproteins (LDL) were measured by monitoring the formation of conjugated dienes at a λ of 234 nm at 37 °C for 6 h using a BioTek Synergy HT spectrophotometer (BioTek Synergy, Winooski, VT, USA) [51]. Serum oxidized LDL (OxLDL) levels were determined using a murine OxLDL sandwich ELISA (cat# SEA527Mu-96T, Cloud-Clone Corp, Houston, TX, USA). Cellular cholesterol efflux induced by human high-density lipoprotein (HDL) was determined in vitro using [^{3}H]-cholesterol-labeled J774A.1 mouse macrophages (ATCC® TIB67™, Manassas, VA, USA), as previously described [52]. Radioactivity counts were measured in both the cell culture medium and cell extract, and the percentage of cholesterol efflux was calculated.

Proximal aortic atherosclerotic lesions in mice were evaluated in serial cryosections of optimal cutting temperature (OCT) compound-embedded preparations. Cryosections were stained with Oil Red O for lipids and the lesion area (surface area stained with Oil Red O) was quantified using AxioVision image analysis software (Zeiss, Jena, Germany). For immunohistochemical staining, thoracic aorta segments were fixed with a 10% neutral buffered formalin solution (cat# HT501128, Merck KGaA, Darmstadt, Germany). Seven-micrometer sections of paraffin-embedded tissue samples were incubated with mouse monoclonal antibodies against IL-10 (cat# GTX632359, diluted 1:50, v:v) and rabbit polyclonal antibodies against TNFα (cat# GTX110520, diluted 1:200, v:v) from GeneTex and stained with diaminobenzidine (DAB) in a Dako Autostainer Link 48 using the Dako EnVision+System-HRP-DAB-kit, according to the manufacturer's protocol. Slides were then dehydrated and coverslipped and images were obtained using a BX61 Olympus bright-field microscope. Images were quantified using ImageJ-Fiji software. RNA was extracted from tissues and reverse transcribed, and the relative mRNA levels of each gene were determined using fluorescence-based quantitative real time PCR (qPCR) (Supplementary Methods (Table S1–S4)).

The data are presented as medians (interquartile ranges). Statistical analyses were performed using GraphPad Prism software (GPAD, version 5.0, San Diego, CA, USA). The effects of NAM administration on gross and biochemical parameters, histological traits, and gene expression levels were determined using a nonparametric Kruskal–Wallis test followed by the Dunn multiple comparison test. Spearman's rho correlation coefficients were calculated to determine the correlations between atherosclerosis and parameters of lipoprotein function, with all parameters considered as nonparametric variables. Differences between groups were considered statistically significant when the p value was <0.05. Additional details about the methods are available in the online version of the paper (Supplementary Methods (Table S1–S4)).

3. Results

3.1. Effect of NAM on Gross Parameters and Systemic Phenotype

Plasma NAM concentrations were increased in a dose-dependent manner (NAM low-dose (LD): ~48-fold, $p < 0.05$; NAM high-dose (HD): ~145-fold, $p < 0.05$) in NAM-treated mice (Table 1). The plasma level of the methylated form of NAM (me-NAM) was also increased (~6-fold, $p < 0.05$) in mice receiving the maximal dose. Body weight gain was significantly reduced only in mice receiving the highest dose of NAM ($p < 0.05$) (Table 1). The latter was not accompanied by changes in daily food intake (Table 1). Supplementation with NAM did not affect the liver size (Table 1). Plasma levels of alanine aminotransferase were reduced (NAM LD: 40%, NAM HD: 56%; $p < 0.05$) in NAM-treated mice compared to untreated mice, while plasma aspartate aminotransferase levels did not differ among groups. Plasma creatinine concentrations did not change with NAM treatment (Table 1). Plasma

glucose and insulin levels in NAM HD-treated mice did not significantly differ from the levels in untreated mice. However, total cholesterol levels were significantly increased in NAM HD-treated mice (~1.8-fold, $p < 0.05$) compared to untreated mice, exclusively due to increased levels of the non-HDL cholesterol fraction (Table 1). Notably, plasma cholesterol and non-HDL cholesterol levels were approximately 0.7-fold lower in NAM LD-treated mice than in NAM HD-treated mice, which should be considered in the interpretation of the analysis of atherosclerosis.

Table 1. Effect of NAM administration on gross parameters and plasma biochemical parameters of male ApoE-deficient mice.

Parameters	Untreated	NAM LD	NAM HD	*p*
Gross parameters				
Body weight (g)	28.7 (27.0; 29.9)	27.4 (23.3; 28.1)	24.6 (22.3; 26.0) *	<0.05
Liver weight (g)	1.3 (1.2; 1.4)	1.3 (1.2;1.4)	1.2 (1.1;1.4)	0.20
Diet intake (g/day)	2.5 (2.4; 2.8)	2.7 (2.6; 2.8)	2.4 (2.1; 2.5) †	<0.05
Water intake (g/day)	3.9 (3.2; 5.1)	4.6 (4.4; 4.8)	4.6 (3.8; 5.4)	0.20
Calculated dose of NAM (g/kg/day)	-	0.5 (0.4; 0.7)	1.9 (1.6; 2.2) †	<0.05
Plasma biochemistry				
NAM (μM)	4.0 (3.3; 5.5)	193.5 (90.5, 248.5)	580.0 (526.0; 605.0) *	<0.05
me-NAM (relative values) ($\times 10^{-3}$) [a]	0.15 (0.13; 0.16)	0.28 (0.19; 0.31)	0.85 (0.45; 0.89) *	<0.05
Glucose (mM)	12.3 (10.6; 15.3)	11.1 (9.4; 11.8)	10.9 (8.4; 12.9)	0.17
Insulin (μg/L)	0.7 (0.6; 0.8)	n. d.	0.7 (0.6; 0.7)	0.32
Triglycerides (mM)	0.5 (0.4; 1.0)	1.8 (1.3; 2.5) *	1.5 (1.1; 2.3) *	<0.05
Total cholesterol (mM)	43.3 (33.7; 47.4)	53.0 (44.2; 55.2)	77.6 (71.6, 82.3) * †	<0.05
Non-HDL cholesterol (mM)	43.1 (33.7; 47.0)	52.6 (43.8; 54.9)	77.3 (71.2, 82.0) * †	<0.05
HDL cholesterol (mM)	0.2 (0.1; 0.4)	0.4 (0.3; 0.4)	0.4 (0.1; 0.5)	0.45
AST (U/L)	61 (24;128)	25 (19; 39)	63 (27; 117)	0.33
ALT (U/L)	16 (7; 48)	5 (3; 6) *	6 (2; 11) *	<0.05
Creatinine (mM)	0.02 (0.01; 0.03)	0.01 (0.01; 0.02)	0.01 (0.01; 0.02)	0.16

Results are reported as medians (interquartile ranges) ($n = 8$ mice per group). All analyses were conducted in three-month-old mice. At two months of age, the mice were challenged with a Western diet, and treated with or without NAM for 1 month. Food and water intake was measured at the end of the study as described in the Materials and Methods section (Supplementary Methods (Table S1–S4)). Plasma concentration of NAM was expressed in μM, whereas that of me-NAM were shown in relative values ($n = 5$–6 mice per group). Plasma levels of the HDL fractions were determined in the plasma supernatants after precipitating with phosphotungstic acid (Roche); the non-HDL fraction was calculated by subtracting the HDL moiety from the total plasma. Statistically significant differences among groups for each variable were determined using a nonparametric a Kruskal–Wallis test followed by Dunn's posttest; differences were considered significant when $p < 0.05$. Specifically, * $p < 0.05$ vs. Untreated group; † $p < 0.05$ vs. NAM LD group. Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; NAM, nicotinamide, me-NAM, methylated form of nicotinamide; NAM LD, low-dose, NAM-treated mice; NAM HD, high-dose, NAM-treated mice, HDL, high-density lipoprotein; n. d., not determined. [a] relative units.

3.2. NAM Administration Prevents the Development of Aortic Atherosclerosis

NAM supplementation significantly decreased the areas of aortic atherosclerotic lesions in treated male mice (NAM LD: 0.55-fold, −45%, $p < 0.05$; NAM HD: 0.45-fold, −55%, $p < 0.05$) compared with untreated mice (Figure 1 and Figure S2, panel (a)). Representative images of the lesions observed in each group are shown. Mice treated with NAM developed less advanced atherosclerotic lesions that were mainly restricted to aortic valves compared with the larger lesions that extended to the free aortic wall in untreated mice. The areas of aortic atherosclerotic lesions were also significantly reduced in female NAM-treated mice (NAM LD: 0.6-fold, $p < 0.05$; NAM HD: 0.3-fold, $p < 0.05$) compared with untreated mice (Figure S2, panels (b)–(d)).

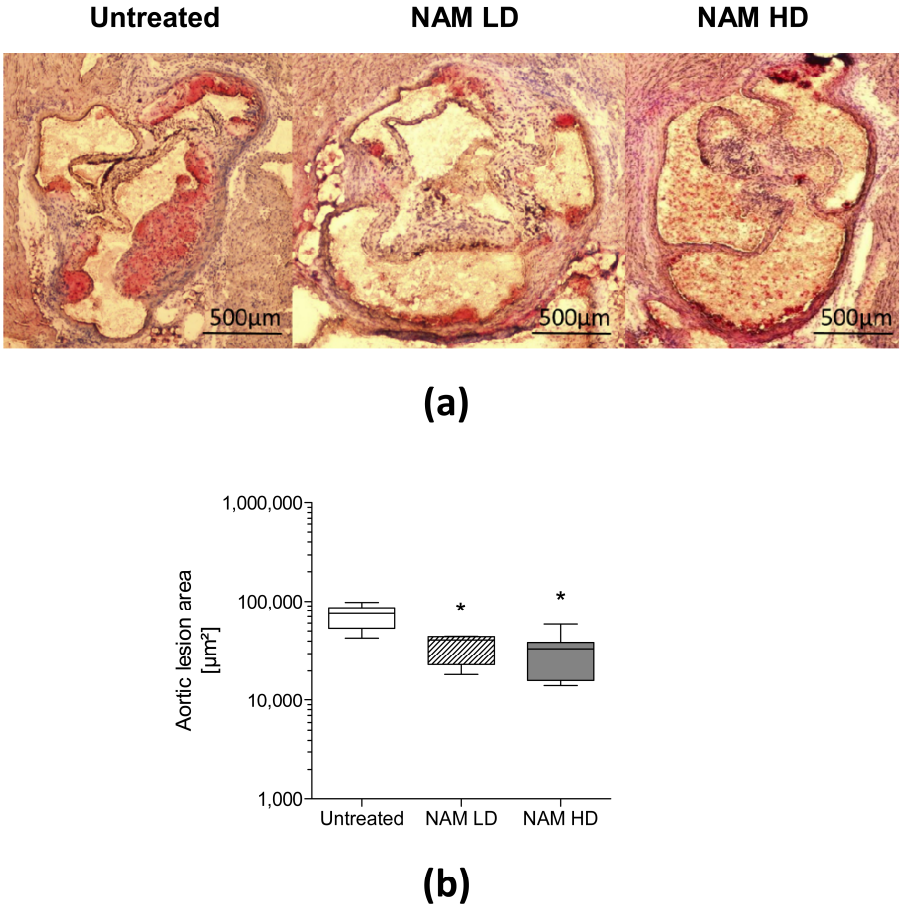

Figure 1. Analysis of proximal aortic lesions in apolipoprotein (Apo)E-deficient mice treated with different doses of NAM. (**a**) Representative images of aortic atherosclerotic lesion in 12-week-old mice challenged to a western diet and NAM over 1 month at 2 months of age. (**b**) Area of proximal aortic lesion quantified from 8 mice per group. Data are expressed as the median (interquartile range) of four consecutive sections throughout the aortic sinus that were obtained every 20 µm when aortic valves became visible. Statistically significant differences among groups for each variable were determined using a nonparametric a Kruskal–Wallis test followed by Dunn's posttest. Differences were considered significant when $p < 0.05$. Specifically, * $p < 0.05$ vs. Untreated group. Abbreviations used: NAM LD, low-dose, NAM-treated mice; NAM HD, high-dose, NAM-treated mice.

The area of atherosclerotic plaques was inversely correlated with the plasma NAM concentration (Spearman's r = −0.45, $p < 0.05$, $n = 24$).

3.3. NAM Administration Directly Protects against the Oxidation of Non-HDL Lipoproteins

The oxidative modification of LDL leads to cholesterol accumulation in macrophages and foam cell formation during atherosclerosis [53]. Notably, the susceptibility of non-HDL particles to oxidation, as measured by conjugated diene formation, was significantly delayed (~2-fold, $p < 0.05$) in NAM HD-treated mice (Figure 2, panels (a) and (b)).

Serum oxLDL concentrations were lower (~35%, $p < 0.05$) in the NAM HD mice than in untreated mice, concomitant with the elevated levels of non-HDL cholesterol in these mice (Figure 2, panel (c)). However, serum oxLDL levels were not associated with the area of atherosclerotic lesions (Figure S3, panel (a)).

The LDL fraction was isolated, and the effect of NAM was analyzed in vitro to determine whether NAM directly prevented LDL oxidation. NAM protected human LDL from oxidation (Figure 2, panel (d)), as observed by a dose-dependent decrease in the rate of conjugated diene production.

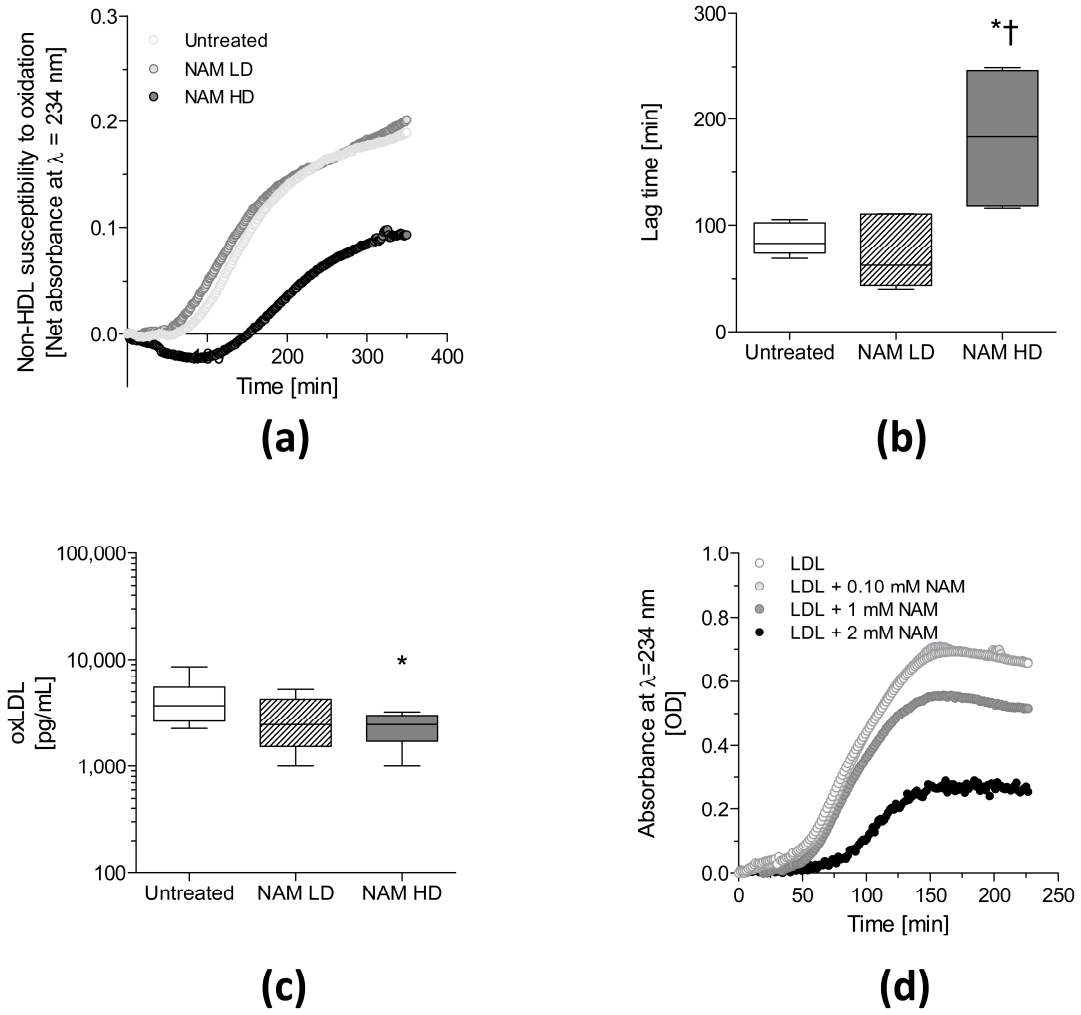

Figure 2. Antioxidant effects of NAM on ApoE-deficient mice. Two-month-old male ApoE-deficient mice were challenged with a Western diet and NAM for 1 month. (**a**) Representative diene formation curves of non-HDL particles. (**b**) Median diene formation lag time calculated from the oxidation curves (n = 3–4 plasma pools/group). (**c**) Serum oxLDL concentration (n = 8 per group). (**d**) Oxidation curves of human LDL incubated at NAM concentration 0.10 mM and 1.0 mM. This experiment was replicated twice with similar outcomes. Oxidation kinetics were carried using plasma lipoproteins isolated by sequential ultracentrifugation from pools of 2–3 mouse plasmas of each experimental group, panels (**a**,**b**), or pooled human plasma, panel (**d**), as appropriate. In panels (**b**,**c**), data are expressed as medians (interquartile ranges). Statistically significant differences among groups for each variable were determined using a nonparametric a Kruskal–Wallis test followed by Dunn's posttest. Differences were considered significant when $p < 0.05$. Specifically, * $p < 0.05$ vs. Untreated group or † $p < 0.05$ vs. NAM LD-treated mice. Abbreviations used: NAM LD, low-dose, NAM-treated mice; NAM HD, high-dose, NAM-treated mice; OD, optical density.

3.4. The NAM Treatment Improves Plasma and Aortic Inflammation

Plasma concentrations of the anti-inflammatory cytokine IL-10 were significantly increased (NAM HD: ~4-fold, $p < 0.05$) in NAM HD-treated mice compared with untreated mice (Figure 3, panel (a)), whereas the circulating IL-4, TNFα and IL-6 levels did not differ significantly among groups.

The gene expression analysis revealed a significant increase (two-fold, $p < 0.05$) in the relative levels of the *Il10* mRNA in the aorta of NAM HD-treated mice compared with untreated mice (Figure 3, panel (b)). However, the levels of the *Tnfa* mRNA were unchanged. The expression of the *Adgre1* mRNA, which encodes the F4/80 macrophage marker, did not differ among groups, suggesting that

macrophage infiltration was not altered by NAM. Both the plasma concentration of IL-10 and relative levels of the *Il10* mRNA in the aorta were inversely correlated with atherosclerosis in ApoE-deficient mice (plasma IL-10 concentration: Spearman's r = −0.46, $p < 0.05$; aortic expression of the *Il10* mRNA: Spearman's r = −0.56, $p < 0.05$) (Figure 3, panels (c) and (d)). Immunohistochemical staining of thoracoabdominal aortas showed an increase in the relative abundance of IL-10 in both NAM LD-(~1.3-fold, $p < 0.05$) and NAM HD-treated mice (~1.2-fold, $p < 0.05$) compared with untreated mice (Figure 3, panel (e)). Importantly, the relative abundance of TNFα in thoracoabdominal aortas was significantly decreased in both groups of treated mice (NAM LD: ~0.7-fold, $p < 0.05$; NAM HD: ~0.5-fold, $p < 0.05$) compared with untreated mice (Figure 3, panel (e)). Negative controls further validated the results of immunohistochemical staining in aortic tissue (Figure S5). These observations suggest the existence of posttranscriptional mechanisms that modulate the levels of IL-10 and TNFα in the aorta. Only the relative thoracoabdominal aortic level of IL-10 showed a marginal trend towards an inverse correlation with atherosclerosis in ApoE-deficient mice (plasma IL-10 level: Spearman's r = −0.47, $p = 0.06$) (Figure S3, panels (b) and (c)). The expression of the *Tnfa*, *Il6* and *Il1b* mRNAs was significantly reduced in lipopolysaccharide (LPS)-activated J774A.1 macrophages incubated with different NAM doses in a dose-dependent manner (Figure 3, panel (f)). Unfortunately, the levels of the *Il10* mRNA were undetectable in these cells.

As a precursor of NAD+ [54], NAM conceivably increases sirtuin (SIRT)1 activity in aortic tissue. Notably, SIRT1 induces liver X receptor (LXR) function [55], which in turn controls the expression of key transporters, i.e., *Abca1* and *abcg1* in macrophages [56,57], involved in cholesterol efflux. Thus, the relative mRNA levels of the abovementioned LXR targets were directly determined in thoracoabdominal aortas and cultured macrophages (Figure S4). *Abca1* was upregulated in NAM LD-(~2-fold, $P < 0.05$) and NAM HD-treated mice (~4-fold, $p < 0.05$), whereas *Abcg1* expression was marginally increased in NAM HD-treated mice (2-fold, $p = 0.07$) (Figure S4, panel (a)). Interestingly, the NAM HD treatment induced *Nr1h2* expression (encoding LXRβ) (1.7-fold, $p < 0.05$) without changing the expression of *Nr1h3* (encoding LXRα) (Figure S4, panel (a)). Only the relative levels of the *Abca1* mRNA were inversely correlated with aortic atherosclerosis (*Abca1* mRNA: Spearman's r = −0.42, $p < 0.05$), while relative levels of the *Abcg1* mRNA only showed a nonsignificant trend towards an association with the area of atherosclerotic lesions (Spearman's r = −0.40, $p = 0.06$) (Figure S4, panels (b) and (c)). Similarly, the *Abca1* mRNA was upregulated in cultured macrophages exposed to NAM (Figure S4, panel (d)); only a marginal effect was observed on *Abcg1* expression. Moreover, the cholesterol efflux capacity induced by a common source of human HDL showed a moderate, but not significant, increasing trend ($p = 0.07$) in NAM-treated cells (at the highest concentration assayed, 10 mM) compared with untreated cells (Figure S4, panel (e)).

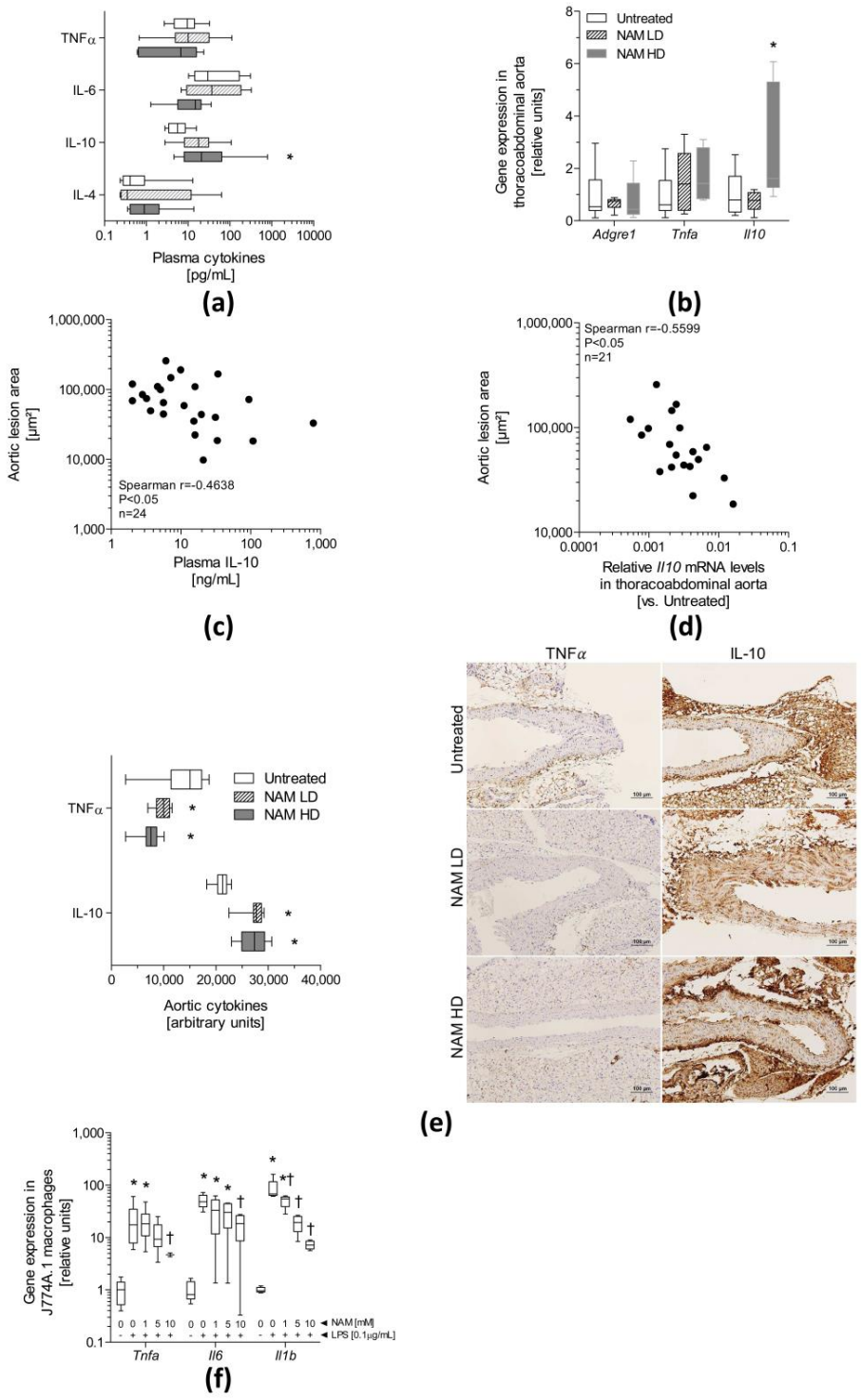

Figure 3. Anti-inflammatory effects of NAM on ApoE-deficient mice and cultured macrophages. Two-month-old male ApoE-deficient mice were challenged with a Western diet and NAM for 1 month. (**a**) Plasma concentration of cytokines (TNFα, IL-6, IL-10, and IL-4) in ApoE-deficient mice (n = 5–6). (**b**) Relative aortic mRNA levels of inflammatory targets. (**c**) Correlation between the area of proximal aortic lesions and circulating IL-10 levels. (**d**) Correlation between the area of proximal aortic lesions and *Il10* aortic mRNA levels. (**e**) Immunohistochemical (IHC) analysis of TNFα and IL-10 levels in the aorta. Left panel, bar chart showing the relative (arbitrary units) expression of TNFα and IL-10 in the aortas from different groups (n = 5–6 mice per group). Right panel, representative images of immunohistochemical staining for IL-10 and TNFα in thoracoabdominal aortas. (**f**) Relative mRNA levels of cytokines assessed in LPS-activated J774A.1 macrophages exposed to different doses of NAM

for 24 h. Data are expressed as the median (interquartile range) of 4 independent experiments. In panels (**a**,**b**,**e**), data are presented as medians (interquartile ranges) (n = 5–6 samples/group) and from 4 independent experiments in panel F. Statistically significant differences among groups for each variable were determined using a nonparametric a Kruskal–Wallis test followed by Dunn's posttest. Differences were considered significant when $p < 0.05$. Specifically, * $p < 0.05$ vs. Untreated group or † $p < 0.05$ vs. NAM HD-treated mice. In panels (**c**,**d**), the relationship between parameters was tested using a nonparametric Spearman's correlation test. Mice of all groups were considered for analysis. Abbreviations used: LPS, Lipopolysaccharide; NAM, nicotinamide; NAM LD, low-dose, NAM-treated mice; NAM HD, high-dose, NAM-treated mice.

4. Discussion

Based on our data, the administration of NAM prevented atherosclerosis and inflammation, despite the substantial concomitant increase in plasma non-HDL-cholesterol levels. Inflammation is frequently characterized by increased plasma concentrations of a number of pro-inflammatory markers (e.g., IL-6 and TNFα) and decreased levels of anti-inflammatory cytokines, such as IL-10 [38]. The hypothesis that NAM is an anti-inflammatory factor has also been supported by different studies [36–45]. As shown in the present study, plasma concentrations of IL-10 were significantly increased in NAM-treated ApoE-deficient mice. Notably, the relative concentrations of the IL-10 and TNFα proteins were significantly altered in the aortas of NAM-treated mice. This finding is consistent with previous data [39] showing an effect of NAM on inhibiting *Tnfa* synthesis and secretion in vitro. As TNFα is mainly released by activated M1 macrophages [58], NAM might predominantly exert its anti-inflammatory effect by reducing TNFα synthesis and increasing IL-10 production in resident macrophages in the aorta. Although the infiltration and activation of immune cells is a characteristic of chronic inflammation processes, such as atherosclerosis [59], the analyses of different molecular surrogates of macrophage infiltration, i.e., *Adgre1* and *Cd68*, in the present study did not provide evidence suggesting the differential accumulation of macrophages in the aortas of ApoE-deficient mice.

Oxidative stress is usually regarded as a pro-inflammatory condition [60]. Conceivably, the anti-inflammatory effect of NAM may also involve antioxidant mechanisms. Indeed, NAM has been defined as an O· radical scavenger and may also inhibit free radical (e.g., NO·, O·, and HCLO·) generation [47], protecting against both protein oxidation and lipid peroxidation induced by reactive oxygen species [46]. Although non-HDL cholesterol accumulated in the plasma of NAM HD-treated, ApoE-deficient mice, mainly due to impaired plasma clearance [61], these lipoproteins were less susceptible to oxidation than the lipoproteins from untreated mice. This direct antioxidant effect of NAM might contribute to preventing atherosclerosis development, despite the increase in the plasma non-HDL level. As previously reported [59], the mechanistic basis for the hypercholesterolemic effect of NAM HD is due to a delayed clearance of non-HDL lipoproteins that appears to depend strongly on the lack of ApoE, as it has not been observed in wildtype mice (Méndez-Lara et al. unpublished data) treated with a similar NAM dosage.

Unlike NAM, the administration of other dietary vitamin B_3-related metabolites (i.e., NMN and NR) has previously been reported to protect the vasculature from oxidative stress [22,23]. As NMN and NR are NAD+ intermediaries [54], this effect might be at least partially attributed to SIRT1 signaling in target tissues [23]. NAM is also a NAD+ precursor [54]; therefore, its administration might provide tissues with an extra source of NAD+ and induce SIRT1 activity in the aortic tissues of treated mice. Although many studies have been designed based on the premise that NAM functions as a potent inhibitor of SIRT1 in vitro, compelling evidence now suggests that NAM may exert the opposite effect in vivo [62]. For instance, NAM, by virtue of its role as an NAD+ precursor [54], would potentially drive SIRT1 activity by increasing cellular NAD + pools [63]. Some key ABC transporters (i.e., *Abca1* and *Abcg1*) involved in the first step of reverse cholesterol transport, cholesterol efflux [64], were positively upregulated in the aortas of NAM-treated mice (Supplementary Figure S4). Interestingly, *Abca1* and *Abcg1* expression are controlled by the nuclear receptor LXR, suggesting that this signaling

pathway might be induced in NAM HD-treated mice [55]. The observation that only the highest dose of NAM exerted the main effects on gene expression suggests that NAM functions as a weak LXR activator. Moreover, the levels of the *Nr1h2* mRNA, which encodes LXRβ, were significantly increased in aortas from NAM HD-treated mice (*Nr1h2:* two-fold, $p < 0.05$) compared with untreated mice. LXRα, which is encoded by the *Nr1h3* gene, may regulate its own expression in human macrophages [65,66], but not the expression of *Nr1h2* [65]. LXRβ activation is sufficient to reduce atherosclerosis [67] and may contribute [68], together with LXRα, to the favorable upregulation of *Abca1* and *Abcg1* expression both in vivo and in vitro.

Cholesterol efflux from plaque macrophages is an important process contributing to the removal of excess cholesterol from the artery wall. However, cholesterol efflux is only the first step of the overall process, and, importantly, macrophage-specific reverse cholesterol transport to feces in vivo (m-RCT) was impaired in NAM HD-treated ApoE-deficient mice [61]. Therefore, delayed plasma clearance of non-HDL might underlie defective m-RCT [61], as observed in LDL receptor knockout mice [69]. Overall, the detrimental effect of NAM HD treatment on m-RCT, in the context of a concomitantly severely worsening hyperlipidemia, highlights the antiatherogenic power of this compound at least in mice. Our data reveal a common NAM-related change in the gene expression pattern of cholesterol transporters and anti-inflammatory cytokines in the aortas of treated mice and macrophages. Accumulating evidence supports the hypothesis of molecular crosstalk between the cholesterol transporters ABCA1/ABCG1 and the immune system that will provide a greater benefit in terms of alleviating inflammation than m-RCT in this case [70–75].

Limitations of the Study

The present study has several limitations. First, most of the experiments described in this study were only performed in male mice. However, we also provided evidence of decreased atherosclerosis in NAM HD-treated female mice. Second, some of the observed changes in mRNA levels without an examination of protein levels or functions do not necessarily reflect changes in the protein content and activity. Third, plasma NAM concentrations were not determined at the beginning of the experiment; however, genetically identical untreated ApoE-deficient mice had significantly lower plasma concentrations of NAM than NAM-treated mice. Fourth, me-NAM, a metabolic product of NAM [54], is also atheroprotective [76,77]. Thus, by increasing the plasma concentration of me-NAM, NAM administration might also contribute to preventing atherosclerosis development in vivo. Although we were unable to exclude a beneficial effect of me-NAM, its individual effect was not directly assessed in the present work. Fifth, the potential involvement of a SIRT1-mediated mechanism was only indirectly revealed by the increased expression of some target LXR genes in aortas of NAM HD-treated mice. Finally, the experimental design used in this study assessed the preventive but not the therapeutic effects of NAM on atherosclerosis. Thus, further studies are warranted to confirm and extend the present observations.

5. Conclusions

NAM supplementation prevents the formation of aortic lesions in ApoE-deficient mice in a dose-dependent manner, which is related to increases in the circulating and relative aortic levels of the IL-10 mRNA and protein, as well as reductions in the level of the TNFα protein in thoracoabdominal aortas, suggesting a switch towards anti-inflammatory macrophages. The susceptibility of non-HDL to oxidation was improved by NAM in vitro and in vivo, thus suggesting another mechanism by which NAM protects against the development of atherosclerosis in NAM-treated mice. The moderate induction of the expression of key cholesterol transporters involved in cholesterol removal in aortas of treated mice might reflect molecular crosstalk, although these changes would not result in increased m-RCT in this mouse model.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3921/9/11/1162/s1, Figure S1: Schemes of the experimental designs used to evaluate the effect of NAM on the development

of atherosclerosis, Figure S2: Analysis of atherosclerosis in proximal aortas of ApoE-deficient mice treated with different doses of NAM, Figure S3: Correlations between the levels of oxidized LDL (OxLDL) and cytokines in the thoracoabdominal aorta with atherosclerotic lesions in the proximal aorta of ApoE-deficient mice, Figure S4: Effect of NAM on the mRNA expression of molecular targets involved in cholesterol transport in the thoracoabdominal aorta of ApoE-deficient mice and in cultured macrophages, Figure S5: Negative controls for immunohistochemical staining and area fraction calculation with ImageJ software, Table S1: Transitions used in QqQ shown by different metabolites, Table S2: List of specific Taqman probes used for gene expression analysis, Table S3: List of primers sequences used for gene expression analysis by SYBR green, Table S4: Characteristics of primers used gene expression analysis by SYBR green, Supplementary methods.

Author Contributions: Conceptualization and design, K.A.M.-L., F.B.-V., and J.J.; methodology and data acquisition, K.A.M.-L., N.L., N.N.-N., N.F., E.M.G.D.-A., E.R.-M., D.S., V.P., J.C.E.-G., T.V.d.O., M.C., and J.J.; interpretation of data, K.A.M.-L., A.F.V., E.L., R.P.C.-M., F.B.-V., and J.J.; formal analysis, K.A.M.-L., A.F.V., R.P.C.-M., and J.J.; writing—original draft preparation, K.A.M.-L., F.B.-V., and J.J.; writing—review and editing, K.A.M.-L., A.F.V., F.B.-V., and J.J.; supervision, F.B.-V., and J.J.; funding acquisition, F.B.-V., A.F.V., and J.J. All authors read and approved the manuscript. F.B.-V. and J.J. are co-corresponding authors.

Funding: This work was funded by a grant from Fundación Española de Arteriosclerosis para Nutrición "Manuel de Oya" 2015 (to J.J.), by Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (Madrid, Spain) grant FIS PI17/00232 (to J.J.), PI18/00164 (to F.B.-V.), FEDER "Una manera de hacer Europa", by Ministerio de Economía y Competitividad (MINECO) (Madrid, Spain) SAF2017-89510-R (to A.F.V.), and by Fundació La Marató de TV3 2016 (303/C/2016) (201602.31) (to J.J.) and (97/C/2016) (201605-31) (to A.F.V.). K.A.M.-L. was recipient of an AGAUR grant FI-DGR2014 (Generalitat de Catalunya). E.M.G.D.-A. was recipient of The National Program of Foreign Postgraduate Scholarships "DON CARLOS ANTONIO LOPEZ" (BECAL) (BCAL04-451-3824321) by Ministerio de Educación (Republic of Paraguay). N.A.L. was recipient of a CONICYT (Comisión Nacional de Investigación Científica y Tecnología) fellowship (PFCHA/DOCTORADO BECAS CHILE/2016-72170639) by Ministerio de Educación (Chile). J.J. was recipient of a Miguel Servet Type 2 contract (CPII18/00004; ISCIII). CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM) is a project of Instituto de Salud Carlos III. J.J. is member of Red de Investigación en "Enfermedades Metabólicas y Cáncer" (RED2018-102799-T), Ministerio de Economía y Competitividad (MINECO), Madrid, Spain. J.C.E.-G., F.B.-V., and J.J.are members of the Quality Research Group 2017-SGR-1149 from Generalitat de Catalunya. K.A.M.-L., J.C.E.-G., and J.J. are members of the Group of Vascular Biology from the Spanish Atherosclerosis Society. Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau is accredited by the Generalitat de Catalunya as Centre de Recerca de Catalunya (CERCA).

Acknowledgments: The English grammar and language was corrected by Scribendi Inc and American Journal Experts.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Piche, M.E.; Poirier, P.; Lemieux, I.; Despres, J.P. Overview of Epidemiology and Contribution of Obesity and Body Fat Distribution to Cardiovascular Disease: An Update. *Prog. Cardiovasc. Dis.* **2018**, *61*, 103–113. [CrossRef] [PubMed]
2. McGill, H.C., Jr. Fatty streaks in the coronary arteries and aorta. *Lab. Investig.* **1968**, *18*, 560–564. [PubMed]
3. Geer, J.C.; McGill, H.C., Jr.; Robertson, W.B.; Strong, J.P. Histologic characteristics of coronary artery fatty streaks. *Lab. Investig.* **1968**, *18*, 565–570. [PubMed]
4. Skalen, K.; Gustafsson, M.; Rydberg, E.K.; Hulten, L.M.; Wiklund, O.; Innerarity, T.L.; Boren, J. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. *Nature* **2002**, *417*, 750–754. [CrossRef]
5. Sampson, U.K.; Fazio, S.; Linton, M.F. Residual cardiovascular risk despite optimal LDL cholesterol reduction with statins: The evidence, etiology, and therapeutic challenges. *Curr. Atheroscler. Rep.* **2012**, *14*, 1–10. [CrossRef]
6. Taylor, A.J.; Villines, T.C.; Stanek, E.J.; Devine, P.J.; Griffen, L.; Miller, M.; Weissman, N.J.; Turco, M. Extended-release niacin or ezetimibe and carotid intima-media thickness. *N. Engl. J. Med.* **2009**, *361*, 2113–2122. [CrossRef]
7. Cannon, C.P.; Braunwald, E.; McCabe, C.H.; Rader, D.J.; Rouleau, J.L.; Belder, R.; Joyal, S.V.; Hill, K.A.; Pfeffer, M.A.; Skene, A.M. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N. Engl. J. Med.* **2004**, *350*, 1495–1504. [CrossRef]
8. Guthrie, R. Niacin or ezetimibe for patients with, or at risk of coronary heart disease. *Clin. Med. Insights Cardiol.* **2010**, *4*, 99–110. [CrossRef]

9. Holzhauser, E.; Albrecht, C.; Zhou, Q.; Buttler, A.; Preusch, M.R.; Blessing, E.; Katus, H.A.; Bea, F. Nicotinic acid has anti-atherogenic and anti-inflammatory properties on advanced atherosclerotic lesions independent of its lipid-modifying capabilities. *J. Cardiovasc. Pharmacol.* **2011**, *57*, 447–454. [CrossRef]
10. Lipton, P.; Michels, J.G. The Effects of Nicotinic Acid on Rabbit Hypercholesterolemia and Atherogenesis. *Geriatrics* **1965**, *71*, 379–387.
11. Lukasova, M.; Hanson, J.; Tunaru, S.; Offermanns, S. Nicotinic acid (niacin): New lipid-independent mechanisms of action and therapeutic potentials. *Trends Pharmacol. Sci.* **2011**, *32*, 700–707. [CrossRef] [PubMed]
12. Orbetsova, V.; Iurukova, T.; Kirov, T. Regression of experimental cholesterol-induced atherosclerosis in rabbits after stopping the atherogenic diet and after treatment with nicotinic acid. *Eksp. Med. Morfol.* **1985**, *24*, 32–38.
13. Strack, A.M.; Carballo-Jane, E.; Wang, S.P.; Xue, J.; Ping, X.; McNamara, L.A.; Thankappan, A.; Price, O.; Wolff, M.; Wu, T.J.; et al. Nicotinic acid and DP1 blockade: Studies in mouse models of atherosclerosis. *J. Lipid Res.* **2013**, *54*, 177–188. [CrossRef] [PubMed]
14. Teixeira, F.; Beja, M.L.; Serra e Silva, P.; Cabrita, S.; Franca, M.B.; Almeida, L.; Morgadinho, M.T. Experimental atherogenesis and vascular noradrenaline content in NZW rabbits and activity of a new nicotinic acid derivative (L 44-0). *J. Lipid Mediat.* **1991**, *3*, 167–175. [PubMed]
15. Kuhnast, S.; Louwe, M.C.; Heemskerk, M.M.; Pieterman, E.J.; van Klinken, J.B.; van den Berg, S.A.; Smit, J.W.; Havekes, L.M.; Rensen, P.C.; van der Hoorn, J.W.; et al. Niacin Reduces Atherosclerosis Development in APOE*3Leiden. CETP Mice Mainly by Reducing NonHDL-Cholesterol. *PLoS ONE* **2013**, *8*, e66467. [CrossRef]
16. Lukasova, M.; Malaval, C.; Gille, A.; Kero, J.; Offermanns, S. Nicotinic acid inhibits progression of atherosclerosis in mice through its receptor GPR109A expressed by immune cells. *J. Clin. Investig.* **2011**, *121*, 1163–1173. [CrossRef]
17. Wu, B.J.; Yan, L.; Charlton, F.; Witting, P.; Barter, P.J.; Rye, K.A. Evidence that niacin inhibits acute vascular inflammation and improves endothelial dysfunction independent of changes in plasma lipids. *Arterioscler. Thromb. Vasc. Biol.* **2010**, *30*, 968–975. [CrossRef]
18. Parwaresch, M.R.; Haacke, H.; Mader, C. Efficacy of hypolipidemic treatment in inhibition of experimental atherosclerosis: The effect of nicotinic acid and related compounds. *Atherosclerosis* **1978**, *31*, 395–401. [CrossRef]
19. Ost, C.R.; Stenson, S. Regression of peripheral atherosclerosis during therapy with high doses of nicotinic acid. *Scand. J. Clin. Lab. Investig.* **1967**, *99*, 241–245.
20. Superko, H.R.; Zhao, X.Q.; Hodis, H.N.; Guyton, J.R. Niacin and heart disease prevention: Engraving its tombstone is a mistake. *J. Clin. Lipidol.* **2017**, *11*, 1309–1317. [CrossRef]
21. Kiss, T.; Giles, C.B.; Tarantini, S.; Yabluchanskiy, A.; Balasubramanian, P.; Gautam, T.; Csipo, T.; Nyul-Toth, A.; Lipecz, A.; Szabo, C.; et al. Nicotinamide mononucleotide (NMN) supplementation promotes anti-aging miRNA expression profile in the aorta of aged mice, predicting epigenetic rejuvenation and anti-atherogenic effects. *Geroscience* **2019**, *41*, 419–439. [CrossRef] [PubMed]
22. Hawrylyshyn, K.M. Nicotinamide Riboside Delivery Gener y Generates NAD+ Reserves to Protect Vascular Cells against Oxidative Damage. Ph.D. Thesis, Graduate Program in Biochemistry, The University of Western Ontario, London, ON, Canada, 2015. Available online: https://ir.lib.uwo.ca/etd/2891 (accessed on 22 June 2015).
23. De Picciotto, N.E.; Gano, L.B.; Johnson, L.C.; Martens, C.R.; Sindler, A.L.; Mills, K.F.; Imai, S.; Seals, D.R. Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice. *Aging Cell* **2016**, *15*, 522–530. [CrossRef] [PubMed]
24. Das, A.; Huang, G.X.; Bonkowski, M.S.; Longchamp, A.; Li, C.; Schultz, M.B.; Kim, L.J.; Osborne, B.; Joshi, S.; Lu, Y.; et al. Impairment of an Endothelial NAD(+)-H2S Signaling Network Is a Reversible Cause of Vascular Aging. *Cell* **2018**, *173*, 74–89.e20. [CrossRef] [PubMed]
25. Yoshino, J.; Baur, J.A.; Imai, S.I. NAD(+) Intermediates: The Biology and Therapeutic Potential of NMN and NR. *Cell Metab.* **2018**, *27*, 513–528. [CrossRef] [PubMed]
26. Waetzig, G.; Seegert, D. Pharmaceutical Composition Containing Nicotinic Acid, Nicotinamide, and/or Tryptophan for Positively Influencing the Intestinal Microbiota. 2015. Available online: https://patents.google.com/patent/EP2861229A1/en (accessed on 22 April 2015).

27. Shalita, A.R.; Smith, J.G.; Parish, L.C.; Sofman, M.S.; Chalker, D.K. Topical nicotinamide compared with clindamycin gel in the treatment of inflammatory acne vulgaris. *Int. J. Dermatol.* **1995**, *34*, 434–437. [CrossRef]
28. Emanuele, E.; Bertona, M.; Altabas, K.; Altabas, V.; Alessandrini, G. Anti-inflammatory effects of a topical preparation containing nicotinamide, retinol, and 7-dehydrocholesterol in patients with acne: A gene expression study. *Clin. Cosmet. Investig. Dermatol.* **2012**, *5*, 33–37. [CrossRef]
29. Fabbrocini, G.; Cantelli, M.; Monfrecola, G. Topical nicotinamide for seborrheic dermatitis: An open randomized study. *J. Dermatol. Treat.* **2014**, *25*, 241–245. [CrossRef]
30. Ashkani Esfahani, S.; Khoshneviszadeh, M.; Namazi, M.R.; Noorafshan, A.; Geramizadeh, B.; Nadimi, E.; Razavipour, S.T. Topical Nicotinamide Improves Tissue Regeneration in Excisional Full-Thickness Skin Wounds: A Stereological and Pathological Study. *Trauma Mon.* **2015**, *20*, e18193. [CrossRef]
31. O'Sullivan, B.M.; Blakemore, W.F. Acute nicotinamide deficiency in pigs. *Vet. Rec.* **1978**, *103*, 543–544. [CrossRef]
32. Varma, L.P. Mental Symptoms in Pellagra and Nicotinic Acid Deficiency. *Ind. Med. Gaz.* **1943**, *78*, 543–546.
33. Mc, F.A. Dermatitis as a monosymptomatic manifestation of nicotinic acid deficiency. *Glasgow Med. J.* **1947**, *28*, 103–115.
34. Valdes, V.A.; Bolo, P.O. Nicotinic acid deficiency without pellagra; radiological changes in colon due to deficiency. *Prensa Med. Argent.* **1953**, *40*, 2622–2628. [PubMed]
35. Zheng, M.; Cai, J.; Liu, Z.; Shu, S.; Wang, Y.; Tang, C.; Dong, Z. Nicotinamide reduces renal interstitial fibrosis by suppressing tubular injury and inflammation. *J. Cell. Mol. Med.* **2019**, *23*, 3995–4004. [CrossRef] [PubMed]
36. Weiss, R.; Schilling, E.; Grahnert, A.; Kolling, V.; Dorow, J.; Ceglarek, U.; Sack, U.; Hauschildt, S. Nicotinamide: A vitamin able to shift macrophage differentiation toward macrophages with restricted inflammatory features. *Innate Immun.* **2015**, *21*, 813–826. [CrossRef] [PubMed]
37. Hiromatsu, Y.; Sato, M.; Tanaka, K.; Ishisaka, N.; Kamachi, J.; Nonaka, K. Inhibitory effects of nicotinamide on intercellular adhesion molecule-1 expression on cultured human thyroid cells. *Immunology* **1993**, *80*, 330–332. [PubMed]
38. Hiromatsu, Y.; Sato, M.; Yamada, K.; Nonaka, K. Inhibitory effects of nicotinamide on recombinant human interferon-gamma-induced intercellular adhesion molecule-1 (ICAM-1) and HLA-DR antigen expression on cultured human endothelial cells. *Immunol. Lett.* **1992**, *31*, 35–39. [CrossRef]
39. Biedron, R.; Ciszek, M.; Tokarczyk, M.; Bobek, M.; Kurnyta, M.; Slominska, E.M.; Smolenski, R.T.; Marcinkiewicz, J. 1-Methylnicotinamide and nicotinamide: Two related anti-inflammatory agents that differentially affect the functions of activated macrophages. *Arch. Immunol. Ther. Exp.* **2008**, *56*, 127–134. [CrossRef] [PubMed]
40. Fukuzawa, M.; Satoh, J.; Muto, G.; Muto, Y.; Nishimura, S.; Miyaguchi, S.; Qiang, X.L.; Toyota, T. Inhibitory effect of nicotinamide on in vitro and in vivo production of tumor necrosis factor-alpha. *Immunol. Lett.* **1997**, *59*, 7–11. [CrossRef]
41. Ungerstedt, J.S.; Blomback, M.; Soderstrom, T. Nicotinamide is a potent inhibitor of proinflammatory cytokines. *Clin. Exp. Immunol.* **2003**, *131*, 48–52. [CrossRef]
42. Ungerstedt, J.S.; Heimersson, K.; Soderstrom, T.; Hansson, M. Nicotinamide inhibits endotoxin-induced monocyte tissue factor expression. *J. Thromb. Haemost.* **2003**, *1*, 2554–2560. [CrossRef]
43. Grange, P.A.; Raingeaud, J.; Calvez, V.; Dupin, N. Nicotinamide inhibits Propionibacterium acnes-induced IL-8 production in keratinocytes through the NF-kappaB and MAPK pathways. *J. Dermatol. Sci.* **2009**, *56*, 106–112. [CrossRef] [PubMed]
44. Lappas, M.; Permezel, M. The anti-inflammatory and antioxidative effects of nicotinamide, a vitamin B(3) derivative, are elicited by FoxO3 in human gestational tissues: Implications for preterm birth. *J. Nutr. Biochem.* **2011**, *22*, 1195–1201. [CrossRef] [PubMed]
45. Yanez, M.; Jhanji, M.; Murphy, K.; Gower, R.M.; Sajish, M.; Jabbarzadeh, E. Nicotinamide Augments the Anti-Inflammatory Properties of Resveratrol through PARP1 Activation. *Sci. Rep.* **2019**, *9*, 10219. [CrossRef] [PubMed]
46. Steven, S.; Frenis, K.; Oelze, M.; Kalinovic, S.; Kuntic, M.; Bayo Jimenez, M.T.; Vujacic-Mirski, K.; Helmstadter, J.; Kroller-Schon, S.; Munzel, T.; et al. Vascular Inflammation and Oxidative Stress: Major Triggers for Cardiovascular Disease. *Oxid. Med. Cell. Longev.* **2019**, *2019*, 7092151. [CrossRef] [PubMed]
47. Ogata, S.; Takeuchi, M.; Teradaira, S.; Yamamoto, N.; Iwata, K.; Okumura, K.; Taguchi, H. Radical scavenging activities of niacin-related compounds. *Biosci. Biotechnol. Biochem.* **2002**, *66*, 641–645. [CrossRef] [PubMed]

48. Kamat, J.P.; Devasagayam, T.P. Nicotinamide (vitamin B3) as an effective antioxidant against oxidative damage in rat brain mitochondria. *Redox Rep.* **1999**, *4*, 179–184. [CrossRef]
49. Hong, G.; Zheng, D.; Zhang, L.; Ni, R.; Wang, G.; Fan, G.C.; Lu, Z.; Peng, T. Administration of nicotinamide riboside prevents oxidative stress and organ injury in sepsis. *Free Radic. Biol. Med.* **2018**, *123*, 125–137. [CrossRef]
50. Mendez-Lara, K.A.; Santos, D.; Farre, N.; Ruiz-Nogales, S.; Leanez, S.; Sanchez-Quesada, J.L.; Zapico, E.; Lerma, E.; Escola-Gil, J.C.; Blanco-Vaca, F.; et al. Administration of CORM-2 inhibits diabetic neuropathy but does not reduce dyslipidemia in diabetic mice. *PLoS ONE* **2018**, *13*, e0204841. [CrossRef]
51. Ribas, V.; Palomer, X.; Roglans, N.; Rotllan, N.; Fievet, C.; Tailleux, A.; Julve, J.; Laguna, J.C.; Blanco-Vaca, F.; Escola-Gil, J.C. Paradoxical exacerbation of combined hyperlipidemia in human apolipoprotein A-II transgenic mice treated with fenofibrate. *Biochim. Biophys. Acta* **2005**, *1737*, 130–137. [CrossRef]
52. Escola-Gil, J.C.; Lee-Rueckert, M.; Santos, D.; Cedo, L.; Blanco-Vaca, F.; Julve, J. Quantification of In Vitro Macrophage Cholesterol Efflux and In Vivo Macrophage-Specific Reverse Cholesterol Transport. *Methods Mol. Biol.* **2015**, *1339*, 211–233.
53. Maor, I.; Hayek, T.; Coleman, R.; Aviram, M. Plasma LDL oxidation leads to its aggregation in the atherosclerotic apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **1997**, *17*, 2995–3005. [CrossRef] [PubMed]
54. Bogan, K.L.; Brenner, C. Nicotinic acid, nicotinamide, and nicotinamide riboside: A molecular evaluation of NAD+ precursor vitamins in human nutrition. *Annu. Rev. Nutr.* **2008**, *28*, 115–130. [CrossRef] [PubMed]
55. Li, X.; Zhang, S.; Blander, G.; Tse, J.G.; Krieger, M.; Guarente, L. SIRT1 deacetylates and positively regulates the nuclear receptor LXR. *Mol. Cell* **2007**, *28*, 91–106. [CrossRef] [PubMed]
56. Repa, J.J.; Turley, S.D.; Lobaccaro, J.A.; Medina, J.; Li, L.; Lustig, K.; Shan, B.; Heyman, R.A.; Dietschy, J.M.; Mangelsdorf, D.J. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science* **2000**, *289*, 1524–1529. [CrossRef] [PubMed]
57. Kennedy, M.A.; Barrera, G.C.; Nakamura, K.; Baldan, A.; Tarr, P.; Fishbein, M.C.; Frank, J.; Francone, O.L.; Edwards, P.A. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. *Cell Metab.* **2005**, *1*, 121–131. [CrossRef]
58. Elenkov, I.J.; Chrousos, G.P. Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. *Ann. N. Y. Acad. Sci.* **2002**, *966*, 290–303. [CrossRef]
59. Wolf, D.; Ley, K. Immunity and Inflammation in Atherosclerosis. *Circ. Res.* **2019**, *124*, 315–327. [CrossRef]
60. Mittal, M.; Siddiqui, M.R.; Tran, K.; Reddy, S.P.; Malik, A.B. Reactive oxygen species in inflammation and tissue injury. *Antioxid. Redox Signal.* **2014**, *20*, 1126–1167. [CrossRef]
61. Mendez-Lara, K.A.; Santos, D.; Farre, N.; Nan, M.N.; Pallares, V.; Perez-Perez, A.; Alonso, N.; Escola-Gil, J.C.; Blanco-Vaca, F.; Julve, J. Vitamin B3 impairs reverse cholesterol transport in Apolipoprotein E-deficient mice. *Clin. Investig. Arterioscler.* **2019**, *31*, 251–260.
62. Hwang, E.S.; Song, S.B. Nicotinamide is an inhibitor of SIRT1 in vitro, but can be a stimulator in cells. *Cell. Mol. Life Sci.* **2017**, *74*, 3347–3362. [CrossRef]
63. Liu, D.; Gharavi, R.; Pitta, M.; Gleichmann, M.; Mattson, M.P. Nicotinamide prevents NAD+ depletion and protects neurons against excitotoxicity and cerebral ischemia: NAD+ consumption by SIRT1 may endanger energetically compromised neurons. *Neuromol. Med.* **2009**, *11*, 28–42. [CrossRef] [PubMed]
64. Lee-Rueckert, M.; Escola-Gil, J.C.; Kovanen, P.T. HDL functionality in reverse cholesterol transport—Challenges in translating data emerging from mouse models to human disease. *Biochim. Biophys. Acta* **2016**, *1861*, 566–583. [CrossRef] [PubMed]
65. Whitney, K.D.; Watson, M.A.; Goodwin, B.; Galardi, C.M.; Maglich, J.M.; Wilson, J.G.; Willson, T.M.; Collins, J.L.; Kliewer, S.A. Liver X receptor (LXR) regulation of the LXRalpha gene in human macrophages. *J. Biol. Chem.* **2001**, *276*, 43509–43515. [CrossRef] [PubMed]
66. Laffitte, B.A.; Joseph, S.B.; Walczak, R.; Pei, L.; Wilpitz, D.C.; Collins, J.L.; Tontonoz, P. Autoregulation of the human liver X receptor alpha promoter. *Mol. Cell. Biol.* **2001**, *21*, 7558–7568. [CrossRef]
67. Bradley, M.N.; Hong, C.; Chen, M.; Joseph, S.B.; Wilpitz, D.C.; Wang, X.; Lusis, A.J.; Collins, A.; Hseuh, W.A.; Collins, J.L.; et al. Ligand activation of LXR beta reverses atherosclerosis and cellular cholesterol overload in mice lacking LXR alpha and apoE. *J. Clin. Investig.* **2007**, *117*, 2337–2346. [CrossRef]
68. Ma, A.Z.; Song, Z.Y.; Zhang, Q. Cholesterol efflux is LXRalpha isoform-dependent in human macrophages. *BMC Cardiovasc. Disord.* **2014**, *14*, 80. [CrossRef]

69. Cedo, L.; Metso, J.; Santos, D.; Garcia-Leon, A.; Plana, N.; Sabate-Soler, S.; Rotllan, N.; Rivas-Urbina, A.; Mendez-Lara, K.A.; Tondo, M.; et al. LDL Receptor Regulates the Reverse Transport of Macrophage-Derived Unesterified Cholesterol via Concerted Action of the HDL-LDL Axis: Insight From Mouse Models. *Circ. Res.* **2020**, *127*, 778–792. [CrossRef]
70. Yvan-Charvet, L.; Wang, N.; Tall, A.R. Role of HDL, ABCA1, and ABCG1 transporters in cholesterol efflux and immune responses. *Arterioscler. Thromb. Vasc. Biol.* **2010**, *30*, 139–143. [CrossRef]
71. Han, X.; Kitamoto, S.; Lian, Q.; Boisvert, W.A. Interleukin-10 facilitates both cholesterol uptake and efflux in macrophages. *J. Biol. Chem.* **2009**, *284*, 32950–32958. [CrossRef]
72. Wang, Y.F.; Yang, X.F.; Cheng, B.; Mei, C.L.; Li, Q.X.; Xiao, H.; Zeng, Q.T.; Liao, Y.H.; Liu, K. Protective effect of Astragalus polysaccharides on ATP binding cassette transporter A1 in THP-1 derived foam cells exposed to tumor necrosis factor-alpha. *Phytother. Res.* **2010**, *24*, 393–398. [CrossRef]
73. Reiss, A.B.; Carsons, S.E.; Anwar, K.; Rao, S.; Edelman, S.D.; Zhang, H.; Fernandez, P.; Cronstein, B.N.; Chan, E.S. Atheroprotective effects of methotrexate on reverse cholesterol transport proteins and foam cell transformation in human THP-1 monocyte/macrophages. *Arthritis Rheum.* **2008**, *58*, 3675–3683. [CrossRef] [PubMed]
74. Hao, X.R.; Cao, D.L.; Hu, Y.W.; Li, X.X.; Liu, X.H.; Xiao, J.; Liao, D.F.; Xiang, J.; Tang, C.K. IFN-gamma down-regulates ABCA1 expression by inhibiting LXRalpha in a JAK/STAT signaling pathway-dependent manner. *Atherosclerosis* **2009**, *203*, 417–428. [CrossRef] [PubMed]
75. Zhu, X.; Lee, J.Y.; Timmins, J.M.; Brown, J.M.; Boudyguina, E.; Mulya, A.; Gebre, A.K.; Willingham, M.C.; Hiltbold, E.M.; Mishra, N.; et al. Increased cellular free cholesterol in macrophage-specific Abca1 knock-out mice enhances pro-inflammatory response of macrophages. *J. Biol. Chem.* **2008**, *283*, 22930–22941. [CrossRef] [PubMed]
76. Mateuszuk, L.; Jasztal, A.; Maslak, E.; Gasior-Glogowska, M.; Baranska, M.; Sitek, B.; Kostogrys, R.; Zakrzewska, A.; Kij, A.; Walczak, M.; et al. Antiatherosclerotic Effects of 1-Methylnicotinamide in Apolipoprotein E/Low-Density Lipoprotein Receptor-Deficient Mice: A Comparison with Nicotinic Acid. *J. Pharmacol. Exp. Ther.* **2016**, *356*, 514–524. [CrossRef]
77. Jiang, N.; Wang, M.; Song, J.; Liu, Y.; Chen, H.; Mu, D.; Xia, M. N-methylnicotinamide protects against endothelial dysfunction and attenuates atherogenesis in apolipoprotein E-deficient mice. *Mol. Nutr. Food Res.* **2016**, *60*, 1625–1636. [CrossRef]