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Abréviations

ACC : acetyl-CoA carboxylase

ACL : ATP citrate lyase

AGPAT : 1-acylglycerol-3-phosphate acyltransferase

AGPI : acides gras polyinsaturés à longue chaine

AMPK : adenosine monophosphate-activated protein kinase

AP-1 : activator protein 1

API6 : antiapoptotic apoptosis inhibitor 6

APOB : apolipoprotéine B

ASC-2 : activating signal cointegrator 2

ATGL : adipose triglycéride lipase

BCL-XL : B-cell lymphoma-extra large

BIRC1A : baculoviral IAP repeat-containing 1α

BSEP : bile salt export pump

CaMKIIγ : calcium/calmodulin-dependent protein kinase IIγ

CAR : constitutive androstane receptor

ChoRE : carbohydrate response element

ChREBP : carbohydrate-responsive element-binding protein

COL1A1 : collagen α1(I):α-smooth muscle actin α-SMA

CORO2A : coronin 2A

COX-2 : cyclooxygénase 2

CPT1 : carnitine palmitoyl-transferase 1

CTα : CTP: phosphocholine acetyltransferase α

DAMP : damage-associated molecular pattern

DAX1 : congenital homolog

DBD : DNA binding domain

DGAT : diacylglycerol acyltransferase

DOS : dioxydosqualène

ELOVL : elongation of very long chain proteins

FAS : fatty acid synthase

FGF : fibroblast growth factor

FoxO1 : forkhead box-‘‘Other’’1 transcription factor

FXR : farnesoid X receptor

G-CSF : granulocyte colony-stimulating factor

GPAT : glycerol-3-phosphate acyl transferase

GR : glucocorticoid receptor

GSM : glucose sensing domain

HDAC3 : histone deacetylase 3

HDL : high density lipoprotein

HMGB1 : high mobility group gel box 1

HMGCR : 3-hydroxy-3-methylglutaryl CoA reductase

IDOL : inducible degrader of LDLR

IL : interleukine

iNOS : nitric oxide synthase

LBD : ligand binding domain

LIPIN : phosphatidate phosphatase

LPA : acide lysophosphatidique

LPS : lipopolysaccharide

LXR : liver X receptor

LXRE : LXR response element

MCP : monocyte chemotactic protein

MDR2 : multidrug resistant protein 2

MERK : mer receptor tyrosine kinase

MLX : max like protein

MOS : monooxydosqualène

mTOR : mammalian target of rapamycin

MTTP : microsomale triglycérides transfert protein

NAFLD : non alcoholic fatty liver disease

NASH : non alcoholic steatohepatitis

NCOR : nuclear receptor co-repressor

NF-κB : nuclear factor κB

NPC : nimann-pick C

NPC1L1 : niemann-pick C1-like protein 1

OSC : oxydosqualene cyclase

PAMP : pathogen-associated molecular pattern

PC : phosphatidyl-choline

PEMT : phosphatidylethanolamine N-methyl transferase

PGC-1β : peroxisome proloferator-activated receptor γ coactivator-1β

PKA : protein kinase A

PKB : protein kinase B

PLIN2 : perilipin 2

PP2A : protein phosphatase 2A

PPAR : peroxisomal proliferator-activated receptor

PXR : pregnan X receptor

RIP140 : receptor-integrating protein 140

RN : récepteur nucléaire

RXR : retinoid X receptors

SCD : stearoyl CoA desaturase

SE : squalene epoxydase

SHP : short heterodimer partner

SIK2 : seronine/threonine kinase salt-inducible kinase 2

SIN3 : stress-activated MAP kinase interacting protein 3

SM : squalene monooxygenase

SMRT : silencing mediator of retinoid and thyroid receptors

SRB1 : scavenger receptor classe B member 1

SRE : sterol response element

SREBP : sterol responsive element binding protein

TGF-1β : transforming growth factor-1β

TICE : trans-intestinal cholestérol excretion

TLR : toll like receptor

TNF-α : tumor necrosis factor-α

VDR : vitamin D receptor

VLDL : very low density lipoprotein

X5P : xylulose-5-phosphate

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Situation du sujet

Chez les mammifères, la lipogenèse ou synthèse *de novo* des acides gras joue un rôle essentiel à l'homéostasie énergétique. Elle est particulièrement active dans le foie et cette voie métabolique est très finement régulée au niveau transcriptionnel en réponse à des stimuli hormonaux et environnementaux.

Le Liver X Receptor ou LXR est un facteur de transcription de la famille des récepteurs nucléaires, qui intervient dans de nombreux processus biologiques dont la régulation de la lipogenèse hépatique. Or, la dérégulation de cette voie métabolique est associée à diverses atteintes hépatiques telles que les stéatoses non alcooliques. La prévalence de ces pathologies augmente, parallèlement à l'épidémie d'obésité, ce qui en fait un problème majeur de santé publique.

Dans cette introduction bibliographique, nous nous intéresserons tout d'abord aux stéatoses non alcooliques et à l'implication de la famille des récepteurs nucléaires dans le développement de ces pathologies. Nous verrons ensuite quels sont les étapes de la lipogenèse hépatique et de la synthèse de triglycérides. Puis, nous nous intéresserons à la structure, au mode d'activation, ainsi qu'aux ligands activateurs de LXR. La partie suivante traitera de l'implication de LXR dans le métabolisme du cholestérol, dans la régulation de l'inflammation et dans le développement de pathologies extra-hépatiques. Nous nous intéresserons enfin à la régulation de la lipogenèse par LXR, à la régulation de son activité par des facteurs hormonaux et nutritionnels ainsi qu'à son possible rôle physiopathologique.

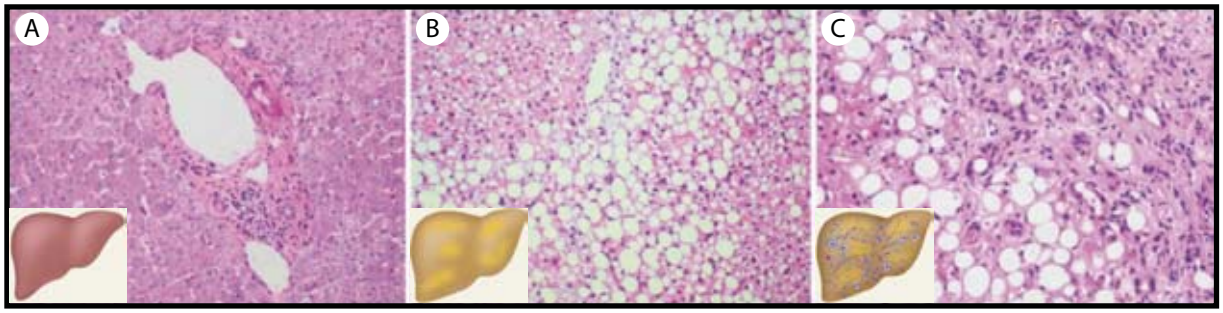


Figure 1. Coupes histopathologiques de foie humain (Adapté de Hebbard and George 2011). (A) Foie sain. (B) Stéatose identifiée par l'accumulation de gouttelettes lipidiques dans le cytoplasme des hépatocytes. (C) Stéatohépatite non alcoolique présentant une stéatose marquée avec une inflammation associée à une fibrose.

Partie I : Physiopathologie du foie et récepteurs nucléaires

I.1. La stéatose hépatique non alcoolique : une épidémie en lien avec l'obésité

En constante augmentation, la prévalence du syndrome métabolique est un problème majeur de santé publique et représente un fardeau socio-économique pour les systèmes de santé. La prévalence de ce syndrome est de 34% dans la population adulte américaine (Marchesini, Marzocchi et al. 2005) et de 14 à 20 % dans la population adulte française (Balkau, Vernay et al. 2003). Les maladies non alcooliques du foie gras ou « non alcoholic fatty liver diseases » (NAFLD) sont considérées comme la manifestation hépatique du syndrome métabolique (Marchesini, Brizi et al. 1999). Ce syndrome est caractérisé par la présence chez le patient d'au moins trois des cinq diagnostiques suivants : i) une hyperglycémie (>150 mg/dL), ii) une hypertension (>130/85 mm/Hg), iii) une dyslipidémie (HDL <40 mg/dL chez l'homme et <50 mg/dL chez la femme), iv) une obésité abdominale (circonférence au niveau de la taille >102 cm chez l'homme et >88 cm chez la femme) et une hyperglycémie à jeun (>110mg/dL) (2001).

Les NAFLD sont caractérisées par une variété de pathologies hépatiques allant d'une accumulation aberrante de triglycérides dans le foie, ou stéatose hépatique (Unger, Clark et al. 2010), à une stéatohépatite non alcoolique ou « non alcoholic steatohepatitis » (NASH) caractérisée par la présence d'un état inflammatoire et fibrotique, allant jusqu'à une cirrhose et plus rarement un hépatocarcinome (**Figure 1**).

Le modèle de l'évolution des NAFLD le plus communément accepté est un modèle linéaire en deux étapes. La première étape est le développement d'une stéatose hépatique. La stéatose est un état physiopathologique bénin et réversible mais peut évoluer à la suite d'une deuxième étape en hépatostéatite non alcoolique. Aux Etats Unis, 15% à 46 % des adultes présentent une stéatose hépatique et 10% à 30% de ces patients développent une NASH. La NASH peut ensuite évoluer en cirrhose et, potentiellement en carcinome hépatocellulaire dans des proportions variables.

Le développement de la stéatose peut se produire à la suite de différents stimuli (**Figure 2**). Tout d'abord, par les acides gras d'origine hépatique qui peuvent provenir des

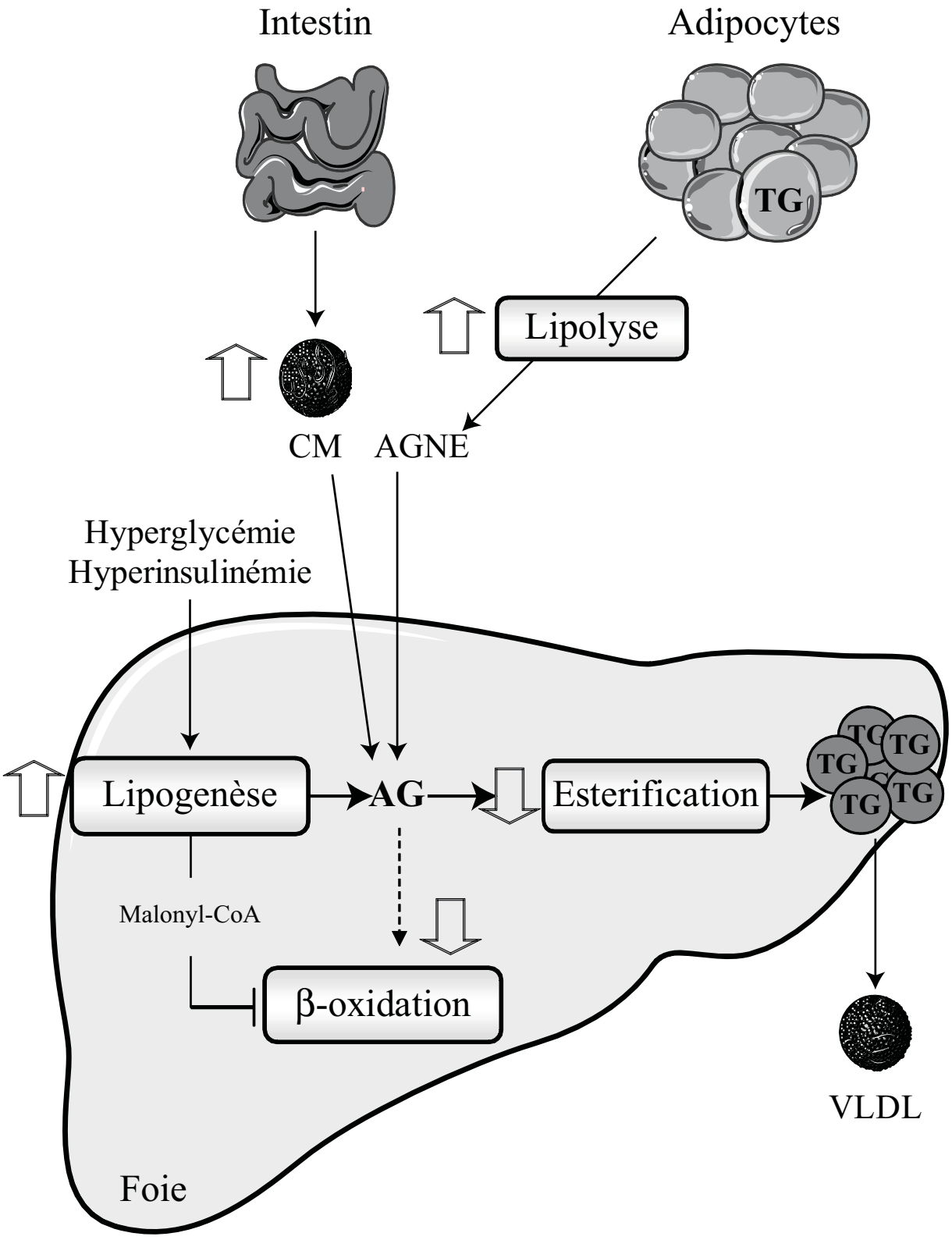


Figure 2. Origines de la stéatose hépatique (Adapté de Postic and Girard 2008). Les origines de la stéatose hépatique peuvent être multiples. L'accumulation d'acides gras (AG) dans le foie peut résulter d'un apport exogène à partir des chylomicrons (CM) *via* l'intestin ou d'acides gras non estérifiés (AGNE) *via* la lipolyse accrue dans le tissu adipeux blanc dans le cas, par exemple, d'une alimentation riche en graisse ou de la résistance à l'insuline respectivement. Un défaut dans la β-oxydation ou de la synthèse de triglycérides mais également un défaut de la sécrétion des triglycérides dans les VLDL conduit aussi à l'accumulation d'acides gras hépatiques. La synthèse *de novo* d'acides gras, ou lipogenèse, est activée dans le cadre d'hyperglycémie ou d'hyperinsulinémie et peut être une cause de la stéatose hépatique. Un intermédiaire de la lipogenèse, le malonyl-CoA, est un inhibiteur allostérique de l'enzyme limitante de la β-oxydation des acides gras ce qui renforce ainsi le pouvoir stéatogénique de la lipogenèse.

remnants de lipoprotéines plasmatiques ou de la lipolyse des triglycérides adipocytaires. Les acides gras peuvent également provenir de la synthèse *de novo* appelée aussi lipogenèse, qui est stimulée par des concentrations élevées en glucose et en insuline (Postic and Girard 2008; Postic and Girard 2008). Une étude utilisant des isotopes stables menée chez des patients obèses atteints de NAFLD a permis de quantifier les origines des acides gras hépatiques. Les acides gras hépatiques de ces patients proviennent à 59% de la lipolyse adipocytaire, à 15% d'origine alimentaire et à 26% de la lipogenèse hépatique (Donnelly, Smith et al. 2005).

I.2. Transition entre stéatose et stéatohépatite

La transition entre stéatose et NASH peut dépendre de facteurs alimentaires, de l'implication de différentes populations cellulaires hépatiques ainsi que des interactions avec d'autres tissus. Cependant la hiérarchisation ainsi que la compréhension du rôle de ces acteurs n'est pas encore clairement connue (**Figure 3**).

Une étude épidémiologique de grande ampleur met en évidence une corrélation entre la consommation de cholestérol alimentaire et le développement de la stéatohépatite et de la cirrhose (Ioannou, Morrow et al. 2009). D'autres données épidémiologiques font également le lien entre le cholestérol alimentaire et le développement et la sévérité des NAFLD (Musso, Gambino et al. 2003; Yasutake, Nakamuta et al. 2009). Il est important de noter que dans les NAFLD, le développement de NASH et de fibrose est corrélé à une accumulation de cholestérol dans le foie (Puri, Baillie et al. 2007; Caballero, Fernandez et al. 2009). De plus, le traitement de patients présentant une NASH avec un inhibiteur du « niemann-pick C1-like protein 1 » (NPC1L1), un transporteur responsable de l'absorption du cholestérol au niveau intestinal, permet une amélioration des paramètres inflammatoires et de la stéatose (Yoneda, Fujita et al. 2010; Park, Shima et al. 2011). Dans des modèles animaux de stéatohépatite induite expérimentalement, il apparait aussi que le cholestérol est un facteur clé du développement de cette pathologie (Subramanian, Goodspeed et al. 2011; Van Rooyen, Larter et al. 2011). Il a été récemment montré dans une étude de nutrition que des régimes riches en cholestérol ou en triglycérides, seuls, bien qu'induisant une accumulation de lipides dans le foie, ne pouvaient induire une inflammation et une fibrose hépatique. Cependant, l'association de hautes teneurs en graisses et en cholestérol dans un régime induit, après 30 semaines, une

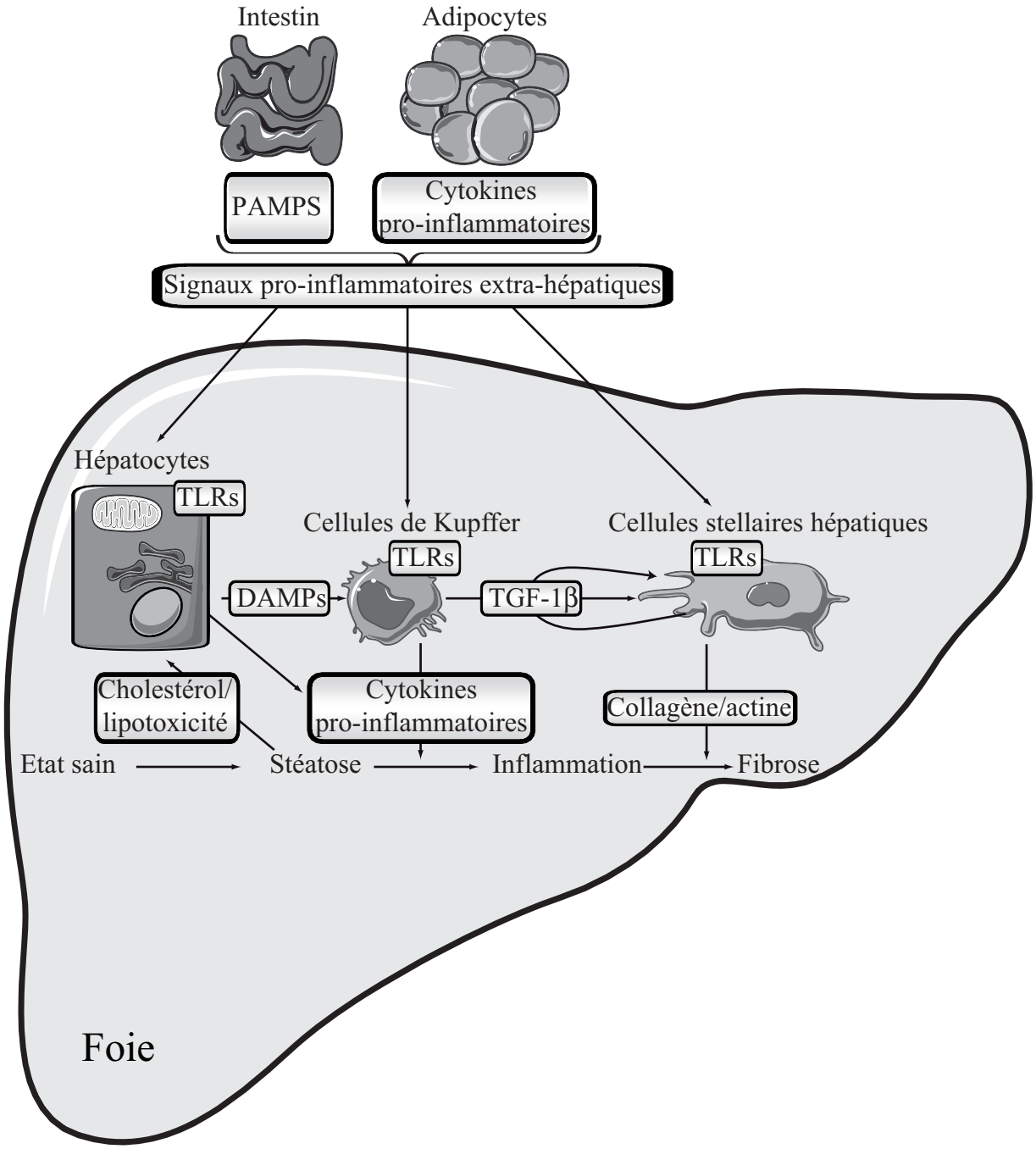


Figure 3. Développement de la stéatohépatite. Le modèle classique de la NAFLD est un modèle linéaire en deux étapes. La première étape est le développement de la stéatose hépatique et permettrait, dans ce modèle, la sensibilisation du foie à des stimuli inflammatoires et fibrotiques conduisant à des pathologies plus sévères de la NAFLD. L'inflammation et la fibrose hépatique sont fortement corrélées avec la quantité de cholestérol. Ce composé, à haute dose, a des effets lipotoxiques et peut conduire à la nécrose des cellules du foie. La mort cellulaire contribue à la libération des « damage associated molecular patterns » (DAMPs) qui sont reconnus par les « toll like receptors » (TLRs) présents sur les cellules hépatiques comme les hépatocytes, les cellules de Kupffer et les cellules stellaires hépatiques. Ces cellules sécrètent alors des cytokines pro-inflammatoires et induisent une inflammation dans le foie. Les cellules de Kupffer qui sont des macrophages résidants dans le foie, vont alors s'activer et sécréter du « transforming growth factor-1β » (TGF-1β). Ce facteur de croissance conduit à la stimulation des cellules stellaires hépatiques responsable de la fibrose. Les cellules stellaires hépatiques sécrètent aussi du TGF-1β qui joue le rôle d'une hormone paracrine. Les stimuli inflammatoires peuvent avoir également une origine extra-hépatique. La perturbation de la perméabilité intestinale ou l'inflammation du tissu adipeux blanc conduisent respectivement à la présence de « pathogens associated molecular patterns » (PAMPs) ou de cytokines pro-inflammatoires dans la circulation sanguine et induisent le déclenchement de l'inflammation dans le foie.

stéatose associée à une augmentation des paramètres inflammatoires et fibrotiques. Ces résultats mettent en lumière la synergie entre ces deux types de lipides alimentaires dans la transition stéatose/NASH (Savard, Tartaglione et al. 2012).

Les cellules de Kupffer et les cellules stellaires hépatiques sont des cellules résidentes du foie et sont impliquées respectivement dans le déclenchement de la réponse inflammatoire (Tosello-Trampont, Landes et al. 2012) et de la mise en place de la fibrose (Matsuoka and Tsukamoto 1990). Les cellules de Kupffer sont des macrophages résidents qui représentent 20% à 25% des cellules hépatiques non parenchymateuses et qui sont la source principale de cytokines pro-inflammatoires et pro-fibrotiques telles que le « tumor necrosis factor-α » (TNF-α) et le « transforming growth factor-1β » (TGF-1β). Elles permettent le recrutement des cellules inflammatoires circulantes au niveau du foie et activent les cellules stellaires hépatiques jouant ainsi un rôle clé dans l'apparition de la NASH (Rivera, Adegboyega et al. 2007; Maher, Leon et al. 2008). L'implication des cellules stellaires hépatiques est clairement établie (Gressner and Bachem 1990; Matsuoka and Tsukamoto 1990). Lors d'atteinte au niveau du foie, les cellules stellaires hépatiques sont activées : elles perdent leur contenu lipidique, prolifèrent et expriment des protéines fibrogéniques comme le « collagen α1(I) » (COL1A1) et l' « α-smooth muscle actin » (α-SMA) (Rockey, Boyles et al. 1992). Les cellules stellaires hépatiques et de Kupffer présentent plusieurs isoformes des « toll like receptors » (TLRs) qui sont des médiateurs des signaux pro-inflammatoires. Les TLRs reconnaissent une large variété de motifs moléculaires issus de pathogènes, les « pathogen-associated molecular patterns » (PAMPs), comme le lipopolysaccharide (LPS), mais également des motifs moléculaires associés à des dommages cellulaires, les « damage-associated molecular patterns » (DAMPs), libérés à la suite de dommages cellulaires, comme, par exemple, le « high mobility group gel box 1 » (HMGB1) (Bianchi 2009), les « heat shock proteins » ou encore des peptides ou de l'ADN mitochondrial (Zhang, Raoof et al. 2010). Parmi les treize TLRs connus, huit sont présents dans le foie des mammifères (TLRs 1,2,4,6-10) et sont exprimés à différents niveaux dans les cellules de Kupffer, les cellules stellaires hépatiques et les hépatocytes (Schwabe, Seki et al. 2006). Cependant, seuls trois isoformes semblent être impliqués dans le développement de la NASH : TLR2 (Miura, Yang et al. 2013), TLR4 (Rivera, Adegboyega et al. 2007; Ye, Li et al. 2012) et TLR9 (Miura, Kodama et al. 2010). Les origines des PAMPs et des DAMPS impliqués dans le développement de la NASH sont multiples. Il a été montré que la NASH est corrélée avec une augmentation de la perméabilité intestinale responsable du passage de PAMPs dans la circulation sanguine (Brun,

Castagliuolo et al. 2007). Les dommages subis par les hépatocytes peuvent conduire *via* plusieurs mécanismes comme le stress du réticulum endoplasmique (Ye, Li et al. 2012) ou la nécrose (Kubes and Mehal 2012) et conduire à la libération de DAMPs, induisant ainsi un état inflammatoire dans le foie. Il a également été montré que l'induction de l'inflammation hépatique pouvait également être le résultat de perturbations extérieures à cet organe. En effet, la production par le tissu adipeux blanc viscéral de cytokines pro-inflammatoires peut induire l'inflammation au niveau hépatique (Stanton, Chen et al. 2011).

Dans le foie, au niveau moléculaire, les lipides sont également impliqués dans le déclenchement du statut inflammatoire. Tout d'abord TLR4 est capable de lier les acides gras saturés et ainsi de relayer certains effets inflammatoires de ces acides gras (Shi, Kokoeva et al. 2006; Huang, Rutkowsky et al. 2012). Il a été également montré qu'un régime riche en graisses pouvait influencer le contenu lipidique des cellules de Kupffer et les sensibiliser à certains types de stimuli inflammatoires (Leroux, Ferrere et al. 2012). De plus, un régime riche en cholestérol induit également l'activation des cellules stellaires hépatiques dans un modèle de fibrose expérimentale (Teratani, Tomita et al. 2012).

I.3 Rôle du microbiote dans le développement des NAFLD

Comme nous l'avons vu précédemment, l'inflammation hépatique peut résulter de la translocation de PAMPs issus de la flore intestinale dans la circulation sanguine. De par son irrigation sanguine *via* la veine porte-hépatique, le foie est le premier organe en contact avec ces PAMPs. L'étude de la flore intestinale a longtemps été négligée et émerge comme un facteur important dans la physiologie globale de l'organisme.

Des dérégulations du microbiote intestinal peuvent être responsables du développement de la stéatohépatite. Le tractus gastro-intestinal contient entre 10 et 100 mille milliards d'organismes répartis entre 500 et 1000 espèces (Guarner and Malagelada 2003). La flore intestinale consiste en un équilibre quantitatif et qualitatif entre ces différentes espèces. Cet équilibre est labile et varie en fonction de l'hôte (différences inter-espèces et inter-individus) mais également de l'âge, de l'alimentation et du style de vie (O'Hara and Shanahan 2006). Plusieurs mécanismes peuvent expliquer les effets stéatotiques et pro-inflammatoires de la flore intestinale. La flore induit une augmentation de l'absorption d'acides gras, de leur

synthèse et de leur quantité au niveau du foie (Backhed, Ding et al. 2004). La perturbation du métabolisme de la choline dans la lumière intestinale a également des effets sur le foie. En effet, le microbiote possède des enzymes permettant la transformation de la choline d'origine alimentaire en diéthylamide et en triméthylamine (Zeisel, Wishnok et al. 1983). Cette biotransformation a pour effet d'induire une déficience en choline (Dumas, Barton et al. 2006) et l'absorption de composés hépatotoxiques pour l'hôte (Lin and Ho 1992). La choline est un nutriment essentiel, nécessaire à l'assemblage et à l'excrétion des lipoprotéines de très basse densité ou « very low density lipotroteins » (VLDLs) (Hebbard and George 2011). Expérimentalement, les régimes déficients en choline sont utilisés pour induire une stéatohépatite non alcoolique. De plus, le microbiote produit de l'éthanol, de l'ammoniaque et de l'acétaldéhyde qui sont métabolisés par le foie et contrôlent l'activité des cellules de Kupffer et la production de cytokines pro-inflammatoires (Nagata, Suzuki et al. 2007). Cependant, le principal composé d'origine bactérienne connu pour être impliqué dans la transition stéatose/NASH est le LPS, un composant des parois des bactéries à Gram négatif. Il a été montré que des souris nourries avec un régime riche en graisses présentaient une augmentation de deux à trois fois de la concentration en LPS plasmatique issu de la translocation à partir de la lumière intestinale (Cani, Neyrinck et al. 2007). Inversement, les souris ne présentant pas de flore intestinale (axéniques) nourries avec un régime riche en graisse ne développent pas d'obésité (Backhed, Manchester et al. 2007), montrant ainsi le rôle important du microbiote dans le développement de cette pathologie et certainement des pathologies associées. Les régimes riches en graisses sont utilisés expérimentalement pour étudier les désordres métaboliques, cependant les effets qu'ils induisent présentent une grande variabilité, même chez des animaux de fond génétique identique (Li, Xie et al. 2008). En effet, le microbiote varie en fonction des individus et il a été montré que le transfert de flore de souris répondant fortement à un régime riche en graisse (i.e. présentant une hyperglycémie et une inflammation systémique) chez des souris axéniques induit le développement d'une stéatohépatite alors que ce n'est pas le cas de souris axéniques recevant la flore de souris répondant faiblement au même régime (i.e. présentant une glycémie normale et un statut inflammatoire faible) (Le Roy, Llopis et al. 2012). Cette différence serait exclusivement due à une composition de la flore qui diffère entre les deux types d'individus donneurs (Le Roy, Llopis et al. 2012). Dans le même sens, l'impact de la flore sur le développement des NAFLD en réponse à un autre type de régime a été étudié. Une forte consommation de fructose est associée au risque de développement des NAFLD (Ouyang, Cirillo et al. 2008) et de NASH (Abdelmalek, Suzuki et al. 2010). Des souris consommant du fructose dans l'eau de boisson

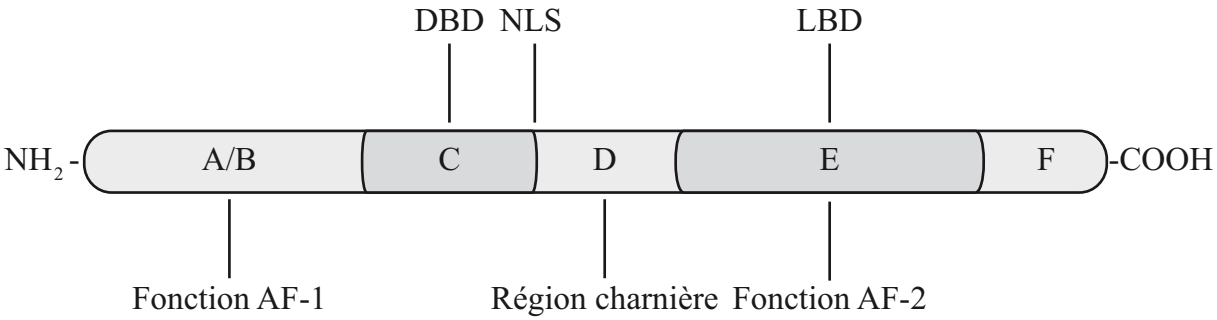


Figure 4. Structure générale des récepteurs nucléaires. Les récepteurs nucléaires sont constitués de six domaines A/B, C, D, E et F. Le domaine A/B est situé à l'extrémité N terminale et contient la fonction activatrice « activation function 1» (AF-1). Le domaine C est composé du domaine de liaison à l'ADN ou « DNA binding domain » (DBD). Le domaine D est un domaine charnière peu conservé. Les domaines C et D possèdent des signaux de localisation nucléaire ou « nuclear localisation signal » (NLS). Le domaine E contient le domaine de liaison au ligand ou « ligand binding domain » (LBD) et porte la fonction de trans-activation dépendante du ligand AF-2. Certains récepteurs nucléaires présentent un domaine F extrêmement variable dont la fonction n'est pas connue.

développent une stéatose plus marquée et présentent des niveaux de LPS circulant et une production de TNF plus importants que des souris consommant du glucose. L'élimination de la flore avec des antibiotiques prévient les effets du fructose (Bergheim, Weber et al. 2008). Enfin, il a été montré que les inflammasomes, des complexes multi-protéiques impliqués dans la reconnaissance des PAMPS et des DAMPs ainsi que dans la maturation des cytokines pro-inflammatoires, régulent la composition de la flore intestinale et la progression de la NASH. En effet, les souris transgéniques déficientes pour différents composants d'inflammasomes présentent une modification de la composition de la flore intestinale associée à une augmentation des paramètres inflammatoires hépatiques. L'élevage de ces souris avec des souris de type sauvage permet une transmission de la flore ainsi que du phénotype inflammatoire chez les souris de type sauvage (Henao-Mejia, Elinav et al. 2012) confirmant le rôle de la modification de la flore intestinale dans le développement de la NASH.

Les récepteurs nucléaires, par les effets qu'ils exercent sur la régulation des gènes impliqués dans le métabolisme hépatique des acides gras, du cholestérol mais également dans la modulation de l'inflammation, sont des cibles pharmacologiques majeures dans le traitement des NAFLD.

I.4. Les récepteurs nucléaires hépatiques

La superfamille des récepteurs nucléaires (RN) comprend 48 membres chez l'homme et 49 chez la souris (Gronemeyer, Gustafsson et al. 2004). Alors qu'ils sont conservés entre l'humain et *C. elegans*, les récepteurs nucléaires n'existent pas chez les plantes et les levures, révélant ainsi une importance primordiale dans les cellules animales. Les récepteurs nucléaires partagent la même organisation structurale à savoir un domaine d'activation N-terminal (AF1), un domaine de liaison à l'ADN ou « DNA binding domain » (DBD), un domaine de liaison au ligand ou « ligand binding domain » (LBD) ainsi qu'un domaine d'activation C-terminal (AF2) (**Figure 4**). Les seules exceptions sont le « congenital homolog » (DAX1) et le « short heterodimer partner » (SHP) qui ne possèdent pas de domaine de liaison à l'ADN fonctionnel. Le domaine de liaison au ligand est la caractéristique de cette famille de facteurs de transcription. Celui-ci est généralement une poche moléculaire hydrophobe dans laquelle des petites molécules peuvent de se fixer. Chaque RN est capable

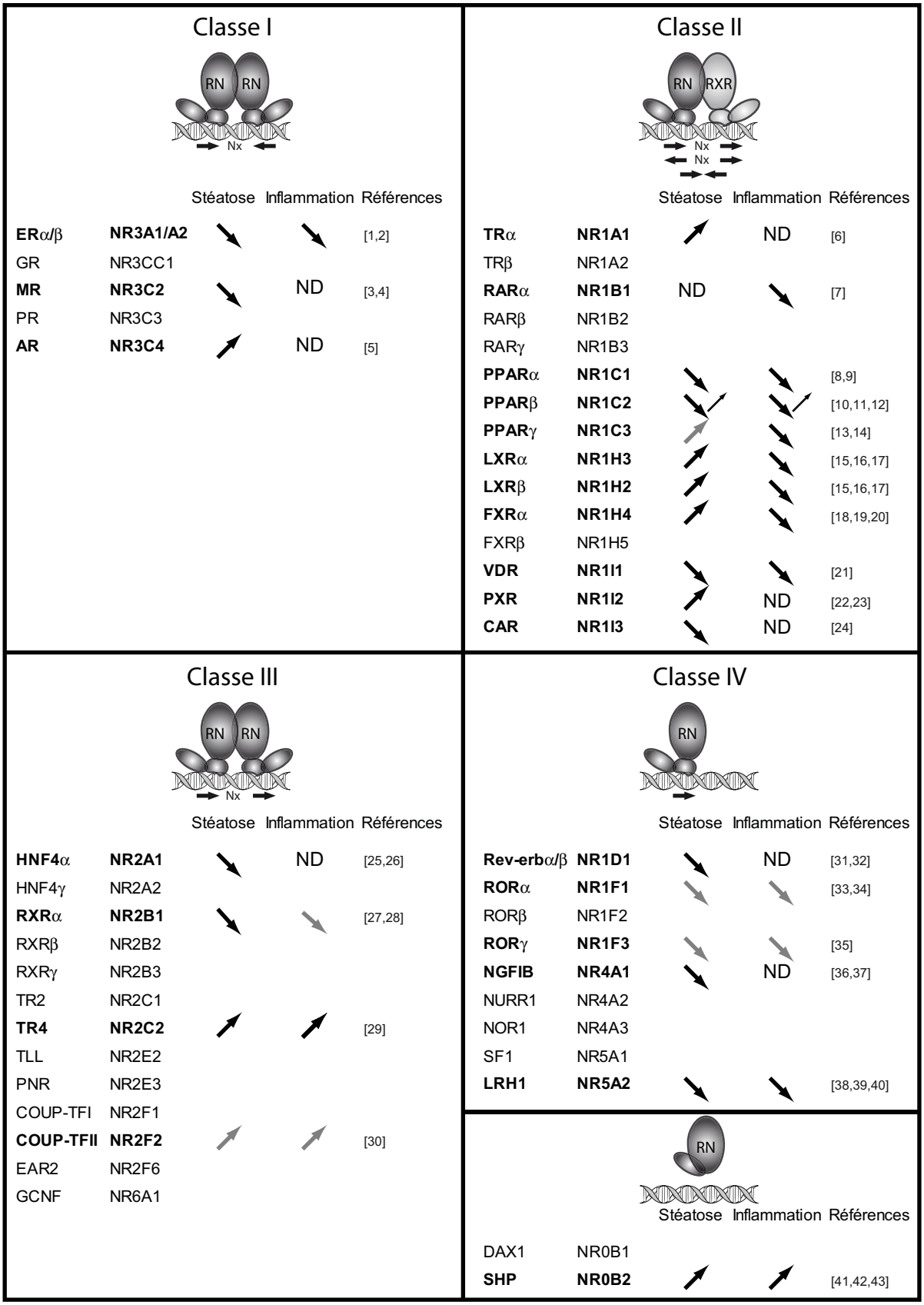


Figure 5. Implication des récepteurs nucléaires dans la stéatose et l'inflammation hépatique. Les récepteurs nucléaires sont classifiés en fonction de leur mode d'action en monomère ou en homo/hétérodimère et de leur mode de fixation sur leur élément de réponse. Les récepteurs impliqués *in vivo* dans la stéatose ou l'inflammation hépatique sont indiqués en gras. Leur rôle positif ou négatif dans le développement de ces états pathologiques est indiqué avec des flèches noires ascendantes ou descendantes respectivement. Lorsque ces deux états sont corrélés avec une modification de l'expression des gènes codant pour les récepteurs nucléaires uniquement, les effets sont alors indiqués avec une flèche grise. ND : non documentés. Correspondance références : [1,2] : (Chow, Jones et al. 2011; Kamada, Kiso et al. 2011); [3,4] : (Wada, Kenmochi et al. 2010; Polyzos, Kountouras et al. 2011); [5] : (Lin, Yu et al. 2008); [6] : (Jornayvaz, Lee et al. 2012); [7] : (Tsuchiya, Ikeda et al. 2012); [8,9] : (Costet, Legendre et al. 1998; Hashimoto, Fujita et al. 1999); [10,11,12] : (Nagasawa, Inada et al. 2006; Barroso, Rodriguez-Calvo et al. 2011; Kostadinova, Montagner et al. 2012); [13,14] : (Matsusue, Haluzik et al. 2003; Yu, Matsusue et al. 2003); [15,16,17] : (Schultz, Tu et al. 2000; Cha and Repa 2007; Liu, Han et al. 2011); [18,19,20] : (Matsukuma, Bennett et al. 2006; Martin, Schmitt et al. 2010; Yang, Shen et al. 2010); [21] : (Barchetta, Carotti et al. 2012); [22,23] : (Zhou, Zhai et al. 2006; He, Gao et al. 2013); [24] : (Gao, He et al. 2009); [25,26] : (Hayhurst, Lee et al. 2001; Guan, Qu et al. 2011); [27,28] : (Wan, An et al. 2000; Kim, Sweeney et al. 2007); [29] : (Kang, Okamoto et al. 2011); [30] : (Ma, Xu et al. 2011); [31,32] : (Cho, Zhao et al. 2012; Solt, Wang et al. 2012); [33,34] : (Lau, Fitzsimmons et al. 2008; Kang, Okamoto et al. 2011); [35] : (Ou, Shi et al. 2013); [36,37] : (Pols, Ottenhoff et al. 2008; Chao, Wroblewski et al. 2009); [38,39,40] : (Venteclef, Smith et al. 2006; Matsukuma, Wang et al. 2007; Oosterveer, Mataki et al. 2012); [41,42,43] : (Boulias, Katrakili et al. 2005; Huang, Iqbal et al. 2007; Hartman, Lai et al. 2009).

de lier sur son LBD un ou plusieurs types de ligands qui lui sont spécifiques. Le mécanisme « classique » d'activation des récepteurs nucléaires implique la fixation d'un ligand spécifique sur le LBD, ce qui induit une modification de la conformation du récepteur et permet ainsi la transition d'un état inactif à un état actif. A l'état basal, le RN est associé à des co-répresseurs interdisant la transcription. Une fois le RN activé par un ligand, les co-répresseurs sont échangés avec des co-activateurs permettant le recrutement de la machinerie transcriptionnelle (notamment l'ARN polymérase II) induisant ainsi l'initiation de la transcription.

La grande variété des différents domaines propres à chaque RN, le grand nombre de co-régulateurs potentiels identifiés (estimé entre 200 et 300 protéines), ainsi que les découvertes grandissantes des possibilités de modifications post-traductionnelles, associés à la régulation fine de la production de ligands spécifiques, complexifient le modèle initial « classique » de l'activation des récepteurs nucléaires (Perissi and Rosenfeld 2005; Han, Lonard et al. 2009; Perissi, Jepsen et al. 2010).

De plus, le fait qu'il ait été mis en évidence la possibilité que les RNs puissent être fixés sur leur élément de réponse en absence de ligand et qu'ils exercent des effets géniques associés aux fonctions non génomiques mis en évidence pour certains d'entre eux ajoute un degré de complexité supplémentaire à la compréhension des fonctions qu'ils peuvent exercer (Gronemeyer, Gustafsson et al. 2004).

La classification des récepteurs nucléaires est réalisée en fonction de similarités phylogénétiques et conduit à la définition de six sous-familles, elles mêmes sous-divisées en 28 groupes, chacun d'entre eux regroupant plusieurs gènes paralogues (Gronemeyer, Gustafsson et al. 2004). De plus, ils peuvent également être regroupés en quatre différentes classes (**Figure 5**). Cette classification est basée sur leur capacité à fonctionner en monomère ou en homo/hétéro-dimère et sur le type d'élément de réponse sur lesquels ils se fixent (Mangelsdorf, Thummel et al. 1995; Germain, Staels et al. 2006).

Parmi les 48 récepteurs nucléaires identifiés chez l'homme, plus d'une vingtaine sont impliqués dans le développement de la stéatose et/ou l'inflammation dans le foie (**Figure 5**).

De plus, il est important de noter que l'expression de la plupart d'entre eux est contrôlée par le rythme circadien (Bookout, Jeong et al. 2006; Yang, Downes et al. 2006). De récents travaux montrent que le rythme circadien contrôle des fonctions hépatiques comme

l'homéostasie glucido-lipidique, ils proposent les récepteurs nucléaires comme les médiateurs essentiels de ces effets (Feng, Liu et al. 2011; Schmutz, Ripperger et al. 2012; Sun, Miller et al. 2012).

I.5. Récepteurs nucléaires dans le traitement des maladies métaboliques hépatiques

Les récepteurs nucléaires participent au fonctionnement hépatique incluant le métabolisme du cholestérol, des lipides, des xénobiotiques, du glucose, des acides biliaires soulignant ainsi leur contribution potentielle dans le syndrome métabolique et dans les maladies hépatiques telles que l'hépatotoxicité médicamenteuse, la cholestase, les calculs biliaires et les NAFLD (Arrese and Karpen 2010; Wagner, Zollner et al. 2011). L'interaction directe des récepteurs nucléaires avec un ligand et l'ADN place ces protéines au centre de la pharmacologie moderne. Les ligands pharmacologiques des récepteurs nucléaires représentent le deuxième type de médicaments vendus avec un budget de cinquante milliards d'euros en 2003 (Gronemeyer, Gustafsson et al. 2004).

L'utilisation de modèles de souris transgéniques ainsi que d'activateurs spécifiques de chaque récepteur nucléaire, a permis d'approfondir leur implication dans le métabolisme lipidique. En effet, certains sont des régulateurs clés de la synthèse d'acides gras, de l'assemblage et la sécrétion de triglycérides, alors que d'autres régulent l'expression de gènes responsables du catabolisme des lipides (Arrese and Karpen 2010; Wagner, Zollner et al. 2011). Ces récepteurs exercent leurs effets *via* la régulation directe ou indirecte de ces processus. De plus, leur implication dans le contrôle de l'accumulation de molécules potentiellement lipotoxiques qui se produit dans les premières étapes des maladies hépatiques en font de bonnes cibles thérapeutiques, par exemple dans la cholestase.

La cholestase est caractérisée par un défaut de sécrétion de bile résultant en son accumulation dans le foie (Trauner, Meier et al. 1998). Les acides biliaires étant potentiellement cytotoxiques, le contrôle de leur concentration intracellulaire et plasmatique doit être finement régulé. Les récepteurs nucléaires comme le « farnesoid X receptor » (FXR) (Makishima, Okamoto et al. 1999), le « pregnan X receptor » (PXR) (Staudinger, Goodwin et al. 2001; Xie, Radominska-Pandya et al. 2001) et le « vitamin D receptor » (VDR) (Makishima, Lu et al. 2002) sont activés par les acides biliaires et d'autres constituants

biliaires. Ils constituent un réseau d'effecteurs permettant de limiter l'accumulation d'acides biliaires en inhibant leur absorption et leur synthèse et en induisant leur détoxication et leur export des hépatocytes (Boyer 2005; Boyer 2007; Wagner, Zollner et al. 2010). Concernant FXR, il induit également la sécrétion de protéines intestinale comme le « fibroblast growth factor 15 » (FGF15 ; FGF19 chez l'humain) qui inhibe la synthèse des acides biliaires dans l'hépatocyte (Inagaki, Choi et al. 2005). De façon intéressante, des variants génétiques des récepteurs nucléaires activés par les acides biliaires sont associés au développement de cholestase pendant la grossesse (Van Mil, Milona et al. 2007). Plusieurs médicaments utilisés pour le traitement de la cholestase fonctionnent en activant ces récepteurs nucléaires. Par exemple, les effets de l'acide ursodésoxycholique peuvent être en partie expliqués par l'activation de FXR (Bramlett, Yao et al. 2000; Schuetz, Strom et al. 2001; Lew, Zhao et al. 2004), de PXR, de VDR (Makishima, Lu et al. 2002) et du « glucocorticoid receptor » (GR) (Weitzel, Stark et al. 2005). Les fibrates, des activateurs du « peroxisomal proliferator-activated receptor α » (PPARα) inhibent la synthèse des acides biliaires et facilitent la sécrétion de phospholipides dans la bile, limitant ainsi la toxicité biliaire (Post, Duez et al. 2001; Roglans, Vazquez-Carrera et al. 2004). Cependant, l'utilisation des fibrates dans le traitement de la cholestase est impossible car ils induisent la formation de calculs biliaires (Caroli-Bosc, Le Gall et al. 2001). L'activation de FXR protège de la formation de calculs biliaires en induisant l'expression de la « bile salt export pump » (BSEP) et de la « multidrug resistant protein 2 » (MDR2), des transporteurs impliqués respectivement dans l'export des acides biliaires et des phospholipides dans la bile (Moschetta, Bookout et al. 2004). De plus, il s'avère que l'acide chénodésoxycholique, utilisé dans la dissolution des calculs biliaires, est un ligand naturel de FXR (Trauner and Halilbasic 2011).

En plus de leurs propriétés dans le contrôle de l'accumulation de molécules potentiellement lipotoxiques dans les premières étapes de la cholestase et des NAFLD, nombre de récepteurs nucléaires régulent les niveaux inflammatoires locaux et systémiques et peuvent ainsi influencer la transition de stéatose/stéatohépatite non alcoolique (**Figure 5**). C'est pourquoi les récepteurs nucléaires ont été largement étudiés comme cibles thérapeutiques potentielles dans des modèles murins de NAFLD (Hebbard and George 2011). Jusqu'à présent, des études précliniques réalisées chez la souris ont montré des résultats prometteurs. Cependant, leur transposition chez l'homme a donné des résultats mitigés voire décevants.

C'est le cas, par exemple, du récepteur activé par les proliférateurs de peroxysomes : PPARα (Issemann and Green 1990). PPARα est impliqué dans l'oxydation des acides gras et est fortement exprimé dans le foie (Braissant, Foufelle et al. 1996) où il régule un ensemble de gènes nécessaire à la prolifération des péroxysomes (Lee, Pineau et al. 1995). PPARα, mais aussi les deux autres isoformes de PPAR (β/δ et γ) sont généralement considérés comme activés par les acides gras et leurs dérivés (Yu, Bayona et al. 1995; Forman, Chen et al. 1997; Kliewer, Sundseth et al. 1997; Krey, Braissant et al. 1997). Cependant, à l'heure actuelle, la spécificité des ligands activateurs des PPARs est loin d'être claire (Ziouzenkova, Perrey et al. 2003; Chakravarthy, Lodhi et al. 2009). Il est évident que PPARα joue un rôle important dans l'oxydation des acides gras hépatiques lors d'un jeûne prolongé (Kroetz, Yook et al. 1998; Kersten, Seydoux et al. 1999), d'une alimentation riche en graisse (Patsouris, Reddy et al. 2006), ou en acides gras polyinsaturés (Martin, Guillou et al. 2007) et dans le diabète de type I (Kroetz, Yook et al. 1998). Au niveau hépatique, PPARα est aussi essentiel pour l'expression de *Fgf21*, une hépatokine importante pour la cétogenèse (Badman, Pissios et al. 2007; Inagaki, Dutchak et al. 2007). Chez les nouveaux nés, l'induction de l'expression hépatique de *Fgf21* par PPARα est essentielle à la thermogenèse (Hondares, Rosell et al. 2010). Les souris transgéniques invalidées pour *Pparα* et nourries avec un régime standard développent en vieillissant une obésité associée à une stéatose hépatique (Costet, Legendre et al. 1998). Les molécules pharmacologiques de la famille des fibrates, activatrices de PPARα sont utilisées en clinique pour le traitement des hyper-triglycéridémies (Berglund, Brunzell et al. 2012). Cependant, si l'utilisation de fibrates réduit la stéatose hépatique chez les rongeurs, elle semble inefficace dans le traitement des NAFLD chez l'homme (Sacks 2008). Il est néanmoins possible que les fibrates puissent participer aux effets protecteurs vis-à-vis de la lipotoxicité dans d'autres organes où PPARα est fortement exprimé tels que le cœur (Haemmerle, Moustafa et al. 2011), le rein, le tissu adipeux brun, le muscle ou l'intestin.

L'expression de l'isoforme γ de PPAR est augmentée dans les foies stéatotiques de rongeurs. PPARγ est une cible pharmacologique dans le traitement du diabète de type II pour lequel des agonistes spécifiques sont utilisés depuis des décennies. Néanmoins, des études cliniques à long terme conduites chez des patients présentant des NAFLD révèlent des effets bénéfiques sur la résistance à l'insuline ainsi que sur des paramètres métaboliques tels que la triglycéridémie, mais également des effets néfastes sur la fibrose, interdisant l'utilisation d'agonistes de PPARγ dans le traitement des NAFLD chez l'homme (Ratziu, Giral et al. 2008; Ratziu, Charlotte et al. 2010; Sanyal, Chalasani et al. 2010). L'activation de PPARβ/δ,

la troisième isoforme des PPARs, réduit le contenu en lipides du foie en induisant le catabolisme du glucose hépatique et la β-oxydation et en diminuant la lipogenèse (Qin, Xie et al. 2008). Cependant, il n'existe pas de ligands de PPARβ/δ utilisable en clinique. FXR, *via* l'induction de SHP, inhibe la lipogenèse hépatique et l'export de triglycérides dans les VLDLs (Watanabe, Houten et al. 2004; Huang, Iqbal et al. 2007). De plus, FXR induit PPARα, ce qui pourrait contribuer aux propriétés hypotriglycéridémiantes des acides biliaires par l'activation de l'oxydation des acides gras (Pineda Torra, Claudel et al. 2003). Un agoniste spécifique de FXR, actuellement testé sur des patients diabétiques présentant une stéatose hépatique, donne des résultats encourageants dans une étude clinique de phase II (Cariou 2008). Enfin FXR contrôle la croissance de la flore intestinale et permet le maintient de l'intégrité de la barrière intestinale en induisant la transcription de plusieurs gènes impliqués dans la protection de la muqueuse de l'intestin vis-à-vis de l'inflammation et des pathogènes (Inagaki, Moschetta et al. 2006). Ce rôle de FXR pourrait expliquer comment les acides biliaires, en plus de leurs propriétés intrinsèques de détergent et de régulation de la flore, réduisent la croissance et la translocation bactérienne et l'endotoxémie chez des rats cirrhotiques (Lorenzo-Zuniga, Bartoli et al. 2003; Hofmann and Eckmann 2006).

D'autres récepteurs nucléaires émergent également comme cibles thérapeutiques pour traiter cette pathologie. Le « constitutive androstane receptor » (CAR) (Dong, Saha et al. 2009) et PXR (He, Gao et al. 2013) en font partie. De récentes études ont également suggéré que le « liver X receptor » (LXR) pourrait être une cible dans le traitement des NAFLD (Griffett, Solt et al. 2012).

LXR, mais aussi PXR (He, Gao et al. 2013) sont impliqués dans la régulation de la lipogenèse *de novo*, une des origines des acides gras pouvant conduire au développement des NAFLD. Dans la partie suivante nous nous intéresserons donc en détail à la synthèse *de novo* d'acides gras ainsi qu'à leur incorporation dans des triglycérides.

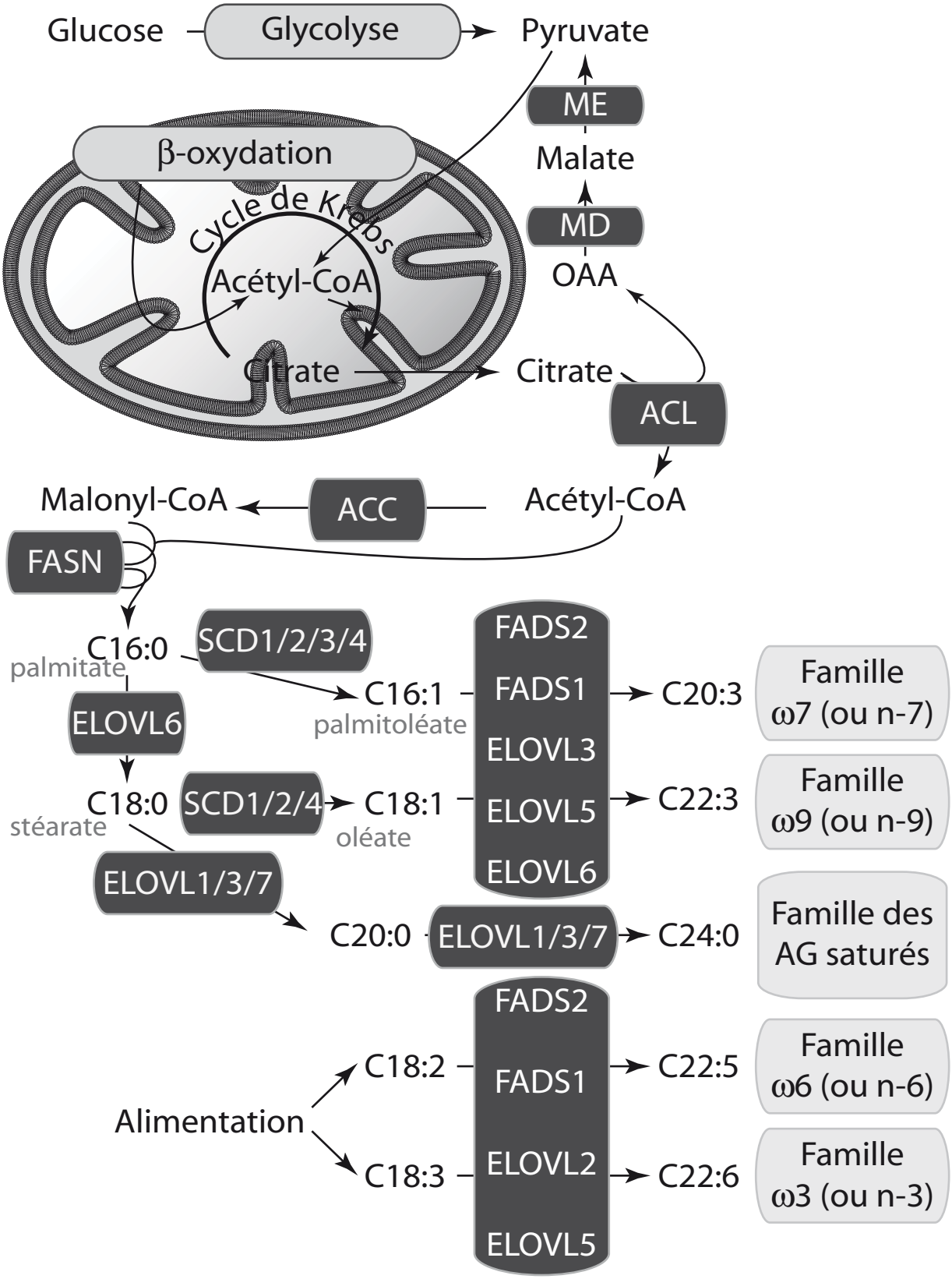


Figure 6. Synthèse hépatique des acides gras. L'acétyl-CoA, produit par la β-oxydation ou par la décarboxylation du pyruvate entre dans le cycle de Krebs pour être transformé en citrate. Le citrate est exporté en dehors de la mitochondrie et l' « ATP citrate lyase » (ACL) active ce composé pour former de l'acétyl-CoA. L' « acetyl-CoA carboxylase » (ACC) carboxyle l'acétyl-CoA pour former du malonyle-CoA qui est ensuite pris en charge par la « fatty acid synthase » (FAS). FAS incorpore plusieurs acétyl-CoA sur une molécule de malonyle-CoA et synthétise l'acide palmitique. Il est soit dessaturé par la « stearoyl-CoA desaturase 1 » (SCD1) ou allongé par l' « elongation of very long chain fatty acids protein 6 » (ELOVL6) pour former respectivement de l'acide palmitique ou stéarique. Le stéarate est lui aussi soit desaturé ou allongé pour former de l'oléate ou du C20:0. Plusieurs isoformes d'élongases (ELOVL) ou de desaturase (FADS) peuvent alors modifier ces acides gras mais également les acides gras polyinsaturés essentiels des familles n-6 et n-3 pour former ainsi une grande variété d'acides gras dans la cellule. Les précurseurs de ces familles sont respectivement le C18:2n-6 et le C18:3n-3 et doivent être apportés par l'alimentation.

Partie II : La lipogenèse *de novo*

La lipogenèse *de* novo est le processus cellulaire conduisant à la synthèse d'acides gras. Elle est finement régulée en réponse au statut hormonal et nutritionnel. Ainsi, une glycémie élevée et une hyperinsulinémie stimulent, *via* la mise en jeu d'importants régulateurs transcriptionnels, l'ensemble des gènes de la lipogenèse et de l'estérification des acides gras sous forme de triglycérides.

II.1. Les étapes critiques de la synthèse d'acides gras

La lipogenèse *de novo* se produit quand l'apport en glucose dans la cellule est élevé. Par exemple, dans le foie, à l'état nourri, les carbohydrates sont utilisés pour régénérer les stocks d'ATP et de glycogène. Si les carbohydrates sont en excès, le flux d'intermédiaires énergétiques est alors redirigé vers la synthèse d'acides gras. La lipogenèse est donc étroitement liée au métabolisme glucidique (Postic and Girard 2008).

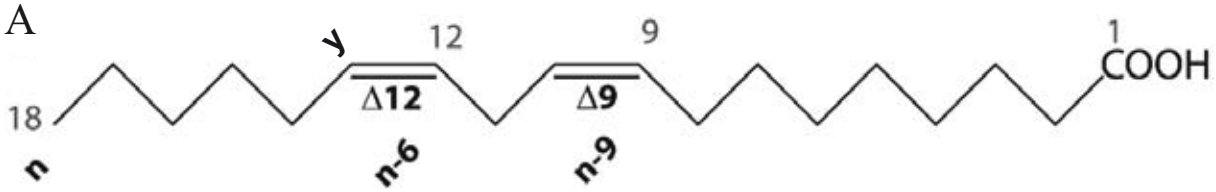
Ainsi, l'oxydation complète du glucose *via* la glycolyse conduit à la synthèse d'acétyl-CoA, qui est ensuite pris en charge par le cycle de Krebs dans la mitochondrie (**Figure 6**). Le citrate, un intermédiaire du cycle de Krebs, est ensuite exporté de la mitochondrie dans le cytosol où il sera transformé en acétyl-CoA, une réaction catalysée par l' « ATP citrate lyase » (ACL). L'acétyl-CoA va servir de substrat à la synthèse *de novo* d'acides gras (**Figure 6**).

L'acétyl-CoA est carboxylé par l' « acetyl-CoA carboxylase » (ACC) pour former du malonyl-CoA. Cette réaction est la première étape limitante de la synthèse d'acides gras. Il existe deux isoformes de ACC : ACCα (ou ACC1) et ACCβ (ou ACC2). ACCα est cytosolique et est principalement exprimée dans des tissus lipogéniques comme le foie et le tissu adipeux blanc. ACCβ a une localisation mitochondriale et est principalement exprimée dans les tissus oxydatifs (Abu-Elheiga, Brinkley et al. 2000). Ces deux isoformes sont codées par deux gènes différents (Abu-Elheiga, Jayakumar et al. 1995; Abu-Elheiga, Almarza-Ortega et al. 1997) et exercent des fonctions distinctes : ACCα est impliquée dans la lipogenèse alors

que ACCβ est plutôt impliquée dans la répression de la β-oxydation en produisant du malonyl-CoA qui est un inhibiteur allostérique de la « carnitine palmitoyl-transferase 1 » (CPT1). En effet, durant la synthèse d'acides gras, une forte production de malonyl-CoA exerce une boucle de rétrocontrôle négatif du catabolisme des acides gras en agissant directement sur CPT1, l'enzyme catalysant l'entrée des acides gras dans la mitochondrie afin que ceux-ci soient oxydés (McGarry and Brown 1997) (**Figure 2**). Les souris transgéniques n'exprimant pas *Accα* ne sont pas viables (Abu-Elheiga, Matzuk et al. 2005). Cependant la délétion spécifique de *Accα* dans le foie a pour conséquence une diminution de la lipogenèse et de l'accumulation de triglycérides sans avoir d'effets sur l'oxydation des acides gras (Mao, DeMayo et al. 2006). Les souris transgéniques déficientes en *Accβ* sont plus maigres que les souris de type sauvage, résistantes à l'obésité induite par un régime riche en graisse et en sucre et présentent une sensibilité accrue à l'insuline. Ces effets résultent d'une augmentation de l'oxydation des acides gras dans le cœur, le muscle squelettique et le foie (Abu-Elheiga, Matzuk et al. 2001; Abu-Elheiga, Oh et al. 2003).

Le malonyl-CoA cytosolique sert de composé de base à la synthèse d'acides gras. Cette réaction est catalysée par la « fatty acid synthase » (FAS) qui est la deuxième enzyme intervenant dans la synthèse d'acides gras. Son principal produit est l'acide gras saturé à 16 carbones, l'acide palmitique (C16:0) (**Figure 6**). FAS utilise le malonyl-CoA comme structure de base, l'acétyl-CoA comme donneur de carbone et du NADPH comme agent réducteur (Chirala and Wakil 2004). FAS est une protéine cytoplasmique composée de deux polypeptides multifonctionnels formant un homodimère d'une taille de 260 kDa. Chaque homodimère possède trois sites catalytiques situés dans la partie N-terminale (« β-ketoacyl synthase » [KS], « malonyl/acetyl transferase » [MAT] et « Dehydrase » [DH]), ainsi que quatre sites catalytiques dans la région C-terminale (« enoyl reductase » [ER], « β-ketoacyl reductase » [KR], « acyl carrier protein » [ACP] et « thioesterase » [TE]) (Smith 1994; Chirala, Jayakumar et al. 2001). Les souris transgéniques invalidées pour *Fas* meurent *in utero* (Chirala, Chang et al. 2003). La délétion spécifique de *Fas* dans le foie ne protège pas de l'accumulation d'acides gras hépatiques. En effet, les souris invalidées pour *Fas* dans le foie et nourries avec un régime pauvre en graisses et riche en cholestérol développent une stéatose hépatique. Cette stéatose semble résulter d'un défaut de la β-oxydation des acides gras (Chakravarthy, Pan et al. 2005).

A



n = longueur de chaine = 18 carbones
x = nombre d'insaturations = 2
n-y = position de la première insaturation à partir de la fonction méthyle

Cn:x(n-y) → C18:2(n-6) : acide linoléique

B

Symbole	Nom commun	
C16:0	Acide palmitique	Acides gras saturés
C18:0	Acide stéarique	
C20:0	Acide arachidique	
C22:0	Acide béhénique	
C24:0	Acide lignocérique	
C16:1(n-7)	Acide palmitoléique	Acides insaturés non essentiels
C16:1(n-10)	Acide sapiénique	
C18:1(n-9)	Acide oléique	
C20:1(n-9)	Acide gadoléique	
C22:1(n-9)	Acide érucique	
C20:3(n-9)	Acide eicosatriénoïque	
C18:2(n-6)	Acide linoléique	Acides polyinsaturés essentiels (n-6 et n-3)
C20:4(n-6)	Acide arachidonique	
C22:5(n-6)	Acide docosapentaénoïque	
C18:3(n-3)	Acide α-linolénique	
C20:5(n-3)	Acide eicosapentaénoïque	
C22:6(n-3)	Acide docosahéxaénoïque	

Figure 7. Structure et nomenclature des acides gras (Adapté de Guillou et al. 2010). (A) Exemple de l'acide linoléique. (B) Nomenclature et noms communs de différents acides gras.

II.2. L'élongation et la désaturation des acides gras

Comme précédemment énoncé, le produit de synthèse principal de FAS est l'acide palmitique (C16:0). Celui-ci peut soit être allongé ou desaturé. L'allongement des acides gras consiste en l'ajout de deux carbones sur un acyl-CoA du côté de la fonction acide carboxylique (**Figures 6 et 7**). Cet allongement nécessite un donneur de carbone, le malonyl-CoA ainsi qu'un agent réducteur, le NADPH. Les enzymes catalysant l'allongement des acides gras sont les élongases ou « elongation of very long chain proteins » (ELOVL). Elles sont enchâssées dans la membrane du réticulum endoplasmique. Jusqu'à présent, il a été identifié sept ELOVL(1-7) chez les mammifères. ELOVL6, aussi connue sous les noms de « long chain fatty acyl elongase » (LCE) et « fatty acyl-CoA elongase » (FACE), catalyse l'allongement de l'acide palmitique (C16:0) pour former de l'acide stéarique (C18:0). Le gène codant pour cette protéine a été découvert pour la première fois comme étant surexprimé chez des souris transgéniques surexprimant les « sterol responsive element binding protein » (SREBP)-1c et SREBP-2 (Moon, Shah et al. 2001). Cette découverte a été confirmée par une autre étude qui identifie le gène codant pour ELOVL6 comme étant sous la régulation transcriptionnelle des SREBPs (Matsuzaka, Shimano et al. 2002). ELOVL6 est exprimée dans le foie et catalyse l'allongement de l'acide palmitique (C16:0) et l'acide palmitoléique (C16:1 n-7) pour former respectivement de l'acide stéarique (C18:0) et de l'acide cis-vaccénique (C18:1 n-7) (Moon, Shah et al. 2001; Matsuzaka, Shimano et al. 2002). Il a également été montré que ELOVL6 peut allonger les acides gras composés de douze et quatorze carbones (Moon, Shah et al. 2001; Matsuzaka, Shimano et al. 2002). Les souris transgéniques invalidées pour *Elovl6* ont été générées par l'équipe de Yamada (Matsuzaka, Shimano et al. 2007). Ces souris présentent une réduction de la quantité d'acide stéarique (C18:0) ainsi que d'acide oléique (C18:1 n-9) et sont protégées du développement d'une résistance à l'insuline induite par un régime riche en graisses. Cependant, elles ne présentent pas d'amélioration de l'obésité ou de la stéatose hépatique. Ces souris ont été également croisées avec des souris mutées pour le récepteur à la leptine (*ob/ob*), les nouveaux nés présentent une amélioration de la résistance à l'insuline ainsi qu'une triglycéridémie plus basse que les nouveaux nés *ob/ob*.

Les acides palmitique (C16:0) et stéarique (C18:0) peuvent être desaturés pour former respectivement de l'acide palmitoléique (C16:1 n-7) et oléique (C18:1 n-9). Cette réaction est catalysée par la « stearoyl CoA desaturase 1 » (SCD1, aussi connue sous le nom de la « Δ9

desaturase ») (Ntambi 1999). Il existe plusieurs isoformes de SCD et l'intégralité des séquences codantes ainsi que leur séquence promotrice ont été décrites dans plusieurs espèces. Chez la souris quatre isoformes ont été identifiées (Ntambi, Buhrow et al. 1988; Kaestner, Ntambi et al. 1989; Zheng, Prouty et al. 2001; Miyazaki, Jacobson et al. 2003). *Scd1* est exprimé dans différents tissus incluant le foie et le tissu adipeux blanc, *Scd2* est principalement exprimé dans le cerveau et les tissus neuronaux (Ntambi, Buhrow et al. 1988), *Scd3* est exclusivement exprimé dans les glandes préputiales et de harder ainsi que dans les sébocytes (Zheng, Prouty et al. 2001) et *Scd4* est uniquement exprimé dans le cœur. L'acide palmitoléique et l'acide oléique sont les espèces majoritaires composant les phospholipides membranaires, les triglycérides et les esters de cholestérol. La SCD1 hépatique du rat a été la première desaturase à avoir été purifiée. SCD1 est une protéine de 40 kDa ancrée dans la membrane du réticulum endoplasmique. Elle catalyse la biosynthèse des acides gras mono-insaturés et nécessite un acyl-CoA, du NADH, une NADH réductase, un cytochrome b5, des phospholipides ainsi que de l'oxygène. Les souris transgéniques invalidées pour *Scd1* dans l'ensemble de l'organisme (Ntambi, Miyazaki et al. 2002) ou spécifiquement dans le foie (Miyazaki, Flowers et al. 2007) ont été générées par le groupe de Ntambi. Les souris invalidées pour *Scd1* dans tout l'organisme sont minces et présentent un défaut de synthèse de lipides notamment de triglycérides. De plus, elles sont protégées de l'obésité et de la résistance à l'insuline induites par un régime riche en graisse (Ntambi, Miyazaki et al. 2002) ou par la déficience en leptine (Cohen, Miyazaki et al. 2002). Une diminution de la lipogenèse ainsi qu'une augmentation de l'oxydation des acides gras semblent être à l'origine des effets protecteurs de la déficience en SCD1 (Cohen, Miyazaki et al. 2002; Ntambi, Miyazaki et al. 2002; Dobrzyn, Dobrzyn et al. 2004). Les études utilisant les souris transgéniques invalidées pour *Scd1* dans le foie uniquement montrent que cette enzyme joue un rôle protecteur dans l'obésité et la stéatose induite par un régime riche en carbohydrates et pauvre en graisse (Miyazaki, Flowers et al. 2007).

II.3. La synthèse de triglycérides

Les acides gras libres sont des molécules cytotoxiques à forte concentration et doivent être estérifiées dans des lipides complexes tels que les esters de cholestérol, les phospholipides et les triglycérides. Les triglycérides permettent le stockage énergétique et,

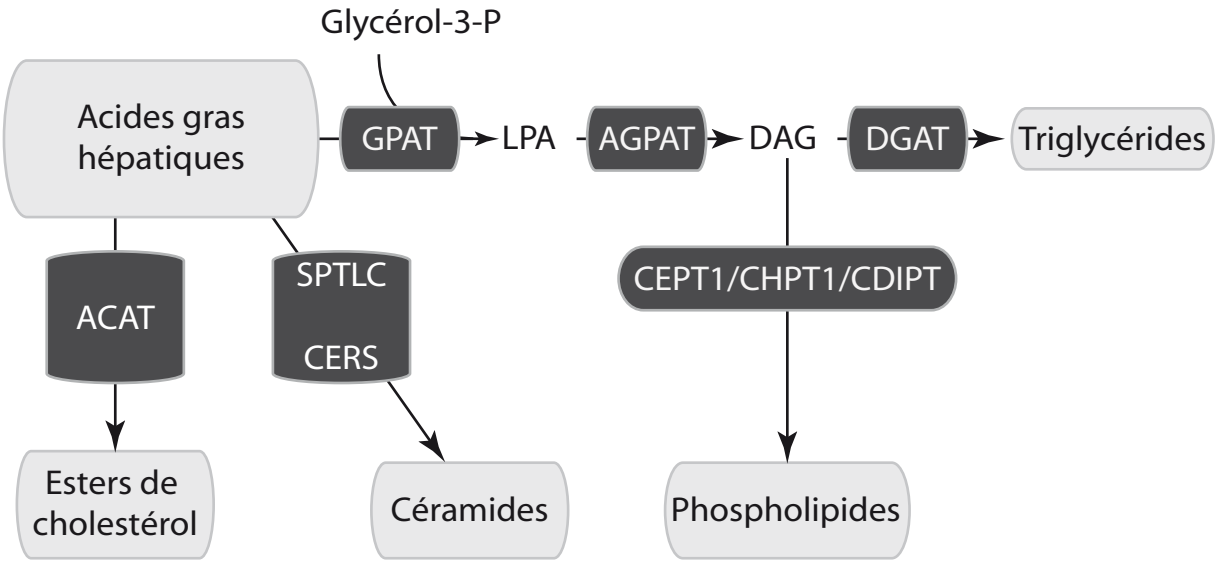


Figure 8. Synthèse des triglycérides hépatiques. La synthèse de triglycérides est réalisée par trois enzymes : la « glycerol-3-phosphate acyltransférase » (GPAT), la « 1-acylglycerol-3-phosphate O-acyltransferase » (AGPAT) et la « diacylglycerol acyltransferase » (DGAT), qui catalysent respectivement l'estérification d'acyl-CoA sur le premier, deuxième et troisième carbone du glycérol-3-phosphate. Les produits de synthèse de la GPAT et de l'AGPAT sont respectivement l'acide lysophosphatidique (LPA) et le diacylglycérol (DAG). Les acides gras peuvent également être incorporés dans d'autres lipides complexes tels que les phospholipides, les céramides et les esters de cholestérol. Les phospholipides sont synthétisés à partir du DAG par différentes enzymes. La « choline/ ethenolaminephosphotransferase 1 » (CEPT1) catalyse la synthèse de phosphatidylecholine et de phosphatidyléthanolamine alors que la « choline phosphotransferase » (CHPT1) catalyse exclusivement la synthèse de phosphatidylecholine. La « CDP-diacylglycerol-inositol-3-phosphatidyltransferase » (CDPIT) catalyse la synthèse de phosphatidyleinositol. Les acides gras peuvent aussi être estérifiés sur du cholestérol par « acetyl-CoA acyltransferase » (ACAT). Les céramides sont synthétisés par l'incorporation de deux acides gras sur une sérine puis sur une sphinganine, respectivement par la « serine palmitoyltransferase » (SPTLC) et la « ceramide synthase » (CERS).

comme nous l'avons vu précédemment, jouent un rôle important dans le développement des NAFLD. Dans cette partie nous verrons les processus enzymatiques conduisant à la synthèse de triglycérides (**Figure 8**).

Les acyl-CoAs à longue chaine produits par la lipogenèse *de novo* peuvent être estérifiés sur un noyau glycérol pour former des glycérolipides. Il y a trois étapes conduisant à l'incorporation de trois acyl-CoAs sur du glycérol-3-phosphate. La première étape est catalysée par la « glycerol-3-phosphate acyl transferase » (GPAT). Elle consiste en l'estérification d'un acyl-CoA sur le carbone en position 1 du glycérol-3-phosphate et conduit à la formation d'acide lysophosphatidique (LPA). Le LPA peut ensuite servir de composé de base à la synthèse de phospholipides ou de triglycérides. Il existe quatre GPATs appartenant à la même famille d'acyltransférases et qui sont codées par quatre gènes différents (Ganesh Bhat, Wang et al. 1999; Cao, Li et al. 2006; Harada, Hara et al. 2007; Wang, Lee et al. 2007; Chen, Kuo et al. 2008; Nagle, Vergnes et al. 2008). GPAT1 et GPAT2 sont localisés dans la membrane externe de la mitochondrie (Lewin, Schwerbrock et al. 2004) alors que GPAT3 et GPAT4 sont présents dans la membrane du réticulum endoplasmique (Gimeno and Cao 2008). Contrairement à GPAT2 et GPAT3, GPAT1 et GPAT4 sont fortement exprimés dans le foie. GPAT1 joue un rôle important dans la synthèse de triglycérides. En effet, les souris transgéniques invalidées pour *Gpat1* présentent une quantité de triglycérides hépatiques, une sécrétion de VLDLs ainsi qu'une triglycéridémie plus faible que les souris de type sauvage (Hammond, Gallagher et al. 2002; Hammond, Neschen et al. 2005). Ces souris sont également protégées contre l'obésité et la résistance à l'insuline induites par un régime riche en graisses (Neschen, Morino et al. 2005). Elles présentent une diminution de 40% et 30% de la proportion d'acide palmitique dans les triglycérides et dans les phospholipides respectivement (Hammond, Gallagher et al. 2002). Cette observation met en lumière la préférence de GPAT1 pour l'acide palmitique comme acide gras à estérifier en position sn-1 sur le glycerol-3-phosphate. L'invalidation de *Gpat1* chez des souris *ob/ob* induit une diminution de la quantité de triacylglycérol et de diacylglycérol dans le foie ainsi qu'une diminution de la glycémie (Xu, Wilcox et al. 2006). Les souris transgéniques invalidées pour *Gpat4* sont aussi protégées de l'obésité induite par un régime riche en graisse et en carbohydrate et par la déficience en leptine (Vergnes, Beigneux et al. 2006). Il semblerait que GPAT4 est moins spécifique que GPAT1 et peut estérifier aussi bien des acides gras saturés que mono-insaturés sur du triglycérol-3-phosphate pour produire de l'acide lysophosphatidique (Chen, Kuo et al. 2008).

L'étape suivante dans la synthèse de triglycérides est l'estérification d'un second acide gras en position sn-2 sur le noyau glycérol du LPA. Cette réaction conduit à la synthèse d'acide phosphatidique (PA) et est catalysée par les enzymes de la famille des « 1-acylglycerol-3-phosphate acyltransferase » (AGPATs). Il existe dix protéines supposées avoir une activité AGPAT (AGPAT1-10) (Leung 2001; Li, Yu et al. 2003; Ye, Chen et al. 2005; Agarwal, Barnes et al. 2006; Tang, Yuan et al. 2006; Agarwal, Sukumaran et al. 2007; Sukumaran, Barnes et al. 2009). Cependant, seuls AGPAT1 et AGPAT2 possèdent une activité enzymatique clairement démontrée (Leung 2001) et il semble que AGPAT2 soit l'isoforme impliquée dans l'acylation du LPA pour former des triglycérides. En effet, AGPAT2 présente une plus forte activité comparé aux AGPAT3-5,9 lors d'essais *in vitro* (Lu, Jiang et al. 2005; Agarwal, Sukumaran et al. 2007). De plus, 80% des souris nouveaux nés transgéniques invalidés pour *Agpat2* meurent dans les trois semaines suivant leur naissance (Cortes, Curtis et al. 2009) confirmant ainsi que AGPAT2 joue un rôle crucial et ne peut être substitué par d'autres isoformes. Les ARN messagers des autres *Agpat* ont été mesurés et la déficience en *Agpat2* n'induit qu'une faible augmentation de l'expression de ces derniers. L'activité totale d'AGPAT est réduite de 90% dans le foie de ces souris, confirmant que AGPAT2 prend en charge la majorité de l'activité AGPAT dans le foie (Cortes, Curtis et al. 2009). Enfin, le substrat préférentiel estérifié sur le LPA par AGPAT2 est l'oleyl-CoA (C18:1 n-9). D'autres substrats sont également incorporés comme les C14:0, C16:0, le C18:2 acyl-CoAs et avec une affinité plus faible les C18:0 et C20:4 acyl-CoAs (Eberhardt, Gray et al. 1997; Hollenback, Bonham et al. 2006). Ces données sont en accord avec la composition en acides gras sur la position sn-2 des triglycérides présentant principalement des groupes acyl monoenoic et dienoic plutôt que des acyl polyenoic généralement enrichis sur la position sn-2 des phospholipides (Glosset 1996).

Afin de synthétiser un triglycéride en estérifiant un acyl-CoA sur la position sn-3 d'un PA, ce dernier doit être déphosphorylé. Cette déphosphorylation est catalysée par une famille de protéines appelées « phosphatidate phosphatase 1 » (aussi appelées LIPIN). Trois enzymes appartiennent à cette famille : LPIN1, LPIN2 et LPIN3 (Carman and Han 2009). La dernière étape de la synthèse de triglycérides est catalysée par la « diacylglycerol acyltransferase » (DGAT). Jusqu'à présent deux protéines possédant une activité DGAT ont été identifiées : DGAT1 et DGAT2 (Coleman and Lee 2004). Ces deux protéines sont codées par deux gènes différents (Cases, Smith et al. 1998; Oelkers, Behari et al. 1998; Cases, Stone et al. 2001; Lardizabal, Mai et al. 2001). Dans des conditions basales DGAT2 est localisée

dans le réticulum endoplasmique. Lors d'un apport en acide oléique, DGAT2 est localisée à proximité de la surface des gouttelettes lipidiques à proximité des mitochondrie (Stone, Levin et al. 2009). DGAT1 est localisée dans le réticulum endoplasmique (Cao, Cheng et al. 2007), mais il a été rapporté qu'il co-localisait avec les gouttelettes lipidiques chez *S. cerevisae* (Sorger and Daum 2002). La surexpression de *Dgat1* ou *Dgat2* a pour conséquence une augmentation de la quantité de triglycérides dans les cellules transfectées. Dans les cellules surexprimant *Dgat1*, l'accumulation de triglycérides se fait sous la forme de petites gouttelettes lipidiques en périphérie de la cellule alors que dans les cellules transfectées avec *Dgat2* les triglycérides s'accumulent dans de grandes gouttelettes lipidiques (Stone, Myers et al. 2004). *Dgat1* et *Dgat2* sont exprimées dans de nombreux tissus dont le foie (Cases, Smith et al. 1998; Cases, Stone et al. 2001). DGAT2 semble avoir une activité plus importante que DGAT1 car les cellules surexprimant *Dgat2* accumulent plus de triglycérides que les cellules surexprimant *Dgat1* (Stone, Myers et al. 2004). De plus, les souris transgéniques invalidées pour *Dgat2* présentent une lipopénie natale létale non compensée par la présence de *Dgat1*. Les souris transgéniques invalidées pour *Dgat1* sont viables mais sont protégées de l'obésité induite par un régime riche en graisses (Smith, Cases et al. 2000).

La synthèse de triglycérides est un moyen de stocker de l'énergie. Les acides gras des différentes familles peuvent cependant être incorporées dans d'autres lipides complexes et leur conférer des propriétés signalisatrices.

II.4. Lipogenèse et signalisation par les acides gras.

Contrairement aux acides gras essentiels de la famille n-6 et n-3, qui ne sont pas synthétisables par les animaux et qui doivent être apportés par l'alimentation, les acides gras synthétisés par la lipogenèse *de novo* sont des acides gras saturés ou insaturés des familles n-7 et n-9 (**Figures 6 et 7**). Les cellules de mammifères possèdent des desaturases et des élongases leur permettant de synthétiser divers types d'acides gras (Guillou, Zadravec et al. 2010). En fonction de la taille de la chaine des acides gras et de leur degré d'insaturation, les acyl-CoAs vont servir de substrat à diverses enzymes qui catalyseront leur incorporation dans différents lipides complexes. L'incorporation sélective des acides gras en fonction de leurs caractéristiques dans différents types de lipides complexes est connue depuis longtemps. Le

développement de nouvelles méthodes d'analyse dans le domaine de la biochimie des lipides (Brown and Murphy 2009; Ivanova, Milne et al. 2009; Shevchenko and Simons 2010; Clark, Anderson et al. 2011) a permis l'analyse plus fine des acides gras composant les lipides complexes et l'émergence de nouvelles interrogations comme la façon dont les acides gras sont sélectionnés et comment ils influencent la signalisation de ces lipides (Chakravarthy, Lodhi et al. 2009; Clark, Anderson et al. 2011).

Récemment, des approches originales basées sur le développement d'animaux transgéniques ainsi que sur des analyses de lipides « nouvelles générations » ont conduit à l'émergence de l'hypothèse selon laquelle la lipogenèse *de novo* ne contribue pas uniquement à la synthèse d'acides gras pour leur stockage sous forme de triglycérides mais qu'elle contribue également à la genèse de molécules lipidiques signalisatrices. Cette hypothèse est en accord avec les différents phénotypes observés dans différents modèles de souris transgéniques invalidées pour les enzymes nécessaires à la synthèse de triglycérides. L'étude de ces voies de signalisation a permis d'introduire le concept de « lipoexpediency » proposant des effets protecteurs de composés issus de la lipogenèse sur les effets délétères induits par la lipogenèse elle-même. Le concept de lipoexpediency au niveau de l'organisme dans son intégralité a fait l'objet de travaux de synthèse (Lodhi, Wei et al. 2011). Ici, nous nous focaliserons sur le concept de « lipoexpediency » dans le foie.

L'acide palmitoléique libre (C16:1 n-7) a été identifié comme une « lipokine » possédant des vertus sur la résistance à l'insuline. Il a été montré qu'une quantité élevée d'acide palmitoléique est corrélée à une sensibilité accrue à l'insuline (Cao, Gerhold et al. 2008). Les souris transgéniques invalidées pour *Elovl6* sont protégées contre l'insulino-résistance provoquée par un régime riche en graisses sans présenter d'amélioration au niveau de l'obésité ou de la stéatose hépatique (Matsuzaka, Shimano et al. 2007). Cette résistance a été attribuée à une amélioration de la signalisation de l'insuline qui serait corrélée à une augmentation de la disponibilité en acide palmitoléique (Matsuzaka, Shimano et al. 2007).

Comme nous l'avons vu précédemment, les souris transgéniques invalidées pour *Fas* dans le foie ne sont pas protégées d'une stéatose hépatique quand elles sont nourries avec un régime pauvre en graisses et riche en carbohydrates. De façon intéressante, ces souris présentent également une diminution de l'expression de gènes cibles de PPARα et un phénotype proche des souris transgéniques n'exprimant pas *Pparα* (Chakravarthy, Pan et al. 2005). PPARα est un récepteur nucléaire important dans la régulation de l'expression de

gènes impliqués dans l'oxydation des acides gras. Chakravarthy *et al*. ont montré en 2009 que la phosphatidyl-choline (PC) estérifiée avec un acide palmitique et un acide oléique, deux acides gras produits de synthèse de FAS, est un ligand potentiel de PPARα (Chakravarthy, Lodhi et al. 2009). La lipogenèse *de novo* semble donc conduire à la production d'un ligand de PPARα qui préviendrait ainsi l'accumulation de graisses *via* l'activation de l'oxydation des acides gras. Cette hypothèse est en accord avec l'observation qu'une augmentation des acides gras libres circulants n'active pas PPARα au niveau hépatique (Sanderson, Degenhardt et al. 2009).

Alors que nous nous sommes focalisés sur la synthèse d'acides gras au niveau du foie et des phénotypes hépatiques en lien avec différentes modèles transgéniques, il est important de mentionner que la lipogenèse se produit également, dans d'autre tissus. La lipogenèse se déroule aussi dans le tissu adipeux blanc. Cependant, chez l'homme, la lipogenèse *de novo* dans le tissu adipeux blanc est considérée comme minoritaire (Bjorntorp and Sjostrom 1978). De plus, à la suite d'un apport en sucre dans l'alimentation, la lipogenèse hépatique est fortement régulée, ce qui n'est pas le cas dans le tissu adipeux blanc (Diraison, Yankah et al. 2003). Chez le rat, il semblerait que la lipogenèse de novo dans le tissu adipeux soit plus importante que chez l'homme (Letexier, Pinteur et al. 2003). Enfin, il est important de rappeler que la lipogenèse n'est pas uniquement importante dans un contexte de stockage énergétique et de production de molécules signalisatrices en lien avec des maladies métaboliques, mais qu'elle est également critique dans de nombreuses autres fonctions cellulaires (Jensen-Urstad and Semenkovich 2012).

II.5. La stéatose hépatique : lipotoxicité ou lipoprotection ?

La stéatose hépatique est associée au diabète de type II dans nombre d'études épidémiologiques. L'hypothèse d'une relation cause à effet entre l'accumulation de triglycérides dans le foie et la résistance à l'insuline a donc été émise (McGarry 1992). L'interrelation entre ces deux pathologies a été schématisée comme un cercle vicieux : d'un coté la résistance à l'insuline induit une hyperglycémie favorisant la lipogenèse et la stéatose hépatique, de l'autre l'accumulation de lipides au cours de la stéatose active différentes voies de signalisation directement ou indirectement *via* l'inflammation (Glass and Olefsky 2012) ou

le stress du réticulum endoplasmique (Fu, Watkins et al. 2012) aggravant la résistance à l'insuline (Chavez and Summers 2012; Samuel and Shulman 2012). Cependant, l'association entre la stéatose hépatique et la résistance à l'insuline est remise en question par plusieurs considérations. Tout d'abord, l'analyse méticuleuse des études cliniques montre que des sujets présentant des degrés similaires de stéatose hépatique peuvent présenter de faible et de forte sensibilité à l'insuline (Stefan, Kantartzis et al. 2008). De plus, des interventions visant à améliorer la résistance à l'insuline n'influent pas forcément sur le niveau de stéatose et inversement (Yu, Murray et al. 2005; Mao, DeMayo et al. 2006; Wendel, Li et al. 2010; Lonardo, Bellentani et al. 2011; Moon, Liang et al. 2012). Dans cette partie, nous allons voir que l'accumulation de lipides dans le foie n'entraine pas forcément une résistance à l'insuline.

A l'état nourri, le foie convertit le glucose d'origine alimentaire en glycogène. Quand les réserves en glycogène hépatique sont constituées (environ 5% de la masse du foie), le glucose en excès est orienté vers la synthèse *de novo* d'acides gras qui sont ensuite estérifiés dans des triglycérides. Ces triglycérides sont incorporés dans les VLDLs et exportés hors du foie. Des patients présentant une mutation du gène codant pour l'apolipoprotéine B (ApoB), une protéine nécessaire à la synthèse des VLDLs, sont incapables d'excréter les triglycérides. Ces patients présentent une stéatose hépatique et une hypotriglycéridémie mais ne développent pas de résistance à l'insuline (Amaro, Fabbrini et al. 2010; Visser, Lammers et al. 2011). Dans le même sens, les souris transgéniques invalidées pour *ApoB* développent également une stéatose hépatique mais restent sensibles à l'insuline (Schonfeld, Yue et al. 2008). Les souris transgéniques n'exprimant pas le gène codant pour la « microsomale triglycérides transfert protein » (MTTP) (Minehira, Young et al. 2008), une protéine impliquée dans l'assemblage des VLDLs, ou pour la « Phosphatidylethanolamine N-methyl transferase » (PEMT) (Jacobs, Zhao et al. 2010) ou la « CTP: phosphocholine acetyltransferase α » (CTα) (Niebergall, Jacobs et al. 2011), deux enzymes impliquées dans la synthèse de phosphatidylcholine, un phospholipide requis pour la synthèse des VLDLs, développent aussi une stéatose hépatique sans changement de la sensibilité à l'insuline. De même, des souris nourries avec un régime déficient en choline et ne pouvant donc pas synthétiser de VLDLs développent une stéatose qui n'est pas accompagnée d'une résistance à l'insuline (Raubenheimer, Nyirenda et al. 2006).

Avant leur incorporation dans les VLDLs, les triglycérides sont stockés dans des gouttelettes lipidiques dans le cytoplasme des hépatocytes. Les gouttelettes lipidiques sont également l'endroit où se déroule la lipolyse, qui consiste en l'hydrolyse des triglycérides

(Lass, Zimmermann et al. 2011). Les souris transgéniques invalidées spécifiquement au niveau du foie pour le gène codant pour l' « adipose triglycéride lipase » (ATGL ou PNPLA2), l'enzyme principale dans l'hydrolyse des triglycérides, développent une stéatose sans modification du statut inflammatoire ni de la sensibilité à l'insuline (Wu, Wang et al. 2011). Les gouttelettes lipidiques sont recouvertes de protéines importantes pour leur structure et la régulation de la lipolyse (Greenberg, Coleman et al. 2011). La « perilipin 2 » (Plin 2) fait partie de ces protéines et inhibe la lipolyse dépendante de ATGL dans le foie (Listenberger, Ostermeyer-Fay et al. 2007). La surexpression de *Plin2* chez la souris induit une stéatose hépatique et est associée à une amélioration de la sensitivité à l'insuline (Sun, Miller et al. 2012). Les gouttelettes lipidiques apparaissent donc, vis-à-vis du développement du diabète de type II, comme des lieux temporaires protecteurs de stockage des triglycérides.

La synthèse de triglycérides est également une façon de protéger les hépatocytes d'une lipotoxicité des acides gras. En effet, les souris transgéniques surexprimant *Dgat2* présentent une augmentation de la quantité hépatique de céramides, de diacylglycérols et de triglycérides mais sans modification de la sensibilité à l'insuline (Monetti, Levin et al. 2007). Comme nous le verrons plus en détails plus loin, SREBP-1c et le « carbohydrate-responsive element-binding protein » (ChREBP) sont deux facteurs de transcription qui, une fois activés, induisent l'expression de gènes impliqués dans la lipogenèse. La surexpression de *Srebp-1c* (Becard, Hainault et al. 2001) ou de *Chrebp* (Benhamed, Denechaud et al. 2012) dans le foie par adénovirus induit une accumulation de triglycérides hépatiques. Néanmoins, surexpression de *Srebp-1c* induit une diminution de la glycémie en dépit de la stéatose (Becard, Hainault et al. 2001) et celle de *Chrebp* empêche le développement de la résistance à l'insuline normalement observée à la suite d'un régime riche en graisse (Benhamed, Denechaud et al. 2012). Chez l'homme les niveaux d'expression de *ChREBP* sont corrélés positivement avec la stéatose hépatique et négativement avec la résistance à l'insuline (Benhamed, Denechaud et al. 2012).

L' « histone deacetylase 3 » (HDAC3) est une enzyme permettant de dé-acétyler les histones et de rendre la chromatine dans une conformation interdisant la transcription de gènes par des facteurs de transcription. L'étude de l'occupation de HDAC3 sur l'ensemble du génome montre un enrichissement de HDAC3 sur les promoteurs de gènes de la lipogenèse (Feng, Liu et al. 2011). L'enrichissement de HDAC3 sur ces gènes est soumis au rythme circadien avec une forte occupation sur les promoteurs durant le jour et une faible occupation durant la nuit (Feng, Liu et al. 2011). Cette régulation est en accord avec la phase de prise de

nourriture des souris durant la nuit et la phase de jeune durant le jour. Ainsi, l'expression des gènes de la lipogenèse durant la nuit permet le stockage d'énergie au cours de la phase d'alimentation. Cette régulation circadienne est directement régulée par les récepteurs nucléaires Rev-erbα et Rev-erbβ, des régulateurs clés du rythme circadien dans l'organisme (Feng, Liu et al. 2011; Bugge, Feng et al. 2012). Les souris transgéniques invalidées pour *Hdac3* dans le foie présentent une augmentation de la lipogenèse, de l'accumulation de triglycérides et de diacylglycérols, une diminution de la néoglucogenèse et une hypersensibilité à l'insuline (Sun, Miller et al. 2012). La diminution du flux néoglucogénique chez les souris déficientes pour *Hdac3* serait le résultat d'une réorientation du métabolisme vers la synthèse de lipides plutôt qu'un défaut des capacités de la néoglucogenèse (Sun, Miller et al. 2012). La lipogenèse et la néoglucogenèse sont donc régulées de façon opposée en fonction des besoins énergétiques du jour ou de la nuit. Dans ce modèle, l'augmentation de la lipogenèse est bénéfique à la signalisation par l'insuline.

LXR est un récepteur nucléaire impliqué dans la régulation transcriptionnelle des gènes de la lipogenèse. L'utilisation d'un activateur pharmacologique de LXR à long terme chez des souris obèses *ob/ob* aggrave la stéatose hépatique mais permet une amélioration de la sensibilité à l'insuline (Archer, Stolarczyk et al. 2013). Cette observation est concomitante avec une redistribution de graisses du tissu adipeux blanc viscéral vers le tissu adipeux blanc sous cutané et une diminution de l'état inflammatoire dans les deux types de tissus adipeux (Archer, Stolarczyk et al. 2013).

LXR possède des fonctions qui paraissent opposées car son activation conduit à une stéatose hépatique bien que LXR possède des propriétés bénéfiques dans le fonctionnement de l'organisme en général. Dans la suite de cette introduction bibliographique, nous nous intéresserons plus en détail à LXR en commençant par l'étude de sa structure, des mécanismes qui régulent son activité et de ses ligands.

Partie III : LXR, un récepteur nucléaire activé par les oxystérols

III.1. LXR Structure et modes d'activation

Les Livers X Receptors sont des facteurs de transcription appartenant à la famille des récepteurs nucléaires, qui comprend 49 membres chez la souris et 48 chez l'homme. Il existe deux isoformes de LXR : LXRα (NR1H3) et LXRβ (NR1H2) qui ont été découvertes en 1995 (Teboul, Enmark et al. 1995; Willy, Umesono et al. 1995). Ce sont des récepteurs nucléaires de classe II fonctionnant en hétérodimère avec le « retinoid X receptors » (RXR), le récepteur de l'acide 9-cis rétinoïque (Repa and Mangelsdorf 2000). L'hétérodimère LXR/RXR se lie sur l'ADN sur des éléments de réponse à LXR (LXREs). Les LXREs sont des éléments de réponse de type « directed repeat » (DR4) composés de deux séquences consensus directes répétées (AGGTCA) et séparées par quatre nucléotides. LXRα est fortement exprimé dans le foie, l'intestin, les reins et les tissus adipeux alors que LXRβ présente une expression ubiquitaire (Auboeuf, Rieusset et al. 1997; Repa and Mangelsdorf 2000).

A l'instar des autres récepteurs nucléaires les LXRs sont organisés en différents domaines fonctionnels (**Figure 4**). Le domaine N-terminal (A/B) est faiblement conservé et contient la fonction de transactivation indépendante du ligand (AF-1) qui est responsable du niveau d'activité basale même en l'absence de ligand. La partie centrale contient le domaine de liaison à l'ADN (DBD). Le DBD est fortement conservé et contient deux motifs en doigt de zinc qui interagissent avec les sites de liaison à l'ADN présents sur les éléments de réponse dans les séquences régulatrices des gènes cibles des LXRs. Enfin, la partie C-terminale de LXR, bien conservée, présente le domaine de liaison avec le ligand (LBD) qui possède la fonction de transactivation ligand dépendante (AF-2). La fixation du ligand entraine une modification des partenaires protéiques capables de se lier à LXR. (Pour une synthèse complète voir (Viennois, Pommier et al. 2011)).

En l'absence de ligand, l'hétérodimère LXR/RXR est lié à l'ADN sur les LXREs présents dans les séquences régulatrices des gènes cibles de LXR, et interagit avec des co-répresseurs protéiques comme le « nuclear receptor co-repressor » (NCoR) ou le « silencing mediator of retinoid and thyroid receptors » (SMRT) (Hu, Li et al. 2003) (**Figure 9**). Les co-

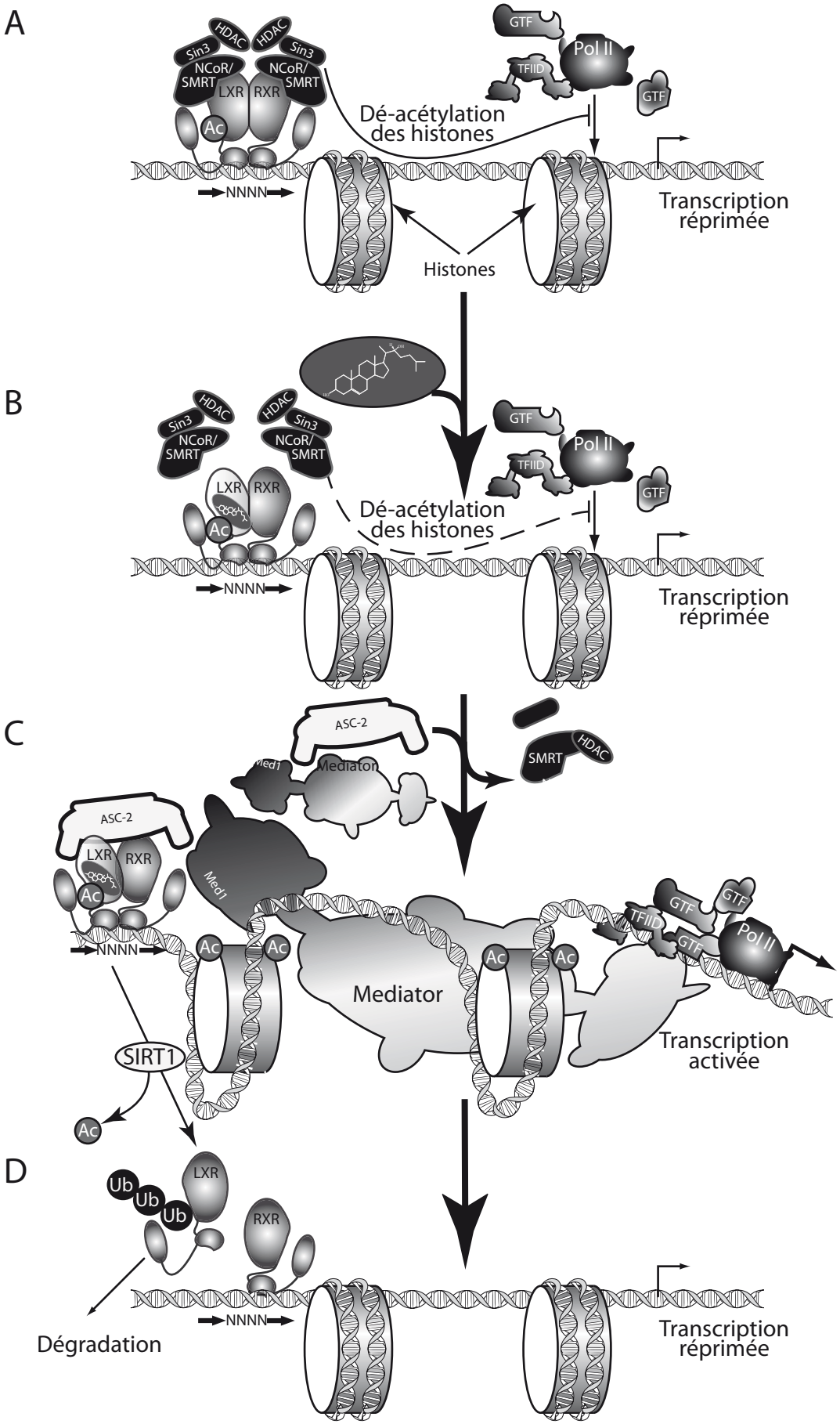


Figure 9. Mécanisme d'activation de LXR. (A) En l'absence de ligand l'hétérodimère LXR/RXR est lié sur son élément de réponse sur le promoteur de ses gènes cibles. Les co-répresseurs, le « nuclear receptor co-repressor » (NCoR) ou le « silencing mediator of retinoid and thyroid receptors) (SMRT), le « stress-activated MAP kinase activated protein 3 » (Sin3) et les « histone deacetylases » (HDACs) sont fixés sur l'hétérodimère et maintiennent la chromatine dans un état de compaction interdisant la transcription. (B) La fixation d'un ligand (ici le 22(R)-hydroxycholestérol) sur le domaine de liaison au ligand de LXR entraine un changement de conformation conduisant au départ des co-répresseurs et au recrutement de co-activateurs, ici l'« activating signal cointegrator-2 » (ASC-2). (C) De façon concomitante, plusieurs mécanismes incluant la modification des histones et le remodelage de la chromatine permettent à la « RNA polymerase II » (RNA PolII) et à la machinerie transcriptionnelle incluant le « General transcription factor » (GTF), le « transcription factor II D » (TFIID) et le complexe mediator d'induire la transcription du gène cible. (D) Ensuite, la « NAD-dependent deacetylase sirtuin-1 » (SIRT1) dé-acétyle LXR ce qui conduit à son ubiquitination et à sa dégradation par le protéasome. Ce mécanisme est important pour commencer un nouveau cycle de transcription du gène cible.

répresseurs recrutent d'autres protéines possédant une activité histone dé-acétylase (HDACs). Ce recrutement se fait par l'intermédiaire de la « stress-activated MAP kinase interacting protein 3 » (SIN3) (Jones, Sachs et al. 2001). L'ADN est alors dans une configuration où la transcription est impossible et la machinerie nécessaire à la transcription ne peut pas interagir avec le site d'initiation. La fixation du ligand sur le LBD induit une modification de la conformation spatiale de LXR (Glass and Rosenfeld 2000) ce qui a pour conséquence la libération des co-répresseurs (Hu, Li et al. 2003) et le recrutement de co-activateurs tels que l'« activating signal cointegrator 2 » (ASC-2) (Lee, Lee et al. 2008) ou le « receptor-integrating protein 140 » (RIP140) (Herzog, Hallberg et al. 2007) sur l'hélice 12 du LBD (Svensson, Ostberg et al. 2003). Les histones sont alors acétylées, la chromatine est dans une configuration propice au recrutement de la machinerie nécessaire à la transcription qui peut alors être initiée. Quand LXR se fixe sur un de ses éléments de réponse, il est acétylé. Une fois la transcription du gène cible achevée, LXR est déacétylé par la déacétylase Sirtuin-1 (SIRT1) (Li, Zhang et al. 2007), ce qui conduit à l'ubiquitination de LXR et à sa dégradation par le protéasome. Cette action de SIRT1 augmente le « turn-over » de LXR *via* des cycles d'activation/dégradation ayant pour effet d'augmenter l'activité de LXR (Li, Zhang et al. 2007).

Ce modèle conventionnel d'activation de LXR a depuis été remis en question à la suite de plusieurs observations (Wagner, Valledor et al. 2003; Boergesen, Pedersen et al. 2012) proposant une fixation de l'hétérodimère LXR/RXR activé sur des éléments de réponse de séquence régulatrices de gènes cibles à la suite d'une modification de marqueurs épi-génétiques révélant ces sites. LXR contribue également à réprimer la transcription de gènes *via* un processus appelé trans-répression, un mécanisme par lequel LXR inhibe les voies inflammatoires (Ghisletti, Huang et al. 2007). Par exemple LXR est capable de stopper la transcription de gènes pro-inflammatoires induits par le facteur de transcription STAT1 (Lee, Park et al. 2009). Ce mécanisme de trans-répression nécessite la SUMOylation de LXR. Une fois SUMOylé, LXR interagit avec les co-répresseurs présents sur les promoteurs de gènes pro-inflammatoires et prévient leur dissociation limitant ainsi la transcription.

LXR a d'abord été considéré comme un récepteur orphelin, ses ligands naturels étant inconnus. Cependant le groupe de Mangelsdorf, en utilisant des approches de gènes rapporteurs, a été le premier à mettre en évidence que des dérivés oxydés du cholestérol, les oxysterols, induisaient l'activation de LXRα (Janowski, Willy et al. 1996).

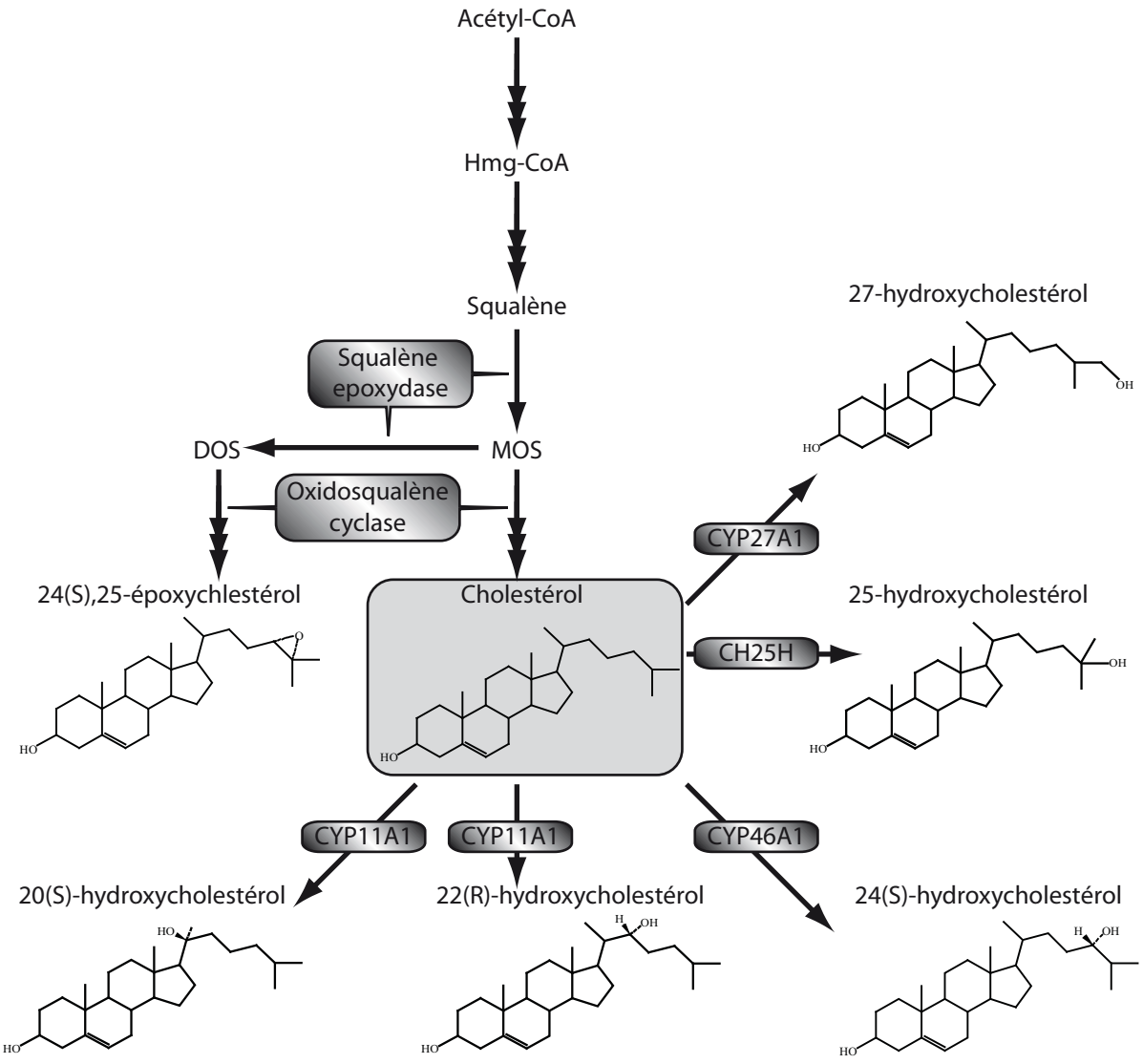


Figure 10. Synthèse et structure des oxystérols. Le 20(S)-hydroxycholestérol, le 22(R)-hydroxycholestérol, le 24(S)-hydroxycholestérol, le 25-hydroxycholestérol et le 27-hydroxycholestérol sont synthétisés à partir du cholestérol. La fonction alcool est branchée sur différents carbones de la chaine latérale du cholestérol en fonction des hydroxycholestérols. Les réactions conduisant à leur synthèse sont catalysées par CYP11A1 pour le 20(S)-hydroxycholestérol et le 22(R)-hydroxycholestérol et par CYP46A1, CH25H et CYP27A1 pour le 24(S)-hydroxycholestérol, le 25-hydroxycholestérol et le 27-hydroxycholestérol. Le 24(S),25-hydroxycholestérol est synthétisé par une voie de synthèse parallèle à celle du cholestérol et qui partage les mêmes enzymes. Deux enzymes sont impliquées dans le contrôle de cette voie de synthèse, la « squalene epoxydase » (SE) synthétise du monooxydosqualène (MOS) et du dioxydosqualène (DOS) et l'« oxydosqualene cyclase » (OSC) catalyse la première réaction des voies conduisant à la synthèse de 24(S),25-hydroxycholestérol et de cholestérol à partir respectivement du DOS et du MOS. Le 24(S),25-hydroxycholestérol possède la même structure que le cholestérol mais possède une fonction epoxy branchée sur les carbones 24 et 25 de la chaine latérale.

III.2. Origines et synthèse des oxystérols

Les oxystérols ont été découverts en 1913 par Lifschutz comme des produits d'auto-oxydation du cholestérol. Ces composés ont d'abord été décrits comme des modulateurs du métabolisme du cholestérol étant donné leur capacité à séquestrer les SREBPs au niveau du réticulum endoplasmique et à limiter l'expression des gènes de la synthèse du cholestérol (pour synthèse (Brown and Jessup 2009)). Les oxystérols sont également responsables de la dégradation de la « 3-hydroxy-3-methylglutaryl CoA reductase » (HMGCR), l'enzyme limitante de la voie de synthèse du cholestérol (Brown and Jessup 2009). Les oxystérols sont aussi des activateurs de LXR (Janowski, Willy et al. 1996; Lehmann, Kliewer et al. 1997). Il existe différents types d'oxystérols, et tous ont en commun la structure du cholestérol et possèdent une fonction chimique contenant un atome d'oxygène telle qu'une hydroxy, kéto ou époxy. Les fonctions chimiques oxygénées peuvent être ajoutées sur le noyau stérol ou sur la chaine latérale du cholestérol. Les oxystérols peuvent être issus d'oxydations enzymatiques ou non enzymatiques.

Il existe une grande variété d'oxystérols. Leurs origines ont fait l'objet de plusieurs travaux de synthèse de la part de différents auteurs (Russell 2000; Schroepfer 2000; Gill, Chow et al. 2008; Brown and Jessup 2009). Les effets spécifiques des différents oxystérols sur LXR dépendent fortement des gènes étudiés et des tissus où ils exercent leurs effets. Par exemple, le 5α,6α-epoxycholestérol exerce des effets à la fois agonistes et antagonistes en fonction des gènes cibles de LXR étudiés et du contexte cellulaire (Berrodin, Shen et al. 2010). Nous avons choisi de nous focaliser sur la synthèse d'oxystérols connus pour être la résultante de réactions enzymatiques (**Figure 10**). Ces oxystérols sont les suivants : le 20(S)-hydroxycholestérol (Janowski, Willy et al. 1996), le 22(R)-hydroxycholestérol (Janowski, Willy et al. 1996), le 24(S)-hydroxycholestérol (Janowski, Willy et al. 1996; Lehmann, Kliewer et al. 1997), le 25-hydroxycholestérol (Janowski, Willy et al. 1996), le 27-hydroxycholestérol (Janowski, Willy et al. 1996; Fu, Menke et al. 2001) et le 24(S),25-époxycholestérol (Lehmann, Kliewer et al. 1997; Svensson, Ostberg et al. 2003). Ils sont généralement admis comme des ligands physiologiques de LXR dans les tissus lipogéniques.

III.3. Biosynthèse des hydroxycholestérols

La formation des hydroxycholestérols est catalysée par différentes enzymes, la plupart appartenant à la famille des cytochromes P450 (**Figure 10**). CYP46A1 est une enzyme microsomale qui catalyse la synthèse de 24(S)-hydroxycholestérol. Cet oxystérol est aussi appelé cérébrosol car il est présent en grande quantité dans le cerveau (Bjorkhem 2007). Le 27-hydroxycholestérol est un intermédiaire de la voie de synthèse des acides biliaires, il est produit par la CYP27A1, une enzyme mitochondriale, et il est le principal oxystérol présent dans la circulation. La cholesterol 25 hydroxylase (CH25H) est responsable de la synthèse du 25-hydroxycholestérol. Contrairement aux autres enzymes responsables de la synthèse des hydroxycholestérols, CH25H n'appartient pas à la famille des cytochromes P450. Elle est localisée dans le réticulum endoplasmique et l'appareil de Golgi et est exprimée à de faibles niveaux dans la plupart des tissus (Russell 2000). Cependant, il a été montré que le 25-hydroxycholestérol pouvait être un produit de synthèse de CYP3A (Honda, Miyazaki et al. 2011) et également apparaître à la suite de réactions non enzymatiques (Smith 1987). Enfin, la CYP11A1 catalyse la formation du 20(S)-hydroxycholestérol et du 22(R)-hydroxycholestérol (Gill, Chow et al. 2008). Ces deux composés sont des intermédiaires de la voie de synthèse des hormones stéroïdes et leur synthèse se produit majoritairement dans les glandes surrénales (Gill, Chow et al. 2008).

Dans le milieu des années 90, il a été démontré par des analyses de gène rapporteur *in vitro* que les hydroxycholestérols activaient LXR, d'abord pour LXRα (Janowski, Willy et al. 1996) puis pour les deux isoformes (Lehmann, Kliewer et al. 1997). En 2007 Chen *et al.* (Chen, Chen et al. 2007) ont apporté *in vivo* une preuve supplémentaire de l'activation de LXR par les oxystérols. En surexprimant la sulfotransférase SULT2B1, une enzyme responsable de la dégradation des oxystérols, ils ont observé une détérioration du fonctionnement de LXR et ce, à la fois *in vitro* et *in vivo*. Ils ont également montré une perturbation de l'activité de LXR chez les souris transgéniques n'exprimant pas *Cyp46a1*, *Ch25h* et *Cyp27a1* et démontré ainsi l'importance *in vivo* des oxystérols dans l'activation de LXR. Cependant l'expression de *Srebp-1c*, un gène cible de LXR, reste élevé en réponse à un régime riche en cholestérol ce qui suggère la présence d'autres ligands endogènes qui ne sont pas synthétisés par les trois enzymes codées par les gènes inactivés. Plusieurs études montrent que le 25-hydroxycholestérol sulfaté, le 25 hydroxycholestérol-3-sulfate n'est pas seulement

un oxystérol inactivé mais un composé avec des propriétés antagonistes sur LXR. En effet, le traitement de cultures cellulaires avec ce composé, ou la surexpression de SULT2B1, l'enzyme catalysant sa synthèse (Li, Pandak et al. 2007), conduit à une diminution de l'activité de LXR (Bai, Xu et al. 2010; Xu, Bai et al. 2010). D'autres oxystérols sulfatés comme le 5α,6α-epoxycholestérol-3-sulfate ou le 7-kétocholestérol-3-sulfate sont des ligands antagonistes de LXR (Song, Hiipakka et al. 2001).

III.4. La voie de synthèse du cholestérol : une autre source de ligands pour LXR.

Le 24(S),25-epoxycholestérol est un autre agoniste de LXR qui a été découvert en 1981 (Nelson, Steckbeck et al. 1981). Comme d'autres oxystérols, le 24(S),25-epoxycholestérol a la capacité de limiter la synthèse de cholestérol en réduisant l'activité de l'HMGCR (Saucier, Kandutsch et al. 1985; Taylor, Kandutsch et al. 1986; Dollis and Schuber 1994), en induisant sa dégradation (Song and DeBose-Boyd 2004) aussi bien qu'en limitant la maturation de SREBP-2 (Janowski, Shan et al. 2001; Wong, Quinn et al. 2006), le facteur de transcription régulant la synthèse du cholestérol. Contrairement aux autres oxystérols, le 24(S),25-epoxycholestérol n'est pas un composé dérivant du cholestérol. Sa synthèse se produit à la suite d'un détournement de la voie de synthèse du cholestérol et qui est ensuite parallèle à cette voie (**Figure 10**). La voie de synthèse du 24(S),25-epoxycholestérol subit donc le même rétro-contrôle que la synthèse du cholestérol (Wong, Quinn et al. 2007). Cette dérivation commence avec le monooxydosqualène (MOS) qui peut être transformé en dioxydosqualène (DOS) par la « squalene epoxydase » (SE), aussi connu comme la « squalene monooxygenase » (SM). La dégradation de cette enzyme est sous le contrôle du protéasome lui même régulé par le cholestérol (Gill, Stevenson et al. 2011). L' « oxydosqualene cyclase » (OSC) peut alors convertir le précurseur du cholestérol (le MOS) et le précurseur du 24(S),25-epoxycholestérol (le DOS) respectivement en lanostérol et en 24(S),25-epoxylanostérol (Nelson, Steckbeck et al. 1981). Ces deux composés sont alors transformés *via* plusieurs réactions communes en cholestérol et en 24(S),25-epoxycholestérol. Les enzymes impliquées dans ces réactions sont communes aux deux voies de synthèse. Plusieurs études montrent que cet oxystérol est un activateur de LXR (Lehmann, Kliewer et al. 1997; Janowski, Grogan et al. 1999). Cependant, ces études consistent en l'ajout de 24(S),25-epoxycholestérol dans des systèmes *in vitro* et des cultures cellulaires. Par la suite

différentes stratégies ont été utilisées pour moduler la quantité de 24(S),25-epoxycholestérol produit de façon endogène. Plusieurs de ces approches sont basées sur le fait que l'OSC possède une meilleure affinité pour le DOS que pour le MOS (Boutaud, Dolis et al. 1992). Les statines, des molécules utilisées pour traiter les hypercholestérolémies et les pathologies cardiovasculaires associées, sont des inhibiteurs de l'HMGCR, l'enzyme catalysant la synthèse de mévalonate. Le traitement de macrophages THP-1 avec des statines conduit à la diminution à la fois du 24(S),25-epoxycholestérol et du cholestérol, mais également à la diminution de l'expression de deux gènes cibles de LXR : *Abca1* et *Abcg1* (Wong, Quinn et al. 2004). La diminution de la réponse LXR est rétablie avec l'ajout exogène de 24(S),25-epoxycholestérol. Les effets inhérents aux statines semblent dépendre de la présence de cholestérol dans les cellules. En effet, la supplémentation en cholestérol annulent les effets des statines sur LXR alors qu'une déplétion en cholestérol a tendance à renforcer les effets des statines (Wong, Quinn et al. 2008). Les statines, utilisées en prétraitement, permettent d'induire, une fois le traitement stoppé, une hyperactivité de la voie du mévalonate. Ce prétraitement sur des lignées d'ovaires de hamster CHO-7 augmente la synthèse de cholestérol, de 24(S),25-epoxycholestérol ainsi que de l'activité de LXR (Wong, Quinn et al. 2008). Dans tous les cas, la diminution ou l'augmentation du flux de la voie du mévalonate, la synthèse de cholestérol ou de 24(S),25-epoxycholestérol apparaissent étroitement liées. Le 24(S),25-epoxycholestérol semble être un composé protégeant la cellule du cholestérol endogène (Wong, Quinn et al. 2007). D'autres travaux ont étudié la possibilité de découpler la synthèse du cholestérol et de celle du 24(S),25-epoxycholestérol pour mieux décrire le rôle de ce dernier. Etant donné que l'OSC présente une meilleure affinité pour le DOS que pour le MOS, l'inhibition partielle de cette enzyme permet d'augmenter la synthèse de 24(S),25-epoxycholestérol aux dépends de celle du cholestérol (Morand, Aebi et al. 1997). L'utilisation de cette inhibition partielle permet de découpler la synthèse de cholestérol de celle de 24(S),25-epoxycholestérol car le MOS produit par la SE s'accumule et peut être catalysée une fois encore par la SE pour former du DOS. Le DOS ainsi formé peut ensuite être transformé en 24(S),25-epoxycholestérol. En effet, l'utilisation d'inhibiteurs de l'OSC induisent une diminution de la synthèse de cholestérol ainsi qu'une augmentation de celle de 24(S),25-epoxycholestérol et de l'activité de LXR dans la lignée de macrophages THP-1 (Wong, Quinn et al. 2004; Beyea, Heslop et al. 2007), une lignée de macrophages murins (Wong, Quinn et al. 2004), des HepG2 (Morand, Aebi et al. 1997) et des CHO-7 (Wong, Quinn et al. 2008). En 2008, Wong *et al.* (Wong, Quinn et al. 2008) ont utilisé une autre stratégie pour étudier les effets du 24(S),25-epoxycholestérol endogène. Etant donné que l'inhibition partielle de l'OSC

induit une augmentation de la synthèse de 24(S),25-epoxycholestérol, ils ont utilisé l'approche inverse, à savoir une augmentation de l'expression de l'OSC humaine dans la lignées cellulaire CHO-7. Ces cellules ne contiennent pas de 24(S),25-epoxycholestérol et présentent une activité LXR réduite comparée aux cellules contrôles. Basée sur l'utilisation de statines, il a été démontré qu'une activation de LXR par un oxystérol intermédiaire de la voie de synthèse du cholestérol était nécessaire à la transcription de *Srebp-1c*. En conclusion, l'ensemble de ces études conforte l'hypothèse que la synthèse de cholestérol *via* la production de 24(S),25-epoxycholestérol peut influencer l'activité de LXR. LXR est donc activé par des dérivés oxydés du cholestérol et s'est donc vu attribué le rôle de senseur de ce composé. Nous allons voir dans la partie suivante que LXR est un acteur majeur de la clairance du cholestérol dans l'organisme.

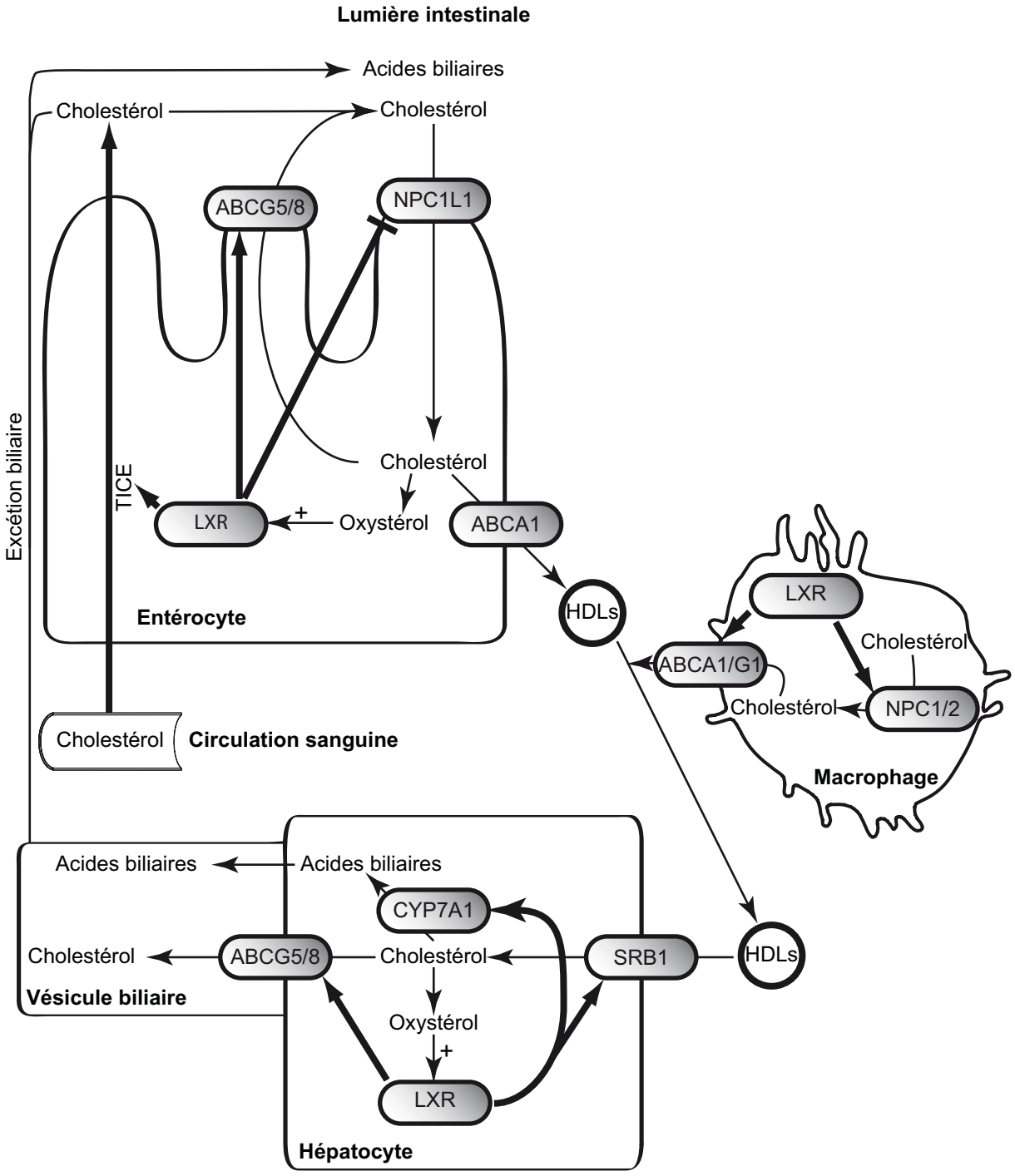


Figure 11. Implication de LXR dans le transport réverse du cholestérol. LXR module l'expression des gènes codant pour les protéines renseignées dans cette figure. Dans l'entérocyte, LXR limite l'absorption du cholestérol en induisant l'expression des ABC transporteurs G5 et G8 (ABCG5/8) et en inhibant le « niemann-pick C1-like 1 » (NPC1L1). LXR induit également l'expression de l'ABC transporteur A1 (ABCA1) qui permet l'excrétion du cholestérol dans les lipoprotéines de haute densité (HDLs). Dans le macrophage LXR, induit l'expression des « niemann-pick C1/2 » (NPC1/2) et des ABC transporteurs A1 et G1 (ABCA1/ABCG1), qui sont impliqués respectivement dans le trafic intracellulaire et l'excrétion du cholestérol dans les HDLs. Dans l'hépatocyte, LXR induit l'expression du « scavenger receptor classe B member 1 » (SRB1) le récepteur des HDL. LXR induit également l'expression des ABC transporteurs G5 et G8 et du Cytochrome P450 7A1 (CYP7A1) impliqués respectivement dans l'excrétion du cholestérol dans la vésicule biliaire et dans la transformation du cholestérol en acides biliaires. L'activation de LXR conduit également à l'augmentation de l'excrétion trans-intestinale du cholestérol (TICE).

Partie IV: Implication de LXR dans le métabolisme du cholestérol et la modulation de l'inflammation

IV.1. Régulation de l'homéostasie du cholestérol

LXR est un des récepteurs nucléaires impliqués dans le métabolisme du cholestérol (Tontonoz and Mangelsdorf 2003). Il est essentiel dans l'excrétion de ce composé en dehors de l'organisme *via* la détection des dérivés oxygénés du cholestérol. Un autre récepteur nucléaire, FXR, senseur des acides biliaires, participe également à l'homéostasie du cholestérol (Calkin and Tontonoz 2012). Il est important de noter que le cholestérol peut être toxique dans la cellule et doit être finement régulé. Cependant, c'est également un composé essentiel pour l'intégrité de la membrane cellulaire et pour plusieurs molécules signalisatrices. Lors d'une déficience en cholestérol alimentaire, ce composé doit être synthétisé par la voie de synthèse du mévalonate, qui est sous la régulation transcriptionnelle du « sterol responsive element binding protein-2 » (SREBP-2) (Brown and Goldstein 1997; Radhakrishnan, Goldstein et al. 2008).

Les études utilisant des modèles de souris transgéniques invalidées pour une (Peet, Turley et al. 1998) ou les deux isoformes de LXR (Repa, Liang et al. 2000) ont permis d'obtenir de nombreuses informations sur le rôle *in vivo* de LXR sur le métabolisme du cholestérol (**Figure 11**). Les souris transgéniques ne possédant pas l'isoforme α de LXR ne peuvent pas excréter le cholestérol et accumulent des esters de cholestérol dans le foie (Peet, Turley et al. 1998). De plus, l'accumulation de cholestérol est plus marquée lorsque ces souris sont nourries avec un régime riche en cholestérol (Kalaany, Gauthier et al. 2005). Ces données mettent en évidence le rôle important de LXR dans le désengorgement du cholestérol provenant de la voie du mévalonate contrôlée par SREBP-2 ou de l'alimentation. Chez la souris ce défaut d'enlèvement du cholestérol résulte de la diminution de l'expression de *Cyp7a1* (Peet, Turley et al. 1998) qui code pour l'enzyme limitante de la dégradation du cholestérol en acides biliaires.

En plus du rôle de LXRα dans la régulation hépatique de *Cyp7a1*, LXR contribue à la réduction de la quantité du cholestérol dans l'organisme de par son rôle dans la régulation de

l'expression de gènes impliqués dans le transport réverse du cholestérol (**Figure 11**). LXR régule aussi l'expression de *Abcg5* et *Abcg8*, deux gènes codant pour deux « demi » transporteurs agissant en dimère qui sont impliqués dans le transport du cholestérol (Yu, York et al. 2003; Yu, Gupta et al. 2005). Ils sont principalement exprimés dans le foie et l'intestin où ils sont respectivement impliqués dans l'excrétion dans la bile et dans la lumière intestinale.

L'utilisation de souris transgéniques invalidées pour LXRα spécifiquement au niveau du foie a permis de montrer que cette isoforme était essentielle au transport réverse, la dégradation et l'excrétion du cholestérol (Zhang, Breevoort et al. 2012). Cependant, l'utilisation d'un agoniste de LXR chez ces souris semble être une bonne stratégie dans le traitement de l'athérosclérose. Une partie de cet effet peut être expliquée par la contribution de LXRβ (Bradley, Hong et al. 2007) ainsi que par l'activité extra-hépatique de LXRα.

Il a été récemment montré que l'intestin pouvait jouer un rôle important dans l'implication de LXR dans la protection vis-à-vis des dommages liés au cholestérol (**Figure 11**). Ainsi, l'expression d'une forme constitutivement active de LXRα dans l'épithélium intestinal diminue l'absorption du cholestérol et induit une augmentation du transport réverse du cholestérol alors que l'activité hépatique de LXR ne modifie pas ce dernier paramètre (Lo Sasso, Murzilli et al. 2010). Il est important de noter que la surexpression de LXRα dans l'intestin améliore non seulement les marqueurs athérogéniques mais également la quantité de lipides hépatiques. En effet, ces souris présentent des niveaux de triglycérides et de cholestérol au niveau du foie plus faibles que des souris de type sauvage après avoir été nourries avec un régime riche en graisses (Lo Sasso, Murzilli et al. 2010). Ces résultats montrent que l'activation spécifique de LXR dans l'intestin peut donc être un levier dans les NAFLD étant donné la modulation de la quantité de lipides hépatiques à la suite d'un régime riche en graisses. Les lipoprotéines de haute densité ou « high density lipoproteins » (HDLs) sont indispensables au transport réverse du cholestérol. Les HDLs sont principalement sécrétées par le foie. ABCA1 promeut le transfert du cholestérol dans l'apolipoprotéine ApoA1, l'étape limitante de la biogenèse des HDLs. *Abca1* possède un LXRE dans son promoteur (Repa, Turley et al. 2000; Venkateswaran, Laffitte et al. 2000). Dans l'intestin, ABCA1 est critique pour la maintenance du taux de cholestérol-HDL révélant l'implication de cet organe dans la production de HDLs et dans la régulation du transport réverse du cholestérol (Brunham, Kruit et al. 2006; Brunham, Kruit et al. 2006).

LXR joue aussi un rôle majeur dans le transport réverse du cholestérol en contrôlant l'efflux du cholestérol des cellules périphériques, notamment les macrophages (**Figure 11**). En effet, LXR régule l'expression de gènes codant pour des ABC transporteurs : *Abca1* et *Abcg1* (Repa, Turley et al. 2000; Venkateswaran, Laffitte et al. 2000). Le cholestérol intracellulaire est donc transféré dans des particules HDLs, transportées jusqu'au foie pour une élimination ultérieure du cholestérol. L'administration *in vivo* d'agonistes de LXR induit dans les macrophages le transport réverse du cholestérol *via* ces deux ABC transporteurs (Naik, Wang et al. 2006; Wang, Collins et al. 2007). Pour être exporté dans les HDLS, le cholestérol doit passer du compartiment endosomal jusqu'à la membrane plasmique, où il pourra être pris en charge par les transporteurs ABC. Ce transport est réalisé par deux protéines : les « nimann-pick C1 » (NPC1) et « nimann-pick C2 » (NPC2). Les ligands de LXR induisent une augmentation de l'expression des gènes codant pour ces protéines (Rigamonti, Helin et al. 2005). LXR induit également l'expression de l' « inducible degrader of LDLR » (*Idol*) conduisant à la dégradation du LDLR et à la limitation de l'absorption du cholestérol dans les tissus périphériques comme les macrophages (Zelcer, Hong et al. 2009). Dans le foie, LXR induit l'expression du gène codant pour le « scavenger receptor classe B member 1 » (SRB1) qui permet le transfert du cholestérol des HDLs jusqu'au foie, une des dernières étapes du transport réverse du cholestérol (Malerod, Juvet et al. 2002).

L'excrétion hépatobiliaire a longtemps été considérée comme la seule voie du transport réverse du cholestérol. Cependant plusieurs études ont montré qu'il existait une voie parallèle d'excrétion du cholestérol du sang jusque dans l'intestin grêle appelée excrétion trans-intestinale du cholestérol. (Trans-intestinal cholestérol excretion : TICE) (Yu, Hammer et al. 2002; Temel, Tang et al. 2007; Temel, Sawyer et al. 2010). LXR est aussi impliqué dans le transport réverse du cholestérol dans cette voie. En effet un traitement par des agonistes de LXR, le T0901317 ou le GW3965, conduit à une induction de deux à trois fois du TICE (Kruit, Plosch et al. 2005; van der Veen, van Dijk et al. 2009).

IV.2. Implication de LXR dans l'inflammation

Dans cette partie, nous verrons comment LXR est impliqué dans diverses voies de signalisation qui couplent le métabolisme du cholestérol à la réponse inflammatoire et au

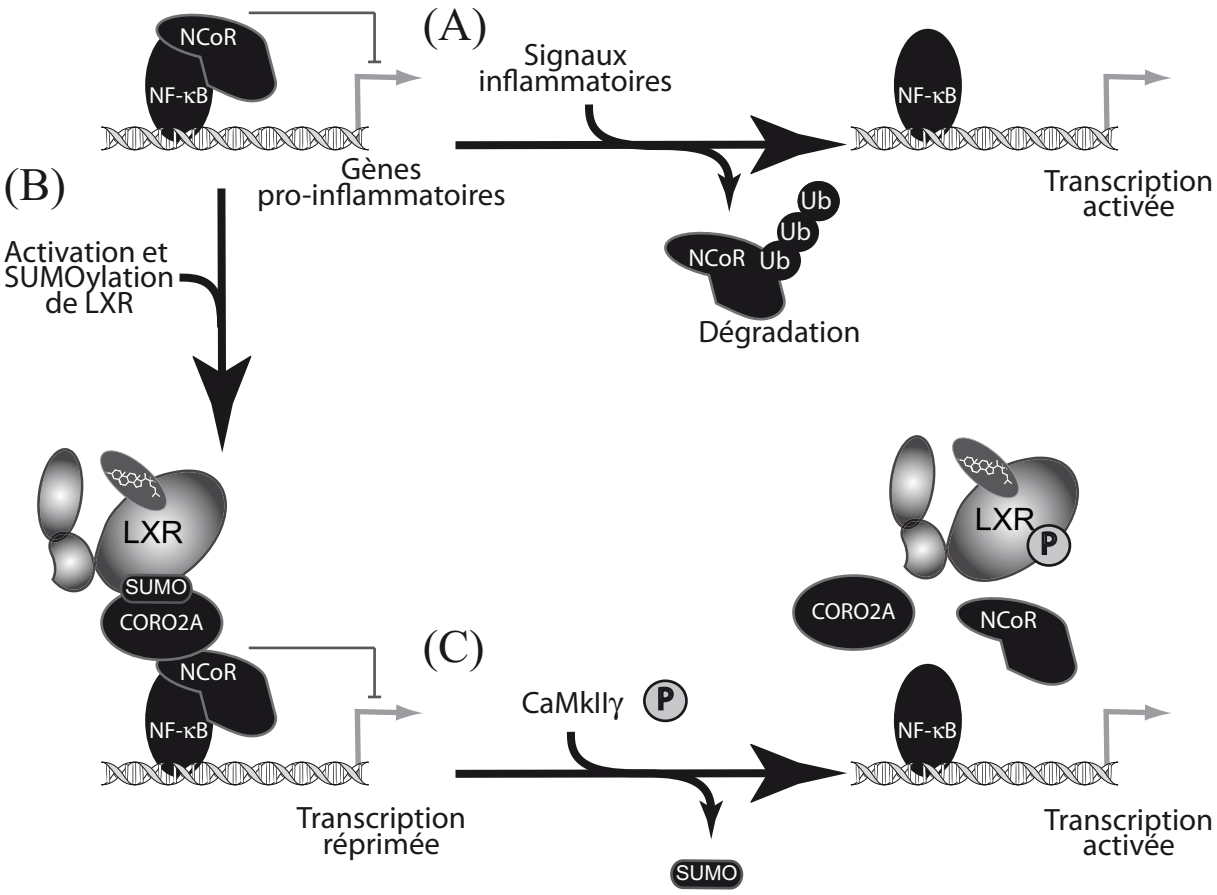


Figure 12. La trans-répression de NF-κB par LXR. Dans un état non inflammatoire, le « nuclear factor of kappa light polypeptide gene enhancer in B cells » (NF-κB) est associé avec des co-répresseurs comme le « nuclear receptor corepressor » (NCoR) interdisant la transcription des gènes pro-inflammatoires. (A) A la suite de stimuli pro-inflammatoires les co-répresseurs sont ubiquitinylés et dégradés par le protéasome et la transcription est activée. (B) L'activation de LXR pendant ce processus stabilise la fixation des co-répresseurs et inhibe la transcription. L'activation et la SUMOylation de LXR permettent la fixation de LXR sur NCoR *via* la « coronin 2A » (CORO2A) stabilisant ainsi le complexe de co-répresseurs. (C) Les signaux pro-inflammatoires dépendants de la « calcium/calmodulin-dependent protein kinase IIγ » (CaMKIIγ) inhibent la trans-répression exercée par LXR en le déphosphorylant. La phosphorylation de LXR entraine sa déSUMOylation et sa dissociation de CORO2A, libérant NFκB qui est alors capable d'induire ses gènes cibles.

maintien des fonctions immunitaires. Parmi les cellules sanguines, les macrophages apparaissent comme une population particulièrement sensible à l'activité de LXR et susceptible de dérégulations affectant non seulement l'inflammation et l'immunité mais aussi la charge des cellules en cholestérol.

Dans les macrophages, l'activation des voies de signalisation de l'inflammation ainsi que la sécrétion de médiateurs pro-inflammatoires sont indispensables dans les mécanismes de l'immunité innée. La première étude liant LXR aux réponses inflammatoires montre que dans les macrophages, LXR antagonise l'expression de gènes pro-inflammatoires (Joseph, Castrillo et al. 2003). Les agonistes de LXR, le T0901317 et le GW3965 induisent une diminution de la synthèse de protéines pro-inflammatoires dans les macrophages de souris préalablement traitées avec du LPS, utilisé expérimentalement pour déclencher des réactions inflammatoires. Parmi ces protéines, on trouve la « nitric oxide synthase » (iNOS), la cyclooxygénase 2 (COX-2), les interleukines 6 (IL-6) et 1β (IL-1β), le « granulocyte colony-stimulating factor » (G-CSF), la « monocyte chemotactic protein 1 » (MCP-1) et la « monocyte chemotactic protein 3 » (MCP-3) (Joseph, Castrillo et al. 2003). L'injection de LPS induit une augmentation de la synthèse de ces protéines plus importante chez les souris transgéniques invalidées pour les deux isoformes de LXR que chez les souris de type sauvage. Les ligands de LXR inhibent l'expression des gènes codant pour ces protéines dans les macrophages de souris de type sauvage ainsi que des souris transgéniques invalidées pour LXRα ou LXRβ. Cependant, ce n'est pas le cas dans les macrophages issus de souris n'exprimant pas les deux isoformes de *Lxr*, révélant ainsi qu'à la fois LXRα et LXRβ possèdent une activité anti-inflammatoire. Les mécanismes induits par LXR qui conduisent à la diminution de l'expression de ces protéines restent encore peu clairement définis. Aucun LXRE n'a été identifié dans les promoteurs des gènes dont l'expression est diminuée en corrélation avec l'activation de LXR. Cependant, les facteurs de transcription pro-inflammatoires « nuclear factor κB » (NF-κB) ou « activator protein 1 » (AP-1) pourraient être inhibés par LXR (Yasuda, Kanno et al. 2005). Cette inhibition des gènes pro-inflammatoires par LXR se déroule selon un mécanisme appelé trans-répression . Contrairement à un mécanisme de répression directe, la trans-répression ne nécessite pas la présence de LXRE sur les promoteurs des gènes trans-réprimés. Cependant, ce mécanisme nécessite une activation de LXR par ses ligands (**Figure 12**). Dans un état sans inflammation, l'expression des gènes cibles de NF-κB est réprimée par le complexe protéique de co-répresseurs interagissant avec ce facteur de transcription au niveau de leur promoteur. En

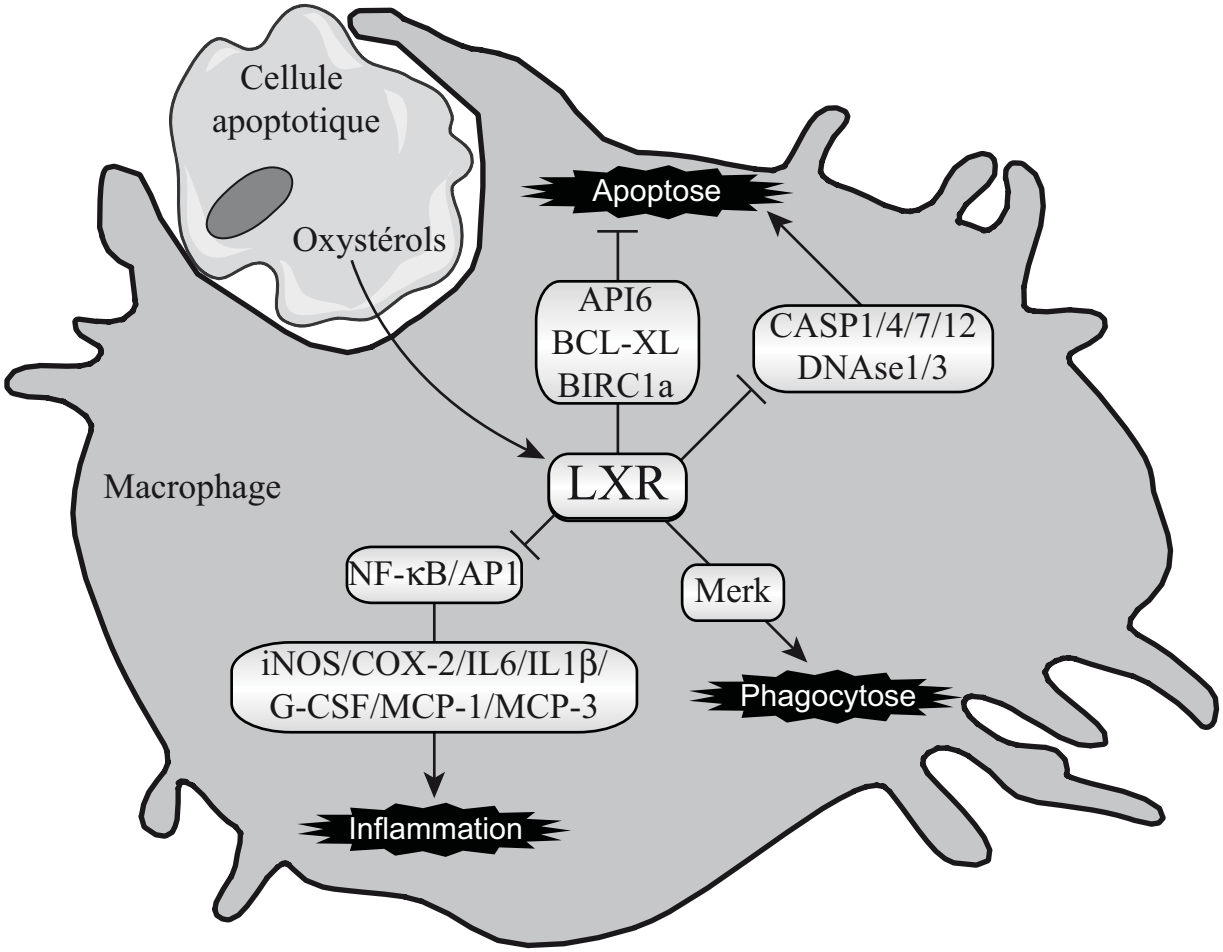


Figure 13. Implication de LXR dans le fonctionnement cellulaire du macrophage. L'activation de LXR dans les macrophages trans-réprime le « nuclear factor of kappa light polypeptide gene enhancer in B cells » (NF-κB) et l'« activator protein 1 » (AP1) et inhibe la production de la « Nitric oxide synthase » (iNOS), de la cyclo-oxygénase 2 (COX-2), de l'« interleukin 6 » (IL6), de l'« interleukin 1β » (IL1β), du « granulocyte colony-stimulating factor » (G-CSF), de la « monocyte chemotactic protein 1 » (MCP-1) et de la « monocyte chemotactic protein 3 » (MCP-3) réduisant ainsi le niveau inflammatoire. L'activation de LXR limite aussi l'apoptose des macrophages en activant les facteurs anti-apoptotiques : « apoptosis inhibitor 6 » (API6), « B-cell lymphoma-extra large » (BCL-XL) et « baculoviral IAP repeat-containing 1a » (BIRC1a) et en inhibant des facteurs pro-apoptotiques : les « caspases1/4/7/12 » (CASP1/4/7/12) et les DNAse1/3. Les cellules apoptotiques phagocytés par les macrophages libèrent des oxystérols qui vont pouvoir activer la « mer receptor tyrosine kinase » (Merk) *via* LXR et faciliter l'initiation de la phagocytose.

réponse aux signaux pro-inflammatoires, le complexe de co-répresseurs est ubiquitinylé et dégradé par le protéasome. L'activation de LXR pendant ce processus bloque l'expression des gènes cibles de NF-κB en maintenant la présence des co-répresseurs au niveau des promoteurs de ces gènes (Kidani and Bensinger 2012). Dans des cultures primaires de macrophages, il a été démontré que l'activation de LXR par un ligand ainsi que sa SUMOylation sont nécessaires afin qu'il se fixe sur les co-répresseurs liés à NF-κB, notamment NCoR, et d'éviter leur dégradation (Ghisletti, Huang et al. 2007). Il a aussi été démontré que l'interaction entre LXR et NCoR était dépendante de la « coronin 2A » (CORO2A). CORO2A est un composant du complexe NCoR et ne possédait pas de fonctions connues. LXR SUMOylé intéragit avec un motif SUMO2/SUMO3 présent dans CORO2A et limite ainsi la clairance de NCoR (Huang, Ghisletti et al. 2011). Les auteurs de cette étude ont également montré que la trans-répression exercée par LXR était inhibée par des signaux pro-inflammatoires dépendants de la « calcium/calmodulin-dependent protein kinase IIγ » (CaMKIIγ) qui phosphoryle LXR (Huang, Ghisletti et al. 2011). La phosphorylation de LXRβ sur le résidu sérine 427 entraine la déSUMOylation de LXR et la rupture de sa liaison avec CORO2A et NCoR. NF-κB est ainsi libre d'exercer son action sur l'induction de gènes cible pro-inflammatoires (Huang, Ghisletti et al. 2011).

LXR est également impliqué dans la survie cellulaire (**Figure 13**). En effet, l'incapacité des macrophages issus de souris transgéniques invalidées pour les deux isoformes de LXR à déclencher une réponse immunitaire suite à l'infection par *Listeria monocytogenes* est corrélée à un taux important d'apoptose de ces macrophages. Cette induction de l'apoptose par les pathogènes est également associée *in vivo* et *ex vivo* à une diminution de l'expression du gène « antiapoptotic apoptosis inhibitor » 6 (*Api6*) (Joseph, Bradley et al. 2004). Il a également été montré que l'activation de l'hétérodimère LXR/RXR par les ligands des deux monomères inhibe l'apoptose des macrophages induite par des signaux apoptotiques, tels qu'un traitement par le cycloheximide, et des infections par *Racillus anthracis*, *Escherichia coli* et *Salmonella typhimurium*. Cet effet est dû à l'induction de l'expression de *Api6* et à d'autres facteurs anti-apoptotiques comme « B-cell lymphoma-extra large » (*Bcl-xl*) et « baculoviral IAP repeat-containing 1a » (*Birc1a*), ainsi qu'à l'inhibition de plusieurs gènes pro-apoptotiques dont les caspases (*Casp*) 1,4,7 et 12 et les *DNAses 1/3* (Valledor, Hsu et al. 2004). Les cellules apoptotiques libèrent des dérivés du cholestérol provenant des fragments de leurs membranes qui agissent comme des ligands de LXR. Il a été démontré que ce mécanisme stimule la clairance des cellules apoptotiques en favorisant la phagocytose par les

macrophages (A-Gonzalez, Bensinger et al. 2009). Cette augmentation de la phagocytose est dépendante de l'activation de la « mer receptor tyrosine kinase » (Merk), une enzyme facilitant l'initiation de la phagocytose par LXR (A-Gonzalez, Bensinger et al. 2009).

Bien que dans cette partie nous nous soyons focalisés sur l'implication de LXR dans la modulation de l'inflammation dans les macrophages, il est important de noter que LXR joue également des rôles inhibiteurs sur l'inflammation dans d'autres types de cellules, qu'elles soient immunitaires ou non. C'est le cas notamment pour les kératinocytes (Schmuth, Jiang et al. 2008), les lymphocytes B (Chang, Zhang et al. 2007; Heine, Dahten et al. 2009), T (Walcher, Kummel et al. 2006; Walcher, Vasic et al. 2010), les monocytes (Myhre, Agren et al. 2008), les cellulaires stellaires (Beaven, Wroblewski et al. 2011; Mallat and Lotersztajn 2011), les cellules de Kupffer (Wang, Dahle et al. 2006), les cellules des ilots pancréatiques (Scholz, Lund et al. 2009), les cellules musculaires (Delvecchio, Bilan et al. 2007), les cellules osseuses (Remen, Henning et al. 2011), et les hépatocytes (Blaschke, Takata et al. 2006; Dai, Ou et al. 2007; Wang, Dahle et al. 2009; Venteclef, Jakobsson et al. 2010; Wang, Ryg et al. 2011).

De façon intéressante, LXR semble également jouer un rôle protecteur vis-à-vis du développement des maladies inflammatoires chroniques de l'intestin. En effet, une étude d'association Danoise incluant 1600 patients révèle une susceptibilité accrue de développer une rectocolite hémorragique chez les porteurs d'un polymorphisme de LXR (Andersen, Christensen et al. 2011). De plus, l'inflammation intestinale peut également contribuer à l'altération de l'expression de LXR ainsi qu'à celle de ses gènes cibles impliqués dans le métabolisme lipidique. En effet, il a été rapporté que la diminution de l'expression de la *FAS* observée chez des patients présentant une colite ulcéreuse pouvait, en partie, être expliquée par une diminution de l'expression de LXR en la présence de cytokines pro-inflammatoires (Heimerl, Moehle et al. 2006).

IV.3. Autres rôles physiopathologiques de LXR

Au regard de l'implication de LXR sur le métabolisme du cholestérol et sur la limitation de l'inflammation, ce récepteur est important dans des maladies telles que l'athérosclérose, le cancer ainsi que la maladie d'Alzheimer.

Plusieurs études ont montré que les agonistes de LXR induisent une réduction des lésions athérosclérotiques. Expérimentalement, les modèles utilisés sont des souris déficientes pour le récepteur aux LDLs, *Ldlr*, ou pour l'apolipoprotéine E, *Apoe*. Les souris invalidées pour le *Ldlr* présentent une hypercholestérolémie et développent une athérosclérose quand elles sont soumises à des régimes riches en cholestérol alors que les souris invalidées pour *Apoe* présentent une hypercholestérolémie et des lésions vasculaires. Il a été montré qu'un traitement par le GW3965 induit une diminution de la taille des lésions vasculaires de 50% chez les souris déficientes en *Apoe* ou les mâles déficients en *Ldlr* et de 35% chez les femelles déficientes en *Ldlr.* De façon intéressante, le traitement par les agonistes de LXR induit une augmentation de l'expression des gènes codant pour ABCA1 et ABCG1 ainsi qu'une diminution de ceux responsables de l'inflammation dans les macrophages (Joseph, McKilligin et al. 2002). Inversement, une greffe de moelle osseuse provenant de souris *Lxr-/-* dans des souris *Apoe-/-* ou *Ldlr-/-* entraine une augmentation de la taille des lésions vasculaires (Tangirala, Bischoff et al. 2002). Levin *et al* ont montré que le traitement de souris *Ldlr-/-* avec un agoniste de LXR réduit la taille des liaisons préexistantes et que cette réduction est dépendante de l'activation de LXR dans les macrophages (Levin, Bischoff et al. 2005). Comme nous l'avons vu précédemment, il a également été démontré que la surexpression de LXRα dans l'épithélium intestinal, en diminuant l'absorption du cholestérol et en induisant le transport réverse de ce composé, protégeait de l'athérosclérose ces souris transgéniques croisées avec des souris invalidées pour *Ldlr* et nourries avec un régime athérogénique (Lo Sasso, Murzilli et al. 2010).

LXR est également impliqué dans la maladie d'Alzheimer. La maladie d'Alzheimer est caractérisée par une dégénérescence neuronale progressive associée au développement de plaques extracellulaires de β-amyloide. Le composant majoritaire de ces plaques est le peptide Aβ issue du clivage de l'« amyloid precursor protein » (APP) par une β-secretase. Il a été suggéré qu'il existait un lien entre le cholestérol, la maturation de l'APP, le peptide Aβ et la maladie d'Alzheimer (Martin, Dotti et al. 2010). Le lien entre cholestérol et maladie d'Alzheimer est renforcé par l'utilisation de statines chez des patients présentant cette pathologie (Jick, Zornberg et al. 2000; Wolozin, Kellman et al. 2000). L'utilisation d'agonistes de LXR induit une augmentation de l'expression du transporteur de cholestérol *Abca1* et diminue la production du peptide Aβ dans un modèle murin de la maladie d'Alzheimer (Koldamova, Lefterov et al. 2005). L'invalidation de *Lxrα* ou *Lxrβ* dans un modèle murin de cette maladie conduit à l'accumulation du peptide Aβ (Zelcer, Khanlou et al.

2007) alors qu'un traitement par un agoniste de LXR réduit la perte de mémoire observée dans un modèle murin transgénique de la maladie d'Alzheimer (Fitz, Cronican et al. 2010). Il a été également démontré que l'activation de LXR diminue les réponses inflammatoires associées à la maladie d'Alzheimer (Zelcer, Khanlou et al. 2007). Ces données montrent dans l'ensemble le rôle protecteur de LXR dans le développement de la maladie d'Alzheimer.

LXR joue aussi un rôle dans le développement des cancers. Plusieurs études épidémiologiques montrent des corrélations entre le cholestérol et le développement du cancer colorectal (Giovannucci and Michaud 2007; Yasuda, Shimizu et al. 2010) ainsi que du cancer de la prostate (Bravi, Scotti et al. 2006; Magura, Blanchard et al. 2008). De part ses effets sur la clairance du cholestérol et sur la prolifération cellulaire, LXR, semble important dans la limitation de la progression cancéreuse. L'activation de LXR diminue l'expression de marqueurs pro-cancéreux dans une lignée de cellules de colon cancéreuses. Cette modulation est corrélée avec une réduction de la prolifération cellulaire (Uno, Endo et al. 2009). Dans un modèle murin de cancer colorectal la surexpression de *Lxrα* dans l'épithélium intestinal réduit la taille des tumeurs par rapport aux souris de type sauvage en réponse à un stimulus tumoral chimique (Sasso, Bovenga et al. 2013). L'activation de LXR dans une lignée cellulaire humaine de carcinome de prostate (LNCaP) diminue le nombre de cellules en phase S et induit l'expression de *ABCA1*. Il a été également rapporté *in vitro* et *in vivo* que l'activation de LXR induit l'apoptose des cellules tumorales de prostate (Fukuchi, Hiipakka et al. 2004; Fukuchi, Kokontis et al. 2004; Pommier, Alves et al. 2010). De façon intéressante, l'administration *in vivo* d'agonistes de LXR diminue le développement tumoral de cellules LNCaP transplantées dans des souris (Fukuchi, Hiipakka et al. 2004).

L'activation de LXR est donc une cible thérapeutique intéressante pour traiter nombre de pathologies. Cependant, l'utilisation de ligands pharmacologiques de LXR reste controversée en regard de la stéatose hépatique qu'ils induisent.

V Le rôle majeur de LXR dans la lipogenèse hépatique

V.1. LXR, un régulateur direct de la lipogenèse hépatique

Les premières études utilisant le T0901317 montrent que ce composé, *in vivo,* conduit à une stéatose hépatique importante ainsi qu'à une induction de la sécrétion de VLDLs riches en triglycérides (Grefhorst, Elzinga et al. 2002). De plus, les souris transgéniques invalidées pour *Lxrα* présentent une diminution de l'expression de gènes impliqués dans la lipogenèse que sont *Srebp-1c*, *Fas*, et *Scd1* (Peet, Turley et al. 1998). LXR a donc été suspecté d'être un régulateur important de la synthèse des acides gras. Une compréhension fine de la régulation transcriptionnelle de la synthèse des acides gras est primordiale sachant que l'augmentation de la lipogenèse contribue à la progression de la stéatose hépatique non alcoolique (Donnelly, Smith et al. 2005).

Il apparaît que LXR est un régulateur direct de l'expression des gènes de la lipogenèse dans le foie. Il a été cependant montré des effets de LXR spécifiques en fonction de tissus (Korach-Andre, Archer et al. 2011). Dans le tissu adipeux blanc, LXR régule la lipogenèse dans les pro-adipocytes (Darimont, Avanti et al. 2006) et contribue au développement (Gerin, Dolinsky et al. 2005) et à l'accumulation de triglycérides dans l'adipocyte mature (Juvet, Andresen et al. 2003). Il a aussi été rapporté que l'activation de LXR augmente le niveau basal de lipolyse (Stenson, Ryden et al. 2010) et de la β-oxydation (Stenson, Ryden et al. 2009). Cependant, le rôle de LXR est dans la lipogenèse a été beaucoup plus étudié au niveau du foie à cause des effets forts du T0901317 sur la stéatose hépatique. Ces effets limitent l'utilisation thérapeutique des agonistes de synthèse de LXR (Viennois, Pommier et al. 2011).

Des LXREs ont été décrits dans le promoteur de *Fasn* (Joseph, Laffitte et al. 2002), *Acc* (Talukdar and Hillgartner 2006) et *Scd1* (Chu, Miyazaki et al. 2006). Il semble que LXRβ joue un rôle plus faible dans la lipogenèse comparé à LXRα, les souris transgéniques invalidées pour *Lxrα* mais non pour *Lxrβ* présentant une expression plus faible des gènes de la lipogenèse comparé aux souris de type sauvage nourries avec un régime riche en cholestérol (Repa, Liang et al. 2000) (**Figure 14**).

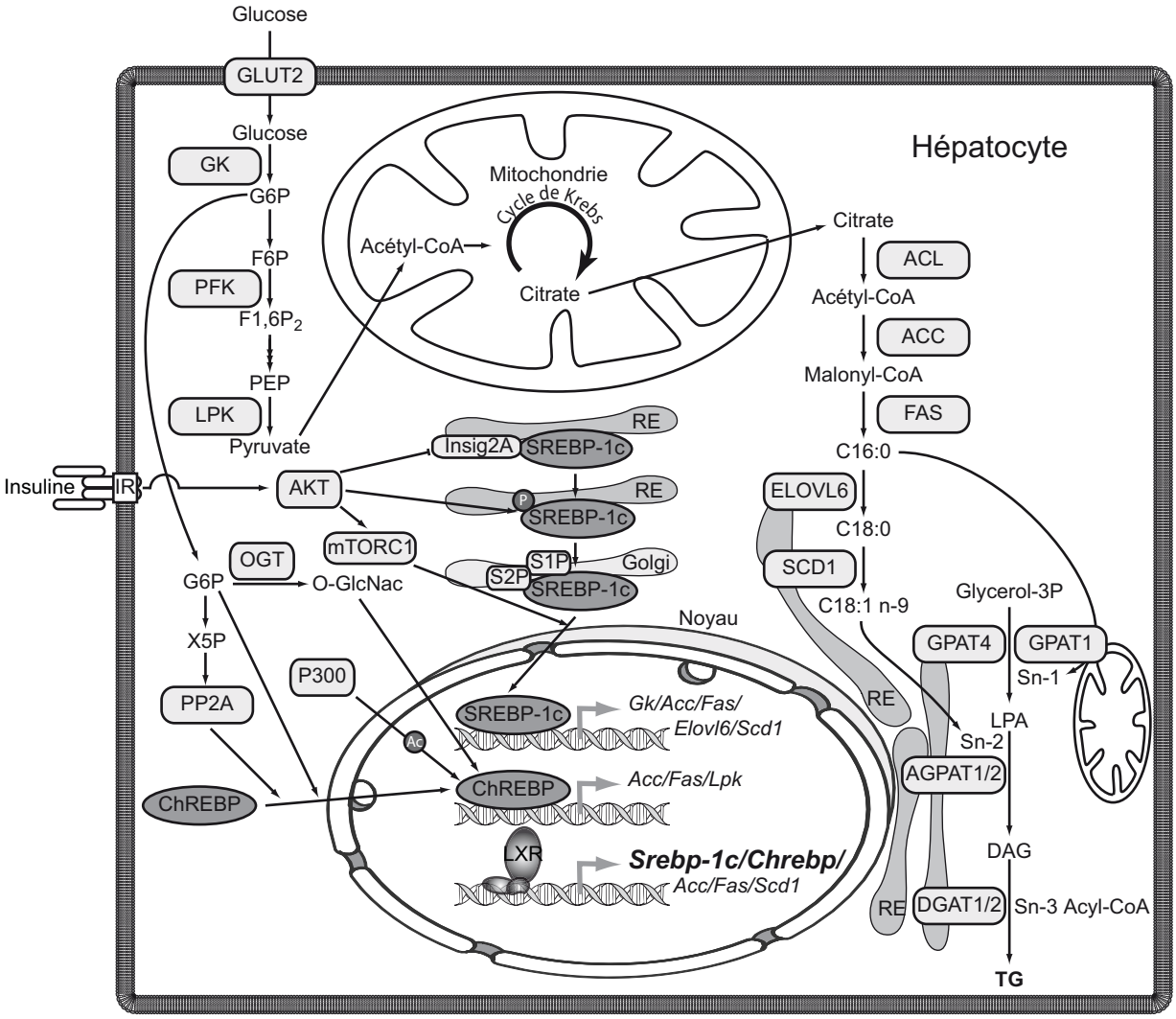


Figure 14. Régulation transcriptionnelle de la lipogenèse par LXR. A l'état nourri, le glucose entre dans l'hépatocyte *via* le transporteur GLUT2. Il est alors catabolisé dans la voie de la glycolyse, qui fait intervenir la « glucokinase » (GK), la « phosphofructokinase » (PFK) et la « liver pyruvate kinase » (LPK), pour donner du pyruvate. Le pyruvate est transformé en acétyl-CoA qui est pris en charge dans le cycle de Krebs. La synthèse d'acides gras et de triglycérides sont respectivement décrites dans la **Figure 6** et la **Figure 8**. L' « ATP citrate lyase» (ACL), l' « acetyl-CoA carboxylase» (ACC) et la « fatty acid synthase » sont des enzymes cytosoliques. La « elongation of very long chain fatty acids protein 6 » (ELOVL6), la « stearoyl-CoA desaturase 1 » (SCD1), la « glycerol-3-phosphate acyltransferase 4» (GPAT4), les « 1-acylglycerol-3-phosphate O-acyltransferase » 1 et 2 (AGPAT1/2) et les « diacylglycerol acyltransferase » 1 et 2 (DGAT1/2) sont associées au réticulum endoplasmique, alors que GPAT1 est localisé au niveau de la membrane externe de la mitochondrie. GPAT1 incorpore spécifiquement de l'acide palmitique (C16:0) en position sn-1 du glycérol-phosphate, alors que les AGPAT1/2 incorporent préférentiellement de l'acide oléique (C18:1 n-9) en position sn-2 de l'acide lysophosphatidique (LPA). Un intermédiaire de la glycolyse, le glucose-6-phosphate (G6P) conduit à l'activation de ChREBP directement et indirectement en phosphorylant et en O-GlcNacétylant ChREBP. Ces deux mécanismes font respectivement intervenir deux enzymes : la « protein phosphatase 2A » (PP2A) et la « O-linked N-acetylglucosamine transferase » (OGT). Le « p300 histone acetyltransferase co-activator » (P300) acétyle ChREBP ce qui induit son activité transcriptionnelle. Le glucose induit une augmentation de la sécretion d'insuline par le pancréas. Au niveau de l'hépatocyte, l'insuline se fixe sur son récepteur (IR), ce qui conduit à la phosphorylation de la « protein kinase B » (AKT) et à la maturation de SREBP-1c *via* l'hinibition de l'expression de l'*Insig2a*, la phosphorylation de SREBP-1c et l'activation de mTORC1. Dans le noyau, des LXREs ont été décrits dans les promoteurs de *Srebp-1c*, *Chrebp*, *Acc*, *Fas* et *Scd1*. Des SRE et des ChoRE ont aussi été respectivement décrits dans les promoteurs de *Gk*, *Acc*, *Fas*, *Elovl6*, *Scd1* et de *Acc*, *Fas*, *Lpk*.

V.2. LXR, un régulateur de l'expression de SREBP-1c et de ChREBP

Il a été également montré que LXR contrôle l'expression génique de deux facteurs de transcription impliqués dans la lipogenèse : *Srebp-1c* (Repa, Liang et al. 2000) et *Chrebp* (Cha and Repa 2007). LXR joue donc à la fois des rôles directs et indirects dans la régulation transcriptionnelle de la lipogenèse (**Figure 14**). Deux LXREs ont été identifiés dans le promoteur de S*rebp-1* (Chen, Liang et al. 2004), alors qu'usuellement les gènes cibles de LXR ne possèdent qu'un seul LXRE dans leur promoteur (Costet, Luo et al. 2000). Les SREBPs sont des facteurs de transcription appartenant à la famille des facteurs de transcription possédant des motifs en hélice-tour-hélice et en glissière de leucine (bHLH/LZ) et sont localisés sous forme mature dans le réticulum endoplasmique. Afin de moduler l'expression de leurs gènes cibles, les SREBPs subissent un double clivage afin de libérer la partie N-terminale qui est alors relocalisée dans le noyau (Wang, Sato et al. 1994). Chez les mammifères il existe trois isoformes de SREBP : SREBP-1a, SREBP-1c et SREBP-2. SREBP-1a et SREBP-1c sont codés par le même gène et leur ARN ne diffère seulement que par leur premier exon (Yokoyama, Wang et al. 1993; Shimomura, Shimano et al. 1997). SREBP-2 est encodé par un gène différent (Hua, Yokoyama et al. 1993; Miserez, Cao et al. 1997). SREBP-2 est impliqué dans la régulation de la transcription des gènes de la cholestérogenèse. En effet, la surexpression d'un dominant positif, une forme tronquée de la partie N-terminale, induit une augmentation de l'expression des gènes de la synthèse de cholestérol ainsi qu'une augmentation de la synthèse de cholestérol (Horton, Shimomura et al. 1998). SREBP-1c joue un rôle dans la lipogenèse, en effet, la surexpression d'une forme tronquée, dominant positif, de cette protéine conduit à une augmentation de l'expression des gènes de la lipogenèse ainsi qu'à une accumulation de triglycérides dans le foie (Shimano, Horton et al. 1997; Shimano, Yahagi et al. 1999). Les souris transgéniques surexprimant la forme tronquée et active de SREBP-1a présentent une augmentation de la synthèse de triglycérides et de cholestérol (Shimano, Horton et al. 1997), suggérant ainsi que SREBP-1a partage les fonctions communes avec les deux autres isoformes de SREBP. La forme tronquée de SREBP-1a présente des effets plus marqués que celle de SREBP-1c sur la lipogenèse (Shimano, Horton et al. 1997). Cependant, chez la souris, le rat, le hamster et l'homme SREBP-1a n'est pas exprimé chez l'adulte (Shimomura, Shimano et al. 1997) suggérant ainsi qu'*in* vivo, SREBP-1c est la seule isoforme régulant la lipogenèse chez l'adulte. La maturation post-traductionnelle de SREBP-1a et SREBP-2 en réponse à une déplétion en

cholestérol dans la cellule est bien décrite (Goldstein, DeBose-Boyd et al. 2006; Brown and Goldstein 2009). La maturation de SREBP-1c ne se produit pas en réponse à de faibles concentrations en cholestérol. Elle résulte d'une stimulation par l'insuline (Yabe, Komuro et al. 2003; Hegarty, Bobard et al. 2005; Howell, Deng et al. 2009) ou par le stress du réticulum endoplasmique (Kammoun, Chabanon et al. 2009). Il est néanmoins clair que l'axe LXR/SREBP-1c est d'une importance primordiale dans la médiation des effets lipogéniques de LXR. En effet les souris transgéniques invalidées pour *Srebp-1c* spécifiquement dans le foie diminue fortement la réponse induite par un agoniste spécifique de LXR ainsi que par une épreuve de jeûne-renourriture (Liang, Yang et al. 2002). L'axe LXR/SREBP-1c est fortement régulé. Chez l'homme, SREBP-1c régule l'expression d'un miRNA qui exerce un rétrocontrôle sur l'auto-régulation de *Lxrα* (Ou, Wada et al. 2011).

De la même façon que SREBP-1c, ChREBP est un facteur de transcription de type bHLH/LZ qui contribue à la régulation transcriptionnelle de la lipogenèse (Denechaud, Girard et al. 2008) qui a été découvert par le groupe de Uyeda en 2001 (Yamashita, Takenoshita et al. 2001). Le gène codant pour ChREBP est principalement exprimé dans le foie, l'intestin grêle, les reins et dans les tissus adipeux blanc et brun (Iizuka, Bruick et al. 2004). Il forme un hétérodimère avec la « max like protein » (Mlx) (Stoeckman, Ma et al. 2004; Ma, Robinson et al. 2006). A l'instar de SREBP-1c, ChREBP doit subir une maturation post-traductionnelle pour être relocalisé dans le noyau et devenir actif afin de réguler l'expression de ses gènes cibles. Lorsque la concentration en glucose dans la cellule est faible, ChREBP est phosphorylé et est retenu dans le cytosol. Lorsque la concentration en glucose dans la cellule est forte, ChREBP est déphosphorylé et transloque dans le noyau. *Chrebp* est sous le contrôle transcriptionnel de LXR. Cependant, ChREBP est activé uniquement par le glucose (Denechaud, Bossard et al. 2008). Jusqu'à présent, deux mécanismes d'activation de ChREBP ont été décrits. L'un d'eux requiert la déphosphorylation de ChREBP sur le résidu sérine 196 par la « protein phosphatase 2A » (PP2A) (Kabashima, Kawaguchi et al. 2003). Cette phosphatase est activée par un métabolite de la voie des pentoses phosphate alimentée par le glucose, le xylulose-5-phosphate (X5P) (Kabashima, Kawaguchi et al. 2003). L'autre mécanisme de régulation implique un domaine sur la partie N-terminale de ChREBP et conservé au cours de l'évolution : le « glucose sensing domain » (GSM) (Li, Chang et al. 2006). Ce domaine GSM requiert le glucose-6-phosphate pour être activé (Li, Chen et al. 2010). L'activation de ChREBP en réponse au glucose semble nécessiter sa glucosylation (Sakiyama, Fujiwara et al. 2010). Enfin il existe un autre mécanisme moléculaire qui régule

l'activité de ChREBP. Il nécessite le « p300 histone acetyltransferase (HAT) co-activator » qui co-active l'induction par le glucose de l'expression des gènes de la glycolyse et de la lipogenèse en acétylant ChREBP ainsi que les histones (Bricambert, Miranda et al. 2010). Les auteurs de cette étude ont également identifié la « seronine/threonine kinase salt-inducible kinase 2 » (SIK2) comme un régulateur de p300 en amont de ChREBP.

Une fois activés, SREBP-1c et ChREBP transloquent dans le noyau où ils se fixent sur leurs éléments de réponse, respectivement les « sterol response element » (SRE) et « carbohydrate response element » (ChoRE), de leurs gènes cibles et modulent leur expression. Des SREs ont été identifiés dans le promoteur de la majorité des gènes impliqués dans la lipogenèse tels que *Acc* (Lopez, Bennett et al. 1996), *Fas* (Latasa, Moon et al. 2000), *Elovl6* (Kumadaki, Matsuzaka et al. 2008) et *Scd1* (Tabor, Kim et al. 1999). De façon similaire des ChoREs ont été identifiés dans les promoteurs d'*Acc* (O'Callaghan, Koo et al. 2001) et *Fas* (Rufo, Teran-Garcia et al. 2001). De plus les souris transgéniques invalidées pour *Srebp-1c* ou *Chrebp* ainsi que pour *Lxr*α/*Lxr*β nourries avec un régime standard présentent une diminution des niveaux d'expression des gènes de la lipogenèse comparées aux souris de type sauvage (Repa, Liang et al. 2000; Liang, Yang et al. 2002; Iizuka, Bruick et al. 2004; Cha and Repa 2007). Les facteurs de transcription LXR, SREBP-1c et ChREBP constituent un réseau de facteurs senseurs des nutriments qui sont impliqués dans le contrôle de la synthèse d'acides gras hépatique (**Figure 14**).

V.3. La régulation hormonale et nutritionnelle de LXR

La signalisation par l'insuline est essentielle pour maintenir l'homéostasie glucidique et lipidique à l'état nourri. Il a été suggéré que l'insuline induisait l'activation de LXR (Tobin, Ulven et al. 2002; Chen, Liang et al. 2004). De plus, l'invalidation de LXR perturbe l'augmentation dépendante de l'insuline de l'expression des gènes de la lipogenèse et de la cholestérogenèse (Tobin, Ulven et al. 2002). L'utilisation de techniques de gène rapporteur a permis de montrer que l'induction de l'expression de *Srebp-1c* par l'insuline nécessitait la présence des deux LXREs dans son promoteur (Chen, Liang et al. 2004). Il a également été suggéré, sur la base d'expériences réalisées sur des cellules en culture, qu'une activation de LXR par un ligand endogène était indispensable au maintient de l'expression de *Srebp-1c*

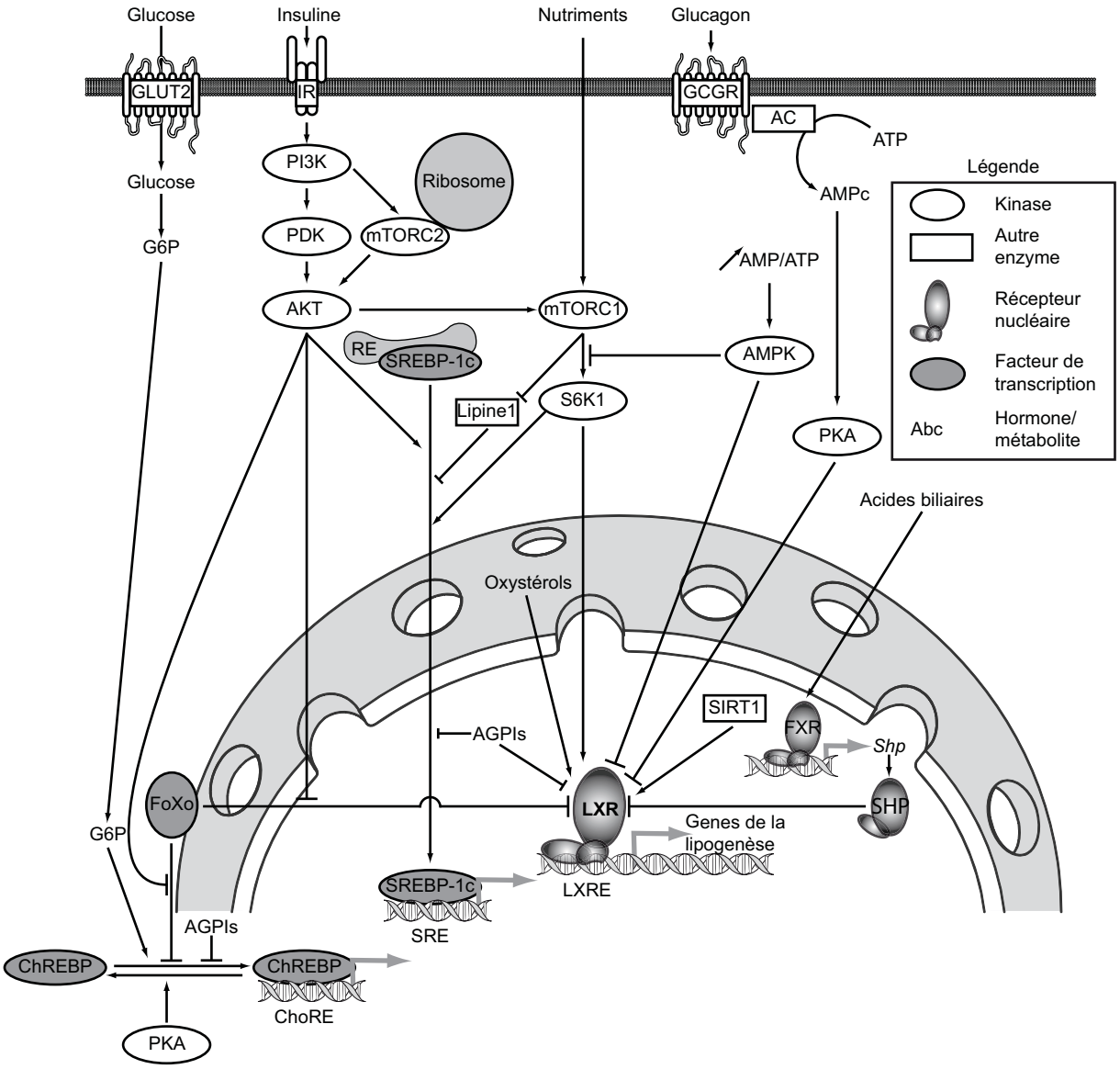


Figure 15. Les régulations nutritionnelles et hormonales de LXR. Les gènes codant pour les deux facteurs de transcription, le « sterol regulatory element binding protein 1c » (SREBP-1c) et le « carbohydrate responsive element binding protein » (ChREBP) sont sous le contrôle transcriptionnel de LXR. ChREBP est activé en réponse à de fortes concentrations en glucose, qui entre dans l'hépatocyte grâce au transporteur GLUT2, *via* un métabolite intermédiaire de la glycolyse, le glucose-6-phosphate (G6P). L'activité de LXR est induite par ses ligands, les oxystérols. A l'état nourri, l'insuline régule l'expression des gènes de la lipogenèse incluant SREBP-1c. Cependant il semble que cette régulation dépende de la présence de LXR. L'insuline, *via* l' « insulin receptor » (IR), la « phosphatidylinositol 3-kinase » (PI3K), la « phosphoinositide-dependent kinase » (PDK) et la « protein kinase B » (AKT) phosphoryle le facteur de transcription « forkhead box O » (FoxO), annulant ainsi la répression qu'il exerce sur LXR ainsi que sur ChREBP. La « p70 Ribosomal S6 kinase 1 » (S6K1) appartient à la voie de signalisation de la « mammalian target of rapamycin » (mTOR). Son activation permet la phosphorylation de LXR sur un résidu sérine et induit sont activité. L'activation de l'« adenosine monophosphate-activated protein kinase » (AMPK), à la suite d'une augmentation du ratio AMP/AKT, inhibe la voie mTORC1/S6K et conduit à la diminution de l'activité de LXRα. L'AMPK inhibe également l'activité de LXRα en le phosphorylant sur un résidu thréonine. AKT, mTORC1, *via* la Lipine1 et S6K1 induisent également la maturation de SREBP-1c. mTORC2 est activé par des facteurs de croissance, dont l'insuline, qui conduisent à son association avec les ribosomes, et induit la lipogenèse *via* SREBP-1c. A l'état de jeune, le glucagon se fixe sur son récepteur (GCGR) induisant ainsi l'activation de l'adénylate cyclase (AC) qui, par l'intermédiaire de l'AMP cyclique (AMPc), active la « protein kinase A » (PKA) qui réprime l'activité de LXR. La PKA inhibe également l'activité de ChREBP en le déphosphorylant. Les acides biliaires activent le « farnesoid X receptor » (FXR) qui active à son tour le « small heterodimer partner » (SHP). SHP interagit avec LXR et réprime l'expression de ses gènes cibles. La « NAD-dependent deacetylase sirtuin-1 » (SIRT1) dé-acétyle LXR et induit sa dégradation, ce qui permet son recyclage et augmente l'expression de ses gènes cibles. Les acides gras poly-insaturés (AGPIs) inhibent l'activité de LXR, SREBP-1c et ChREBP par différents mécanismes.

(DeBose-Boyd, Ou et al. 2001). Le groupe de Brown et Goldstein a également mis en évidence que l'insuline pouvait conduire à la synthèse d'un ligand endogène requis pour l'activation de SREBP-1c par LXR (Chen, Liang et al. 2004). La « p70 ribosomal S6 kinase 1 » (S6K1) est une kinase appartenant à la voie de signalisation de la « mammalian target of rapamycin » (mTOR), une protéine intégrant de nombreuses voies métaboliques. S6K1 phosphoryle LXRα sur un résidu sérine et induit l'augmentation de l'expression de ses gènes cibles (Hwahng, Ki et al. 2009). Les mêmes auteurs ont montré que l'activation de l' « adenosine monophosphate-activated protein kinase » (AMPK), une kinase impliquée dans la régulation du métabolisme énergétique en fonction du statut nutritionnel, inhibe la voie mTOR/S6K et conduit à la diminution de l'activité de LXRα. L'AMPK inhibe également l'activité de LXRα en le phosphorylant sur un résidu thréonine (Hwahng, Ki et al. 2009). L'oltipraz, l'activateur de l'AMPK utilisé dans cette étude, induit aussi une dissociation de LXRα de son élément de réponse (Hwahng, Ki et al. 2009) (**Figure 15**).

Un autre mécanisme d'activation de LXR par l'insuline a été proposé. La surexpression dans le foie d'une forme constitutivement active du « forkhead box-''Other''1 transcription factor » (FoxO1) a pour conséquence une diminution de l'expression de *Srebp-1c* (Zhang, Patil et al. 2006). En l'absence de stimulus cellulaire le facteur de transcription FoxO1 est localisé dans le noyau dans lequel il régule l'expression de ses gènes cibles. Les protéines FoxOs sont phosphorylées par une cible en aval du récepteur à l'insuline, la « protein kinase B » (PKB), conduisant à leur inactivation par une relocalisation du noyau jusqu'au cytosol (Birkenkamp and Coffer 2003). Zhang *et al*. (Zhang, Patil et al. 2006) ont émis l'hypothèse qu'une forme constitutivement active de FoxO1 pouvait perturber l'activité de LXR. Il a ensuite été montré que la forme active de FoxO1 empêchait la fixation de LXRα sur les LXREs présents sur le promoteur de *Srebp-1c* (Liu, Qiao et al. 2010). Cette suppression de l'expression de *Srebp-1c* par FoxO1 peut être abolie par l'inactivation de FoxO1 par l'insuline (Liu, Qiao et al. 2010).

Le fructose alimentaire induit une augmentation de la synthèse *de novo* d'acides gras *via* plusieurs mécanismes. Quand le fructose atteint l'hépatocyte il entre dans la glycolyse en aval de la réaction catalysée par la phosphofructokinase, dont l'activité est régulée par les concentrations cytosoliques de citrate et d'ATP (Hellerstein, Schwarz et al. 1996). Le catabolisme du fructose est n'est donc pas soumis au rétrocontrôle négatif de la glycolyse exercé par le statut énergétique et fournit donc des intermédiaires métaboliques qui servent à

la lipogenèse (Nomura and Yamanouchi 2012). De plus la consommation de fructose est associée à l'augmentation de l'expression des gènes de la lipogenèse dont *Srebp-1c* (Nagai, Nishio et al. 2002) *Acc, Fas* et *Chrebp* (Janevski, Ratnayake et al. 2012). Le « peroxisome proliferator-activated receptor γ coactivator-1 β » (PGC-1β) est un co-activateur de plusieurs facteurs de transcription incluant SREBP-1c et LXR (Lin, Yang et al. 2005). Une diminution de l'expression de PGC-1β chez le rat diminue la réponse lipogénique induite par un régime riche en fructose (Nagai, Yonemitsu et al. 2009). Cette diminution est concomitante avec la diminution de la fixation de LXR sur le promoteur de *Srebp-1c* (Nagai, Yonemitsu et al. 2009) ainsi que la diminution de l'expression de gènes cibles de LXR, dont *Cyp7a1* , et révèle l'importance de PGC-1β dans le fonctionnement de LXR.

Il a été proposé que le glucose pouvait induire l'activation de LXR et que ce dernier pouvait être lui-même un senseur du glucose (Mitro, Mak et al. 2007). Depuis, ces résultats ont été fortement remis en question (Lazar and Willson 2007; Denechaud, Bossard et al. 2008). Anthonisen *et al.* ont plus récemment montré que le glucose induit *in vitro* et *in vivo* la β-N-acétylglucosamination de LXR et que cette modification post-traductionnelle est concomitante avec une augmentation de l'expression de *Srebp-1c*. Cette β-N-acétylglucosamination se produit chez des souris normales mais aussi indépendamment de l'insuline chez des souris traitées avec de streptozotocine (Anthonisen, Berven et al. 2010). Ces résultats restent en désaccord avec l'étude menée par le groupe de Catherine Postic montrant que le glucose nécessite ChREBP et non LXR pour induire les effets lipogéniques du glucose (Denechaud, Bossard et al. 2008). L'activité de ChREBP n'a pas été prise en en compte dans l'étude de Anthonisen *et al* mais aurait permis de caractériser l'interaction de ChREBP et de LXR en lien avec cette modification post-traductionelle, d'autant plus que ChREBP subit également ce genre de modification (Guinez, Filhoulaud et al. 2011).

Pendant le jeûne, les cellules α des îlots de langerhans pancréatiques sécrètent du glucagon en réponse à une concentration faible de glucose dans le sang. Dans le foie, le glucagon induit une augmentation de la quantité d'AMPc et, en aval, l'activation de la « protein kinase A » (PKA). Cette kinase est impliquée dans plusieurs mécanismes cellulaires *via* la phosphorylation de ses protéines cibles. La PKA peut phosphoryler LXR sur son domaine de liaison au ligand et sur son domaine d'hétérodimèrisation (Yamamoto, Shimano et al. 2007). Ces phosphorylations ont pour conséquence une diminution de l'activité de LXR

(Yamamoto, Shimano et al. 2007). Cette découverte est en accord avec l'inhibition de la lipogenèse par le glucagon (**Figure 15**).

Les acides gras essentiels, aussi connus sous le nom d'acides gras polyinsaturés à longue chaine (AGPIs) de la famille n-3 et n-6 peuvent moduler le métabolisme lipidique. L'acide linoléique (C18:2 n-6) et l'acide α-linolénique (C18:3 n-3), les précurseurs des familles n-6 et n-3 respectivement, ne peuvent pas être synthétisés chez les animaux et doivent être donc apportés dans l'alimentation. Ces précurseurs peuvent ensuite être allongés ou desaturés pour produire des AGPIs à très longue chaine comme l'acide arachidonique (C20:4 n-6) ou l'acide docohexaénoïque (C22:6 n-3) (Guillou, Zadravec et al. 2010) (**Figure 7**). Il a été montré que la présence d'AGPIs dans l'alimentation conduisait à une diminution de l'expression de gènes de la lipogenèse comme *Acc*, *Fas* et *Scd1* (Jump and Clarke 1999). La déficience en acides gras essentiels alimentaires (Sekiya, Yahagi et al. 2003; Alwayn, Javid et al. 2004) ou une perturbation de la voie de synthèse des AGPIs à très longue chaine (Moon, Hammer et al. 2009) conduisent à une augmentation de la lipogenèse et à une accumulation de triglycérides dans le foie. Les AGPIs répriment l'expression de *Srebp-1c* (Ou, Tu et al. 2001) ainsi que sa maturation post-traductionnelle (Hannah, Ou et al. 2001). De plus les AGPIs inhibent aussi la maturation de ChREBP (Dentin, Benhamed et al. 2005). Les AGPIs sont également connus pour se lier et activer certains récepteurs nucléaires comme PPARα (Gottlicher, Widmark et al. 1992; Martin, Guillou et al. 2007). Il a été montré *ex vivo* que les AGPIs se liaient à LXR et pouvaient agir en tant qu'antagonistes (Ou, Tu et al. 2001; Svensson, Ostberg et al. 2003). Les AGPIs peuvent donc réprimer la lipogenèse *via* SREBP-1c et ChREBP mais également *via* LXR (Ou, Tu et al. 2001). Cependant, cette possibilité reste encore assez controversée. En effet, plusieurs études utilisant des approches en cultures cellulaires (Pawar, Xu et al. 2002; Pawar, Botolin et al. 2003) et *in vivo* (Pawar, Botolin et al. 2003; Takeuchi, Yahagi et al. 2010) présentent des données invalidant cette possibilité. Au delà des effets antagonistes des AGPIs sur LXR, il a été montré que certains de ces AGPIs pouvaient réprimer l'expression de *Srebp-1c* en réduisant l'activité de LXR (Howell, Deng et al. 2009) (**Figure 15**).

D'autres mécanismes moléculaires senseurs de nutriments et influençant l'activité de LXR sur la promotion de la lipogenèse peuvent impliquer le FXR et l'histone déacétylase SIRT1. FXR est un récepteur nucléaire activé par les acides biliaires (Makishima, Okamoto et al. 1999). A la suite de son activation, FXR induit l'expression d'un récepteur nucléaire orphelin à qui il manque le domaine de liaison à l'ADN, le SHP (Goodwin, Jones et al. 2000).

Il est connu comme étant un co-répresseur de beaucoup de récepteurs nucléaires. Il a été montré que SHP interagit avec l'hélice 12 de LXR et réprime l'expression de ses gènes cibles mais également son activité dans des expériences de gène rapporteur (Brendel, Schoonjans et al. 2002) (**Figure 15**).

SIRT1 agit en fonction de la disponibilité alimentaire et régule le métabolisme glucidique et lipidique (Feige and Auwerx 2007; Hou, Xu et al. 2008; Ponugoti, Kim et al. 2010). Comme nous l'avons vu précédemment SIRT1 déacétyle LXR ce qui conduit à son ubiquitination et sa dégradation par le protéasome (Li, Zhang et al. 2007). Cette dégradation est importante pour le recyclage de LXR et pour le maintien de son activité. Les souris transgéniques invalidées pour *Sirt1* présentent des caractéristiques communes avec les souris invalidées pour *Lxr,* à savoir une diminution du cholestérol-HDL et des triglycérides plasmatiques (Kalaany, Gauthier et al. 2005; Li, Zhang et al. 2007) (**Figure 15**).

De nombreuses maturations post-traductionnelles de LXR en lien avec le statut hormonal et nutritionnel permettent de réguler l'activité de LXR. Ces facteurs hormonaux et nutritionnels pourraient être des cibles opportunes dans le cadre du traitement des NAFLD, comme pourrait l'être LXR

V.4. LXR : une cible potentielle dans le traitement des NAFLD

Il n'existe pas de traitement pharmacologique des NAFLD. Jusqu'à présent une amélioration de l'hygiène alimentaire ainsi qu'un changement de style de vie (exercice physique) sont les seuls leviers permettant de réduire le développement de ces maladies (Carvalhana, Machado et al. 2012). Comme nous l'avons vu dans la partie I, le modèle classique du développement des NAFLD suit un schéma en deux étapes, la première consistant en une accumulation de triglycérides dans le foie prédisposant les cellules hépatiques à une inflammation conduisant à l'apparition d'une stéatohépatite. Si l'on se conforme à ce modèle il apparait adéquat d'envisager un traitement pharmacologique dès la première étape permettant de limiter l'accumulation de triglycérides dans le foie.

Dans ce but, un agoniste inverse de LXR (SR9238) a été récemment mis au point et testé sur des souris nourries avec un régime riche en graisses (Griffett, Solt et al. 2012). Le

SR9238 agit sur les deux isoformes de LXR. Ce composé module l'activité de LXR uniquement au niveau du foie et de n'inhibe pas l'activité de LXR dans les tissus extra-hépatiques. Les auteurs de l'étude montrent que le SR9238 inhibe la lipogenèse et l'accumulation de lipides dans le foie dans un modèle de NAFLD induit par un régime riche en graisses. Le traitement de ces souris avec le SR9238 n'a pas d'effet sur les paramètres inflammatoires hépatiques ni sur les niveaux de cholestérol circulant. Ces données confortent le fait que des agonistes inverses hépato-spécifiques de LXR peuvent être utilisés judicieusement dans les premières étapes des NAFLD. L'absence d'inflammation ou de dommages hépatiques suggère que la prévention de la stéatose induite par la lipogenèse n'est pas associée à une diminution de la clairance du cholestérol hépatique. Cette caractéristique du SR9238 est primordiale étant donné que l'accumulation de cholestérol libre au niveau du foie est un déterminant dans la transition de la stéatose à la NASH (Musso, Gambino et al. 2013).

Cependant cette stratégie se heurte à plusieurs problèmes. Tout d'abord, la stéatose hépatique est une pathologie asymptomatique difficile à diagnostiquer (Tiniakos, Vos et al. 2010) et ce sont surtout les pathologies plus développées qui sont détectées. Cette caractéristique de la stéatose rend donc la plage de traitement potentielle difficile à établir. De plus, comme nous l'avons vu dans la première partie, d'autres récepteurs nucléaires ainsi que des mécanismes cellulaires sont également des acteurs de l'accumulation de triglycérides dans le foie. Il est aussi admis que l'accumulation de triglycérides en tant que telle protège des dommages cellulaires et ne favorise pas l'apparition d'un phénotype inflammatoire (Listenberger, Han et al. 2003; Yamaguchi, Yang et al. 2007). En effet, ces molécules joueraient plutôt un rôle protecteur vis-à-vis de l'inflammation comparé à d'autres molécules lipidiques comme les acides gras libres, les diacylglycérols ou le cholestérol libre (Farrell, van Rooyen et al. 2012). Il a été montré que l'inhibition de la synthèse de triglycérides, en inhibant la synthèse de DGAT2, réduit le contenu en triglycérides mais conduit à une augmentation des paramètres inflammatoires et fibrotiques du foie dans un modèle de stéatose hépatique induite par un régime déficient en choline et méthionine chez des souris diabétiques *db/db* (Yamaguchi, Yang et al. 2007). Cette augmentation de l'inflammation qui se produit en dépit d'une réduction de la stéatose renforce une théorie émergente qui remet en question le modèle en « deux étapes » du développement des NAFLD. En effet, il est apparu que dans certains cas l'inflammation module l'accumulation de triglycérides au niveau du foie. Par exemple, les patients développant une NASH peuvent présenter ou non une stéatose hépatique

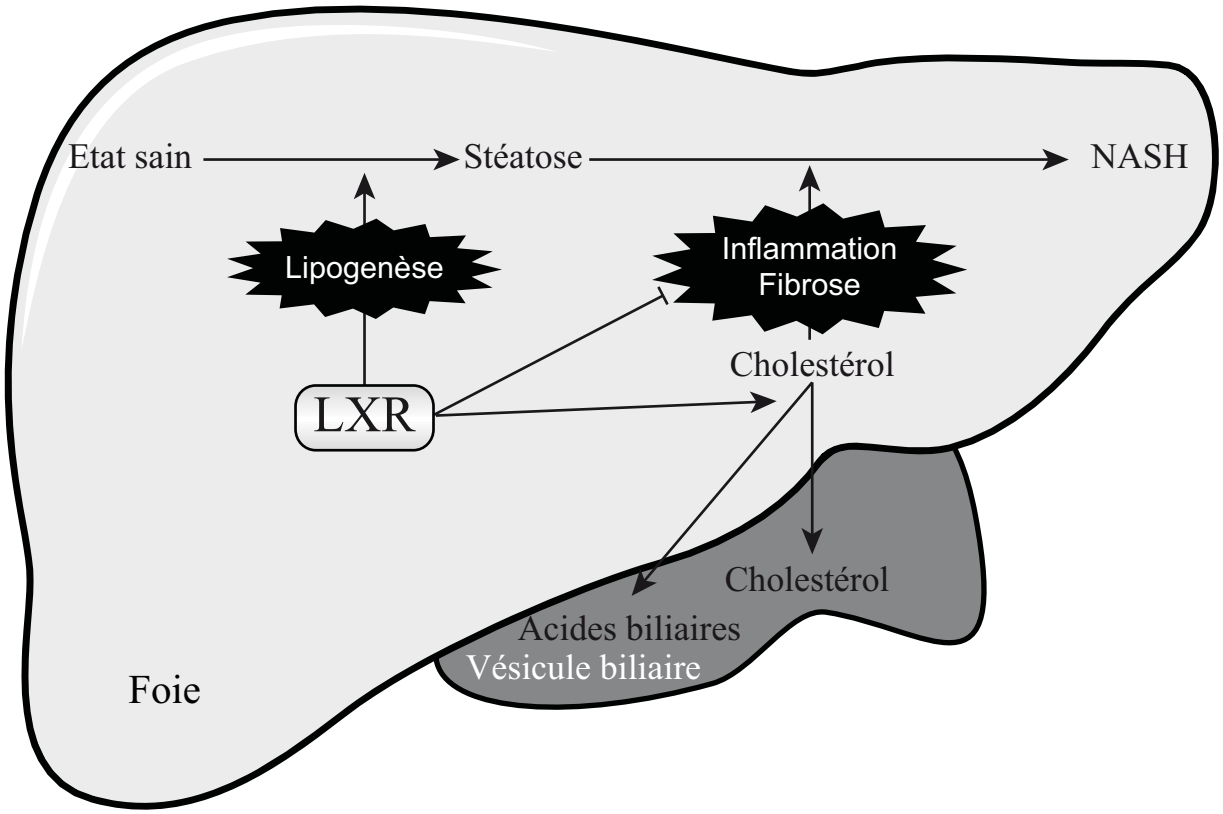


Figure 16. Rôle de LXR dans la transition stéatose/NASH. L'activation de LXR induit la transcription des gènes impliqués dans la lipogenèse et conduit à la stéatose hépatique. Cependant LXR limite la transition entre la stéatose et la stéatohépatite. En effet, LXR est impliqué dans la clairance du cholestérol qui est corrélé avec le développement de la stéatohépatite. LXR réprime aussi les paramètres inflammatoires et fibrotiques dans le foie.

(Tilg and Moschen 2010; Tiniakos, Vos et al. 2010). De plus, la modulation de l'inflammation par des approches pharmacologiques à un effet sur la stéatose. En effet, l'utilisation d'anticorps dirigés contre TNF permet de diminuer la quantité de triglycérides hépatiques chez les souris obèses *ob/ob* (Li, Yang et al. 2003). L'élimination des cellules de Kupffer induit une stéatose *via* la diminution de la sécrétion de l'interleukine 10 (IL10), une interleukine anti-inflammatoire normalement produite par ces cellules (Clementi, Gaudy et al. 2009).

En regard de ces données, une autre stratégie affectant l'activité LXR peut être envisagée. Comme nous l'avons vu précédemment, LXR possède des propriétés anti-inflammatoires et joue un rôle prépondérant dans la clairance du cholestérol qui est fortement impliqué dans le développement de la NASH (**Figure 16**). LXR pourrait donc être une cible pharmacologique intéressante dans l'inhibition de la transition stéatose/NASH. Seulement quelques études ont été conduites dans ce sens. Dans un modèle expérimental de NAFLD induit par un régime riche en graisses, l'activation de LXR permet de limiter les dommages hépatiques résultant d'une injection intra-péritonéale de LPS (Liu, Han et al. 2011). L'activation de LXR par un agoniste, le GW3965, réduit aussi l'infiltration de mastocytes, les taux plasmatiques d'alanine amino-transférase, de bilirubine, de TNF et de prostaglandine E2 dans un modèle d'atteinte hépatique induit par l'administration de LPS et de peptidoglycane chez le rat (Wang, Dahle et al. 2006). Les cultures primaires de cellules de Kupffer démontrent que l'activation de LXR diminue la sécrétion de TNF et de prostaglandine E2 induites par le LPS dans ces cellules (Wang, Dahle et al. 2006). Cependant cet agoniste ne présente des effets anti-inflammatoires uniquement à faible dose (Wang, Dahle et al. 2009). Ces auteurs confirment l'implication de LXR dans le contrôle de la sécrétion de TNF par les cellules de Kupffer car les cellules de Kupffer issues de souris transgéniques n'exprimant pas *Lxrα* produisent plus de TNF que les souris transgéniques invalidées pour *Lxrβ* ou les deux isoformes ou que des souris de type sauvage (Wang, Dahle et al. 2009).

LXR est également impliqué dans la limitation de l'activation des cellules stellaires hépatiques. En effet, l'activation pharmacologique de LXR permet de diminuer les marqueurs fibrotiques dans des cultures primaires de cellules stellaires hépatiques (Beaven, Wroblewski et al. 2011). En parallèle, l'agoniste de LXR induit une augmentation du contenu lipidique de ces cellules. Les cellules stellaires hépatiques issues de souris transgéniques invalidées pour les deux isoformes de *Lxr* présentent des capacités fibrotiques et inflammatoires plus importantes que les cellules issues des souris de type sauvage (Beaven, Wroblewski et al.

2011). D'un point de vu morphologique, elles contiennent une grosse gouttelette lipidique contrairement aux cellules contrôles qui présentent plusieurs petites gouttelettes. Au regard de ces observations sur les cultures primaires, les auteurs se sont intéressés au rôle *in* vivo de LXR dans des modèles expérimentaux de fibrose. L'injection intra-péritonéale de tétra chlorure de carbone ou un régime déficient en choline et en méthionine révèlent que les souris transgéniques invalidées pour les deux isoformes de *Lxr* présentent des paramètres fibrotiques plus marqués que leurs homologues de type sauvage (Beaven, Wroblewski et al. 2011). Cette étude montre donc clairement l'importance de LXR dans le développement de la fibrose hépatique.

Résultats expérimentaux

Chapitre I

Le foie est un organe majeur dans l'homéostasie lipidique de l'ensemble de l'organisme. Le métabolisme lipidique doit être continuellement ajusté afin de répondre aux besoins de l'organisme. Cette adaptation se fait notamment par la modification de l'expression de gènes codant pour des enzymes impliquées dans le catabolisme des acides gras pendant la phase de jeûne et de la synthèse d'acides gras lors d'un apport énergétique excessif. Cette régulation met en jeu des facteurs de transcription tels que les récepteurs nucléaires. Parmi eux, LXR (Schultz, Tu et al. 2000) et l'isoforme α des PPARs (Lee, Pineau et al. 1995; Montagner, Rando et al. 2011), deux récepteurs nucléaires de classe II, sont respectivement impliqués dans la régulation transcriptionnelle de la synthèse et la dégradation des acides gras.

Dans ce chapitre, nous avons d'abord voulu reproduire au laboratoire les effets d'une activation pharmacologique de LXR sur la modulation transcriptionnelle de la lipogenèse hépatique, préalablement décrits dans la littérature (Schultz, Tu et al. 2000) et qui sont au cœur de ces travaux de thèse. Nous avons réalisé une étude pangénomique du transcriptome hépatique des souris mâles de type sauvage et invalidées pour les deux isoformes de *Lxr* (Peet, Turley et al. 1998; Repa and Mangelsdorf 2000), jusqu'alors non décrits dans la littérature.

Cependant, il a été montré qu'il existait des interrelations entre ces deux récepteurs nucléaires et, bien que présentant des propriétés opposées, ils partageaient des fonctions communes. En effet, la lipogenèse, sous le contrôle transcriptionnelle de LXR, conduit à la formation de lipides complexes pouvant activer PPARα (Chakravarthy, Pan et al. 2005; Chakravarthy, Lodhi et al. 2009). De plus, il a été montré que ces deux récepteurs nucléaires partageaient des éléments communs de fixation sur des séquences promotrices de l'ADN (Boergesen, Pedersen et al. 2012). Cependant, des études réalisées en culture cellulaires révèlent des effets antagonistes d'un récepteur sur le fonctionnement de l'autre et inversement (Ide, Shimano et al. 2003; Yoshikawa, Ide et al. 2003).

Dans ce premier chapitre, en utilisant des souris transgéniques invalidées pour *Lxr* ou pour *Pparα* et des activateurs pharmacologiques de ces deux récepteurs nucléaires, nous avons d'abord étudié le rôle de LXR dans la régulation transcriptionnelle de la lipogenèse hépatique. Puis nous nous sommes intéressés aux régulations croisées entre LXR et PPARα.

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Research paper

A systems biology approach to the hepatic role of the oxysterol receptor LXR in the regulation of lipogenesis highlights a cross-talk with PPARα

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Steatosis

ABSTRACT

The Liver X Receptors (LXRs) α and β and the Peroxisome Proliferator-Activated Receptor α (PPARα) are transcription factors that belong to class II nuclear receptors. They drive the expression of genes involved in hepatic lipid homeostasis and therefore are important targets for the prevention and treatment of nonalcoholic fatty liver disease (NAFLD). LXRs and PPARα are regulated by endogenous ligands, oxysterols and fatty acid derived molecules, respectively. In the liver, pharmacological activation of LXRs leads to the over-expression of genes involved in *de novo* lipogenesis, while PPARα is critical for fatty acid catabolism in nutrient deprivation. Even if these two nuclear receptors seemed to play opposite parts, recent studies have highlighted that PPARα also influence the expression of genes involved in fatty acids synthesis. In this study, we used pharmacological approaches and genetically engineered mice to investigate the cross-talk between LXRs and PPARα in the regulation of genes responsible for lipogenesis. We first investigated the effect of T0901317 and fenofibrate, two synthetic agonists of LXRs and PPARα, respectively. As expected, T0901317 and fenofibrate induce expression of genes involved LXR-dependent and PPARα-dependent lipogenic responses. Considering such overlapping effect, we then tested whether LXR agonist may influence PPARα driven response and *vice versa*. We show that the lack of PPARα does not influence the effects of T0901317 on lipogenic genes expression. However, PPARα deficiency prevents the up-regulation of genes involved in ω-hydroxylation that are induced by the LXR agonist. In addition, over-expression of lipogenic genes in response to fenofibrate is decreased in LXR knockout mice as well as the expression of PPARα target genes involved in fatty acid oxidation. Altogether, our work provides *in vivo* evidence for a central interconnection between nuclear receptors that drive hepatic lipid metabolism in response to oxysterol and fatty acids.

Abbreviations: Acc, Acetyl-CoA carboxylase; ANOVA, Analysis of variance; ChREBP, Carbohydrate responsive element binding protein; CPT-1, Carnitine palmitoyl transferase 1; FA, Fatty acids; Fas, Fatty acid synthase; GO, Gene ontology; GPAT1, Glycerol-3-phosphate acyltransferase; LXR, Liver X Receptor; NAFLDalcoholic fatty liver disease, Non; NASHalcoholic steatohepatitis, Non; PPAR, Peroxisome proliferator-activated receptor; qPCR, quantitative polymerase chain reaction; RXR, Retinoid X Receptor; Scd1, Stearoyl-CoA desaturase-1; SREBP1-c, Sterol regulatory element binding protein 1 -c; TBP, TATA-box-binding protein; TG, Triglyceride.

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1. Introduction

With the ongoing obesity epidemic several metabolic diseases have become a major public health issue. Amongst these diseases, Non Alcoholic Fatty Liver Disease (NAFLD) is an increasingly common liver disease worldwide [1]. The hallmark of NAFLD, also called hepatic steatosis, is an abnormally elevated level of hepatic triglycerides (TGs). In itself, NAFLD is not considered as an irreversible health threat but it may favour the development of insulin resistance, Non Alcoholic SteatoHepatitis (NASH), liver fibrosis and hepatocarcinoma. Therefore, understanding the early steps of hepatic fatty acid accumulation as triglycerides is essential for prevention of such liver diseases [2].

Hepatic fatty acids can be taken up from the circulation or originate from *de novo* synthesis, a biosynthetic pathway also named lipogenesis [3]. Since in human NAFLD, steatosis can be associated with up-regulated lipogenesis [4], it is a major issue to understand its control. Moreover, the regulation of lipogenesis is very important, not only because end products of the pathway are incorporated in TGs but also because intermediate metabolites such as acetyl-coA, malonyl-coA, acyl-coAs and other intermediaries in TG biosynthesis are potent signalling molecules [5]. For instance, the malonyl-coA that is produced by acetyl-coA carboxylase is a potent allosteric inhibitor of CPT-1, a rate-limiting enzyme in mitochondrial β-oxidation [6]. Therefore, malonyl-coA is not only an intermediate metabolite in fatty acid synthesis but also a significant brake for fatty acid degradation.

The liver is a central organ in whole-body lipid homeostasis. Hepatic regulation of lipid metabolism largely occurs through metabolic gene programs. These include fatty acid oxidation, ketogenesis and neoglucogenesis during fasting periods, while, in the fed state, glycolysis and lipogenesis are up-regulated. This tight regulation of hepatic fatty acid metabolism depends on transcriptional control by nuclear receptors [7] and other transcription factors [8,9]. During fasting, the α isoform of Peroxisome Proliferator-Activated Receptor (PPARα) has been shown to play a central role in fatty acid catabolism [10]. PPARα is a class II nuclear receptor that drives transcription in response to a variety of lipid ligands by binding to specific DNA sequences as a heterodimer with the Retinoid X Receptor (RXR). It is highly expressed in the liver. Most activating ligands for PPARα are fatty acids or fatty acid derived molecules [11].

In the fed state, three transcription factors cooperate to drive glycolysis and fatty acid synthesis. The carbohydrate responsive element binding protein (ChREBP) and Sterol Regulatory Element Binding Protein 1-c (SREBP1-c) are particularly essential to the effect of glucose [12] and insulin [13] on these pathways. The Liver X Receptors (LXRs) indirectly drive glycolysis and lipogenesis by regulating the expression of ChREBP [14] and SREBP1-c [15]. Like PPARs, LXRs are also class II nuclear receptors [16]. There are two LXR isoforms. LXRα is highly expressed in the liver while LXRβ is ubiquitously expressed [17,18]. LXRs have first been described as oxysterol sensors that play a central role in cholesterol homeostasis [19]. It is now well accepted that LXRs also exert major regulatory functions in hepatic fatty acid metabolism [20].

Both PPARα and LXR-sensitive transcriptional responses are essential for the liver to cope with excess or limiting levels of lipids. Dysregulation of their function may influence the synthesis of lipotoxic lipids and, oppositely, the synthesis of beneficial lipids through *de novo* lipogenesis also called “lipoexpediency” [5,21,22].

Several likely cross-talks between PPARα and LXR signalling have been considered on the basis of different experimental results. When co-expressed in cell culture, LXR was shown to inhibit PPARα transactivation [23] and *vice versa* [24]. Moreover, LXRs regulate the expression of PPARα in the intestine [25]. In addition, a recent publication using ChIP-Seq experiments performed *in vivo* mapped very large overlap between PPARα and LXR binding sites [26]. Therefore, based on these experimental evidences it is quite clear that LXRs and PPARα may either cooperate or have opposite effects in the regulation of various pathways. In this work, we first used microarray to analyze major LXR-sensitive pathways in the liver and we thereby confirmed the central role of LXR in fatty acid metabolism. Next, we used a chronic treatment with pharmacological ligands in transgenic animal models deficient for either LXRs or PPARα to investigate whether one receptor may influence the effect of the other. We specifically focused on hepatic genes involved in fatty acid synthesis. Altogether, our findings further emphasize complex interaction between LXR and PPARα in the regulation of gene expression.

2. Materials and methods

2.1. Animals

LXRαβ double-deficient and wild-type mice with a mix C57BL6J/129SVJ genetic background [15] and PPARα-deficient mice [27] on C57BL6J genetic background [28] were bred at INRA’s transgenic rodent facility at 22 ± 2 °C. Age-matched C57BL6J mice were provided by Charles River (Les Oncins, France) and acclimated to local animal facility condition prior to treatment. All animals used in this study are male mice aged 16–17 weeks. *In vivo* studies were conducted under E.U. guidelines for the use and care of laboratory animals and were approved by an independent ethics committee.

2.2. Chronic treatment with pharmacological agonists

The LXR and PPARα agonist used in this study are T0901317 (Sigma, Saint-Quentin-Fallavier, France) and fenofibrate (Sigma, Saint-Quentin-Fallavier, France), respectively. Concerning the treatment with T0901317, mice received T0901317 (30 mg/kg of body weight per day suspended in 0.5% carboxymethyl-cellulose, 0.5% Tween80) by daily gavage for 4 days. Five animals of each genotype received the vehicle alone as control. Concerning the treatment with fenofibrate, mice received fenofibrate (100 mg/kg of body weight per day suspended in 3% aqueous solution of gum Arabic) by daily gavage for 10 days. Five animals of each genotype received the vehicle alone as control.

2.3. Organs sampling

Following euthanasia, the liver were removed, weighed, dissected, snap-frozen in liquid nitrogen and stored at −80 °C until use.

2.4. Gene expression studies

Total RNA was extracted with TRIzol® reagent (Invitrogen, CergyPontoise, France). Transcriptomic profiles were obtained using Agilent Whole Mouse Genome microarrays (4×44K) following manufacturer’s instruction. Microarray data and all experimental details are available in the Gene Expression Omnibus (GEO) database (accession GSE38083). For real-time quantitative polymerase chain reaction (qPCR), analyses were performed as described elsewhere [29]. Briefly, total RNA samples (2 μg) were reverse-transcribed using High Capacity cDNA (Applied Biosystems, Courtabeuf, France). Primers for SYBR Green assays are presented in Supporting Table 1. Amplifications were performed on an ABI Prism 7300 Real Time PCR System (Applied Biosystems). QPCR data were normalized by TATA-box binding protein (TBP) mRNA levels and analyzed with LinRegPCR [30].

2.5. Biochemical assays

Hepatic lipid contents were determined as described elsewhere [31]. Briefly, following homogenization of tissue samples in methanol/5 mM EGTA (2:1, v/v), lipids corresponding to an equivalent of 1 mg of tissue were extracted according to Bligh and Dyer in chloroform/methanol/water (2.5:2.5:2.1, v/v/v), in the presence of the internal standards: glyceryltrinonadecanoate, stigmasterol and cholesteryl heptadecanoate (Sigma, Saint-Quentin-Fallavier, France). TGs, free cholesterol and cholesterol esters were analyzed by gas–liquid chromatography on a Focus Thermo Electron system using a Zebron-1 Phenomenex fused-silica capillary column (5 m, 0.32 mm i.d., 0.50 mm film thickness). Oven temperature was programmed from 200 to 350 °C at a rate of 5 °C/min, and the carrier gas was hydrogen (0.5 bar). The injector and the detector were at 315 and 345 °C, respectively.

2.6. Statistical analysis

All data were analyzed using R (www.r-project.org). Microarray data were processed with bioconductor packages (www.bioconductor.org) as described in GEO entry GSE38083. Genes with q-value < 0.001 were considered differentially expressed between the two genotypes. The enrichment of Gene Ontology (GO) Biological processes was evaluated using conditional hypergeometric tests (GOstats package). For data other than microrarray data, differential effects were analyzed by analysis of variance (ANOVA) followed by Student's t-tests with a pooled variance estimate. $P < 0.05$ was considered significant.

3. Results

3.1. Central role of LXR in lipogenic gene expression

We first investigated the roles of LXRs using microarrays. We compared the transcriptome of liver samples from wild-type mice and mice lacking both LXR isoforms. The absence of LXRs has a major impact on liver transcriptome. 495 known genes were identified as up-regulated while 355 known genes were shown to be significantly down-regulated (q-value < 0.001, Supplementary Table 2). A heatmap of the average intensities for the corresponding 1088 probes illustrates the strong impact of LXR deficiency (Fig. 1A). Amongst the down-regulated genes, 3 GO categories out of the 12 significantly over-represented (p-value < 0.01, Supplementary Table 3) were highly related to lipid and triglyceride biosynthesis. Amongst the up-regulated genes 95 GO categories out of the 177 significantly over-represented (p-value < 0.01, Supplementary Table 4) are related to inflammation and immune cell biology. We also noticed that several other pathways (Supplementary Table 4) including lipid catabolic process and cholesterol biosynthetic process were found to be up-regulated in LXR-deficient mice. In this work we focused on the contribution of LXR to hepatic triglyceride metabolism. We used qPCR to measure the expression of three genes identified as down-regulated in LXR$^{-/-}$ mice through the microarray analysis and confirmed the down-regulation of Scd1 (Fig. 1B) as well as other lipogenic genes such as Fas and Srebp1-c.

3.2. α And β isoforms of LXR differentially influence lipogenic gene expression

In order to investigate the individual effect of each LXR isoform on the expression of lipogenic genes, we tested the expression of all known genes coding for the main enzymes involved in fatty acid and triglyceride synthesis in wild-type, LXRα$^{-/-}$, LXRβ$^{-/-}$ and LXRαβ$^{-/-}$ mice. Consistent with our previous results, we found in this independent experiment that Srebp1-c, Fas and Scd1 expression is down-regulated in the absence of the two LXR isoforms but not necessarily in the absence of only LXRα or LXRβ (Fig. 2A). Actually, LXRβ deficiency tends to increase the expression of these three genes. Consistent with these data, we found that most genes involved in the early steps of fatty acid synthesis are down-regulated in LXRα$^{-/-}$ mice and in LXRαβ$^{-/-}$ mice while they tend to increase in LXRβ$^{-/-}$ mice (Fig. 2B). Interestingly, the genes involved in the last steps of triglyceride synthesis show a very distinct expression profile compared to the one of fatty acid synthesis and Gpat1 (Fig. 2C). Altogether, our data suggest distinct effect of LXRα and LXRβ on the expression of genes involved in fatty acid synthesis and triglyceride assembly. Finally, no significant effect was measured on liver TG level (Fig. 2D). However, consistent with gene expression, the deficiency for both LXR isoforms tends to reduce the average TG level. Interestingly, while no significant effect was measured on TG level, LXRα$^{-/-}$ and LXRαβ$^{-/-}$ mice show elevated hepatic free cholesterol and cholesterol esters. The absence of LXRβ has a less marked effect on hepatic cholesterol accumulation.

3.3. Pharmacological activation of LXR specifically induces lipogenic genes

As expected T0901317 given to wild-type mice resulted in a marked increase in the expression of fatty acid synthase (Fas) while it has no effect in LXRαβ$^{-/-}$ mice (Fig. 3A). Moreover, several other genes involved in fatty acid synthesis followed the same expression pattern (Fig. 3B). This increase in LXR-sensitive genes is associated with a marked increase in liver triglycerides in response to T0901317 (Fig. 3C).

3.4. Pharmacological activation of PPARα specifically induces lipogenic genes

Another nuclear receptor that regulates lipogenesis is PPARα. When administered fenofibrate, a PPARα agonist, wild-type mice showed significant increase in Fas and Scd1 expression (Fig. 4A). Nevertheless, the response to fenofibrate is more modest than the one to T09013117. However, as seen in Fig. 4B, fenofibrate significantly impacts the expression of the LXR-sensitive genes in fatty acid and triglyceride synthesis in wild-type, but not in PPARα$^{-/-}$ mice. In turn, this does not result in a significant increase in liver triglycerides (Fig. 4C). The strong effect of pharmacological activation of PPARα on oxidative pathways may contribute to prevent lipid accumulation. For instance, Cyp4a14 and Cyp4a10 involved in ω-hydroxylation are markedly up-regulated in response to fenofibrate in wild-type but not in PPARα-deficient mice (Fig. 4D).

3.5. Pharmacological activation of LXR activates the lipogenic response independently of PPARα but induces other PPARα-sensitive genes

Since we identified eight genes involved in fatty acid metabolism that were pharmacologically regulated by both LXR and PPARα, we questioned whether the activation of one receptor may depend on the other. To test this, we first investigated the effect of pharmacological activation of LXR in PPARα$^{-/-}$ mice. We tested whether pharmacological LXR activation may require PPARα to promote lipogenic gene expression. We found that in PPARα$^{-/-}$ mice, the strong lipogenic response to T0901317 was induced similar to what is observed in wild-type mice (Fig. 5A and B). This results in an increased triglyceride level in the liver of mice from both genotypes (Fig. 5C). Finally, we found that pharmacological activation of LXR also induces a significant increase in Cyp4a14 and Cyp4a10. Such increase is markedly reduced in PPARα$^{-/-}$ mice (Fig. 5D).

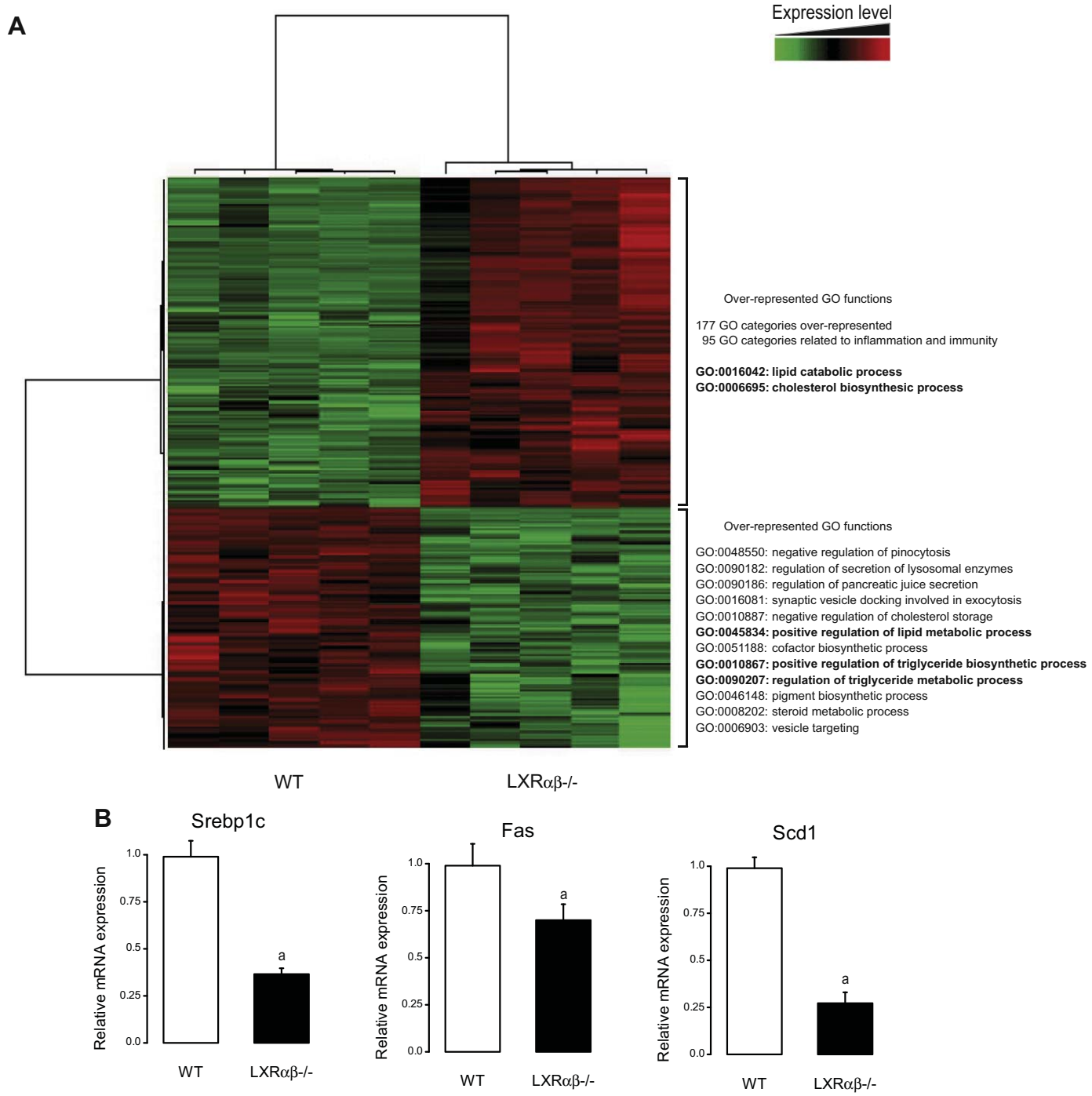

Fig. 1. Central role of LXR in lipogenic gene expression. (A) Transcriptome analysis of WT and LXRαβ$^{-/-}$ livers. mRNAs were extracted from the livers of male WT and LXRαβ$^{-/-}$ mice. Hepatic transcriptomes were analyzed using Agilent Whole Mouse Genome microarrays (4 × 44K). The heatmap for the 1088 probes significantly regulated when comparing wild-type to LXRαβ$^{-/-}$. Red and green colors indicate values above and below the mean, respectively. Black color indicates values close to the mean. Individual values for each group are represented in the heatmap and the hierarchical clustering was obtained from individual values using 1-Pearson correlation coefficient as distance and the Ward's criterion for agglomeration. Analysis of GO biological functions significantly enriched (q-value < 0.001) amongst the down-regulated genes clearly pointed to lipid metabolism as the most robustly enriched in LXRαβ$^{-/-}$. Up-regulated genes clearly pointed to immunity and inflammation as the most robustly enriched GO biological function. Other up-regulated GO biological functions included lipid catabolic process and cholesterol biosynthetic process. (B) Srebp1-c, Fas and Scd1 mRNA quantification was assayed by qPCR. Data are the mean ± SEM of values measured in WT and LXRαβ$^{-/-}$ mice ($n = 9$ animals/group). [a]Significant difference between genotypes.

3.6. LXRs deficiency impairs lipogenic response to pharmacological activation of PPARα and reduces the expression of Cyp4a14 and Cyp4a10

We also examined the effect of pharmacological activation of PPARα in LXR-deficient mice. We found that fenofibrate administration increases the expression of LXR-sensitive genes involved in fatty acid synthesis only in wild-type mice not in LXR$^{-/-}$ mice (Fig. 6A and B). This is consistent with the significant difference in the effect of fenofibrate on hepatic TG measured in wild-type and not in LXR$^{-/-}$ mice (Fig. 6C). Because LXR deficiency reduces the constitutive expression of lipogenic genes, we also

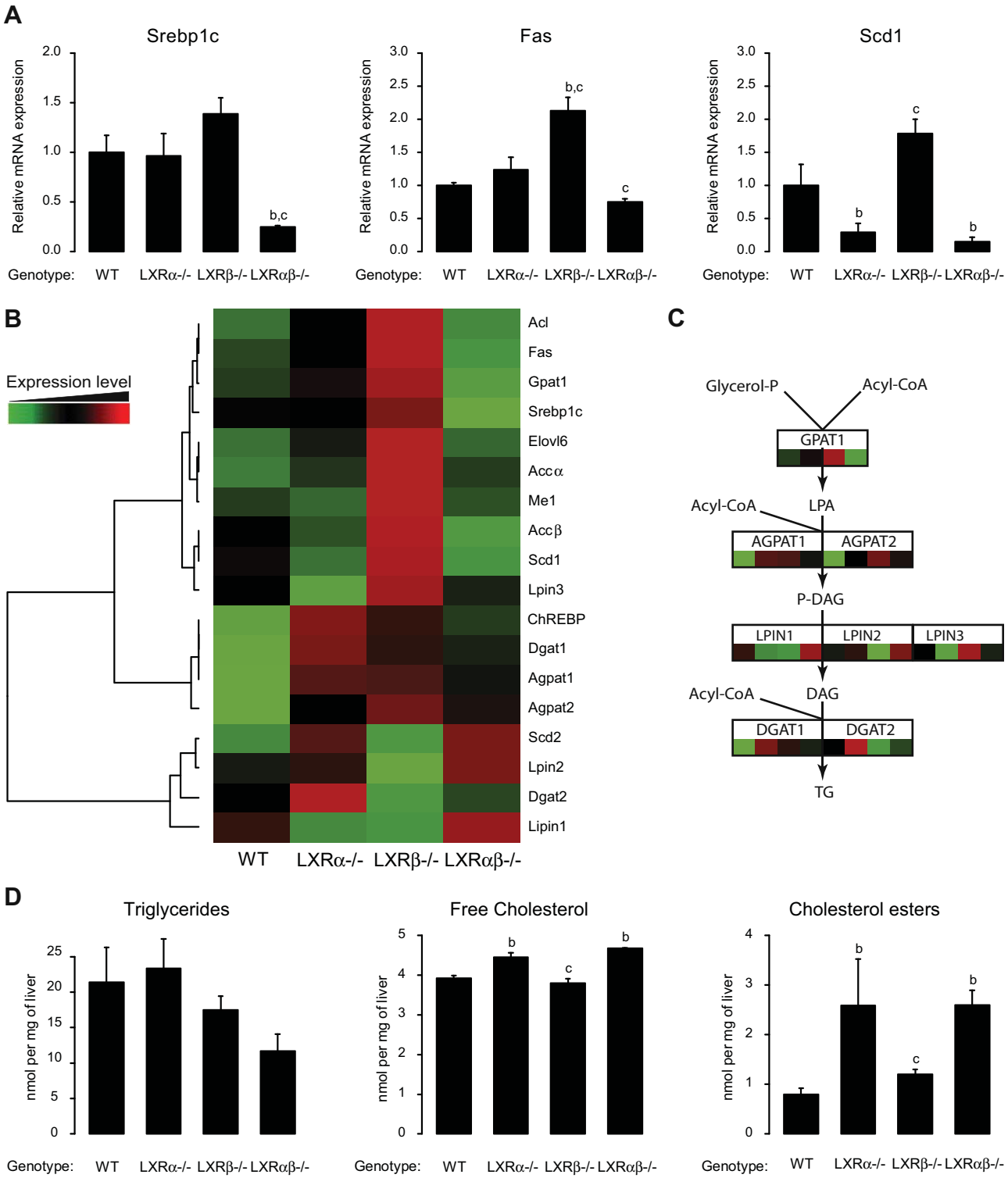

Fig. 2. LXRα and LXRβ deficiency differentially impact lipogenic genes. (A) Srebp1-c, Fas and Scd1 mRNA quantification was assayed by qPCR. Data are the mean ± SEM of values measured in WT, LXRα$^{-/-}$, LXRβ$^{-/-}$ and LXRαβ$^{-/-}$ mice ($n = 4$ animals/group). [b,c]Significant difference compared to WT and LXRα$^{-/-}$, respectively. (B) Hierarchical classification of hepatic gene expression. mRNA level of genes involved in fatty acids and triglycerides synthesis were measured by qPCR. (C) Triglycerides biosynthetic pathway. Expression levels of genes of this pathway were extracted from the heatmap in Fig. 2B. (D) Liver triglycerides, free cholesterol and cholesterol esters were analyzed by gas chromatography. The presence of internal standards enabled to quantify neutral lipids. Data are the mean ± SEM of values measured in WT, LXRα$^{-/-}$, LXRβ$^{-/-}$ and LXRαβ$^{-/-}$ mice ($n = 4$ animals/group).[b,c]Significant difference compared to WT and LXRα$^{-/-}$, respectively.

presented the data as fold-changes within each genotype. Such presentation allowed us to identify that not all the genes show the same pattern (Fig. 6D). They were all sensitive to LXRs deficiency. However, while most genes show reduced sensitivity to PPARα agonist in the absence of LXRs we identified that Scd1 and Accβ were actually more sensitive to fenofibrate when LXRs are lacking. Therefore, we conclude that LXRs have a dual influence on the sensitivity of lipogenic genes to PPARα activation. Some genes such

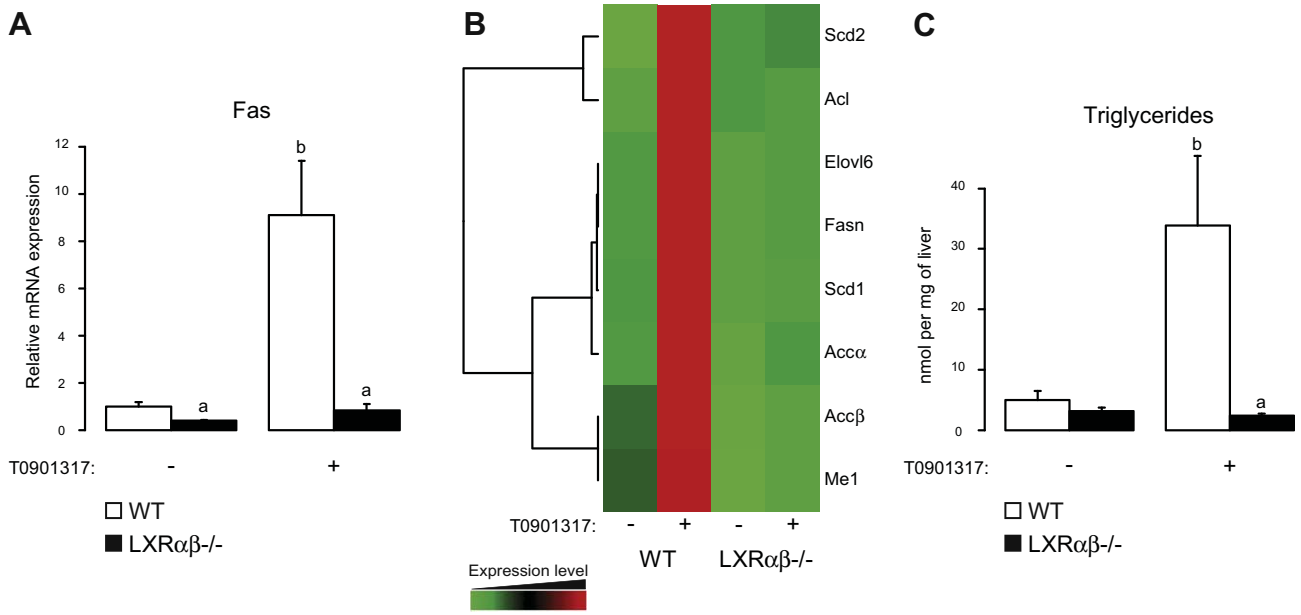

Fig. 3. T0-induced LXR activity promotes most lipogenic genes. (A) Fas mRNA quantification was assayed by qPCR. Data are the mean ± SEM of values measured in WT and LXRαβ$^{-/-}$ mice treated or not with T0901317 ($n = 5$ animals/group). [a]Significant difference between genotypes. [b]Significant difference induced by T0901317 treatment. (B) Hierarchical classification of hepatic gene expression. mRNA level of genes involved in fatty acid synthesis were measured by qPCR. (C) Liver triglycerides were analyzed by gas chromatography. The presence of internal standards enabled to quantify neutral lipids. Data are the mean ± SEM of values measured in WT and LXRαβ$^{-/-}$ mice treated or not with T0901317 ($n = 5$ animals/group). [a]Significant difference between genotypes. [b]Significant difference induced by T0901317 treatment.

as Me1, Acl, Accα and Fas are less sensitive to fenofibrate in the absence of LXRs. Some others, such as Scd1 and Accβ, are hypersensitive to fenofibrate in the absence of LXRs.

3.7. LXRs deficiency impairs other PPARα-sensitive pathways

Finally, we also questioned whether LXR deficiency influences the effect of fenofibrate on other pathways sensitive to LXRs expression. We tested the expression of genes involved in lipid catabolism and in cholesterol biosynthesis. These two pathways were found to be up-regulated in LXR-deficient mice (Fig. 1A). PPARα drives the expression of genes involved in lipid catabolism. The expression of PPARα itself and the expression of three typical target genes involved in lipid catabolism, namely Cyp4a14, Cyp4a10 and Acox1 were measured (Fig. 7A). Interestingly, we found that the absence of LXRs influences the expression of PPARα and its targets in response to fenofibrate. Similarly, the presence of LXR influences the effect of fenofibrate on a number of genes involved in cholesterol biosynthesis (Fig. 7B). In addition, fenofibrate do not show the same effect on hepatic cholesterol esters or on free cholesterol in wild-type mice and in mice lacking LXRs (Fig. 7C).

4. Discussion

Associated to the obesity epidemic, liver diseases such as NAFLD and NASH have become a priority health issue worldwide. Therefore, it is critical to better understand the mechanisms that drive hepatic lipid metabolism including *de novo* fatty acid synthesis [2]. The oxysterol receptor LXR is well known to play a central role in the transcriptional control of hepatic cholesterol metabolism and to regulate myriads of other functions [32,33]. In this work we report the hepatic transcriptome of wild-type and LXR$^{-/-}$ mice. We also provide further evidence for its central role in triglyceride metabolism [19,34] and in inflammation [32]. Consistent with the established role of LXR in cholesterol homeostasis [19,34], we found accumulation of both free cholesterol and cholesterol ester in LXRαβ$^{-/-}$ mice. Interestingly, while triglyceride accumulation is a hallmark for NAFLD, cholesterol level fuels inflammation in the progression of NASH [35]. Therefore, LXRs are central to various hits in NAFLD that includes triglyceride accumulation, inflammation and cholesterol homeostasis. In this work we chose to focus on fatty acid and triglyceride synthesis that may influence the early steps in NAFLD progression. While both α and β isoforms of LXR influence the expression of these genes, they seem to have different effects. The lack of both LXR isoforms results in a reduced expression of genes primarily involved in fatty acid synthesis. In wild-type mice, this set of lipogenic genes is induced by T0901317, a potent pharmacological agonist for LXR [36]. This gene expression profile correlates with elevated hepatic triglycerides. When both isoforms are absent, the lipogenic effect of T0901317 is abolished. This is consistent with the direct role of LXR in the regulation of Accα [37], Fas [38], Scd1 [39] and with the indirect role of LXR in the regulation of lipogenesis through its effect on SREBP1-c [15]. SREBP1-c is a master transcriptional regulator of the expression of fatty acid and triglyceride biosynthesis [40,41]. Various reports have provided strong evidence that PPARα, another nuclear receptor which is essential to the catabolism of hepatic fatty acid during fasting, is also important for lipogenic gene expression during refeeding [42]. We found that pharmacological activation of PPARα by fenofibrate also induces the expression of genes involved in fatty acid synthesis. This result is in agreement with the recent findings that PPARα activation in response to fenofibrate promotes lipogenesis and requires SREBP1-c expression [43].

Interestingly, we found that PPARα deficiency does not impair the lipogenic response to LXR activation. This result also contrasts with data obtained in cell culture that showed mutual interaction between PPARα and LXR that impairs SREBP1-c expression in response to LXR signalling [24]. In addition, we report a PPARα-dependent increase in Cyp4a14 under T0901317. This may be a consequence of increased *de novo* lipogenesis promoting PPARα activity [5,21,22]. Finally, we also evidenced that the presence of LXR influences the fenofibrate-induced PPARα activation. In mice

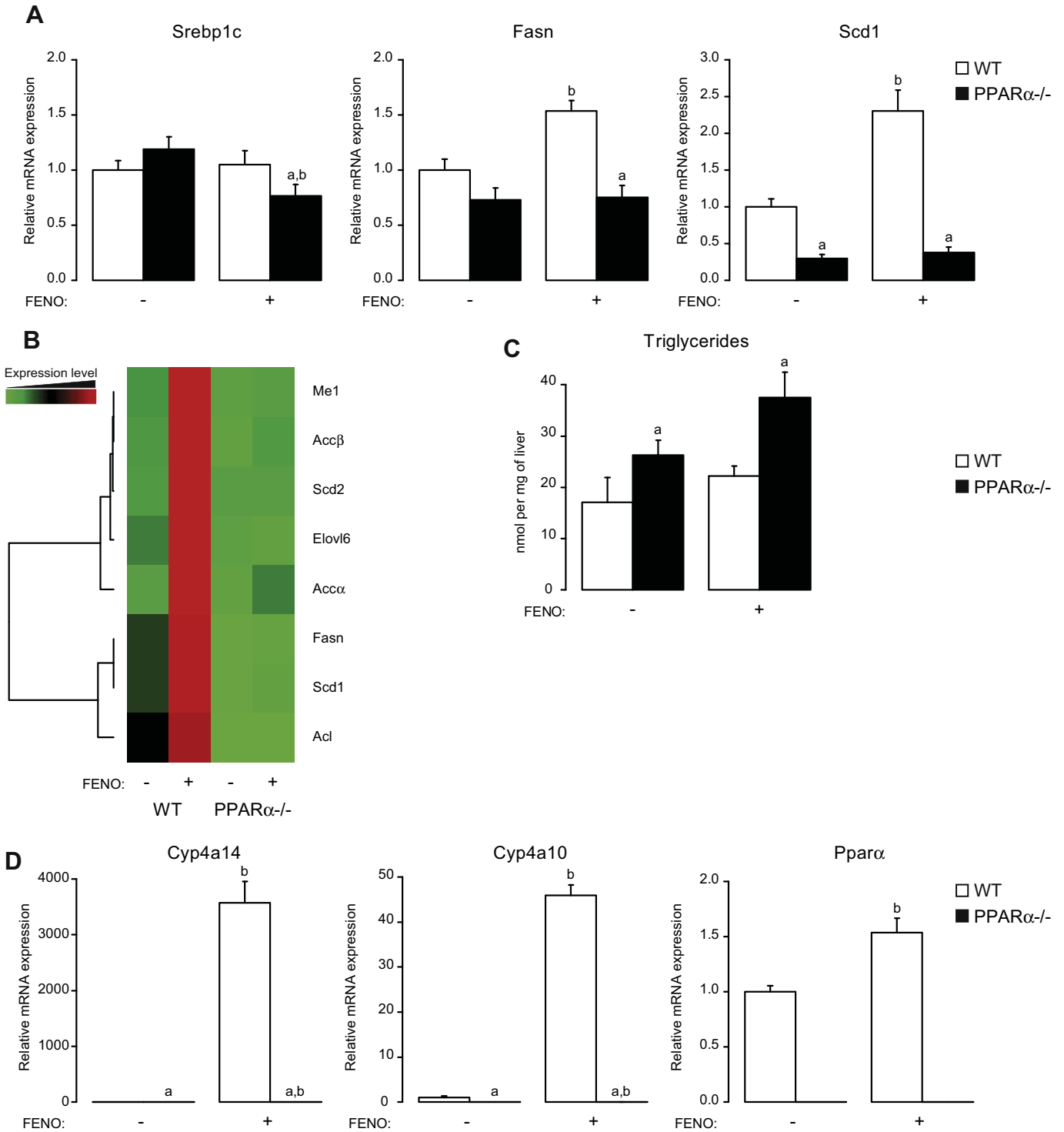

Fig. 4. Pharmacological activation of PPARα by fenofibrate activates lipogenic genes. (A) Srebp1-c, Fas and Scd1 mRNA quantification was assayed by qPCR. Data are the mean ± SEM of values measured in WT and PPARα$^{-/-}$ mice treated or not with fenofibrate ($n = 6$ animals/group). [a]Significant difference between genotypes. [b]Significant difference induced by fenofibrate treatment. (B) Hierarchical classification of hepatic gene expression. mRNA level of genes involved in lipogenesis were measured by qPCR. (C) Liver triglycerides were analyzed by gas chromatography. The presence of internal standards enabled to quantify neutral lipids. Data are the mean ± SEM of values measured in WT and PPARα$^{-/-}$ mice treated or not with fenofibrate ($n = 6$ animals/group). [a]Significant difference between genotypes. [b]Significant difference induced by fenofibrate treatment. (D) Cyp4a14, Cyp4a10 and Pparα mRNA quantification was assayed by qPCR. Data are the mean ± SEM of values measured in WT and PPARα$^{-/-}$ mice treated or not with fenofibrate ($n = 6$ animals/group). [a]Significant difference between genotypes. [b]Significant difference induced by fenofibrate treatment.

lacking LXRs the well-known lipogenic response to fenofibrate [43] was reduced. Amongst lipogenic enzymes, only Me1 [44] and Scd1 [45] have been described as direct PPARα targets with functional PPREs. Therefore the down-regulation of the whole pathway could be a consequence of the reduced expression of SREBP1-c in LXR-deficient mice. Surprisingly, the expression of prototypical PPARα target such as Cyp4a14, Cyp4a10 and Acox1 are also modified in response to fenofibrate when LXRs are lacking. In addition, the

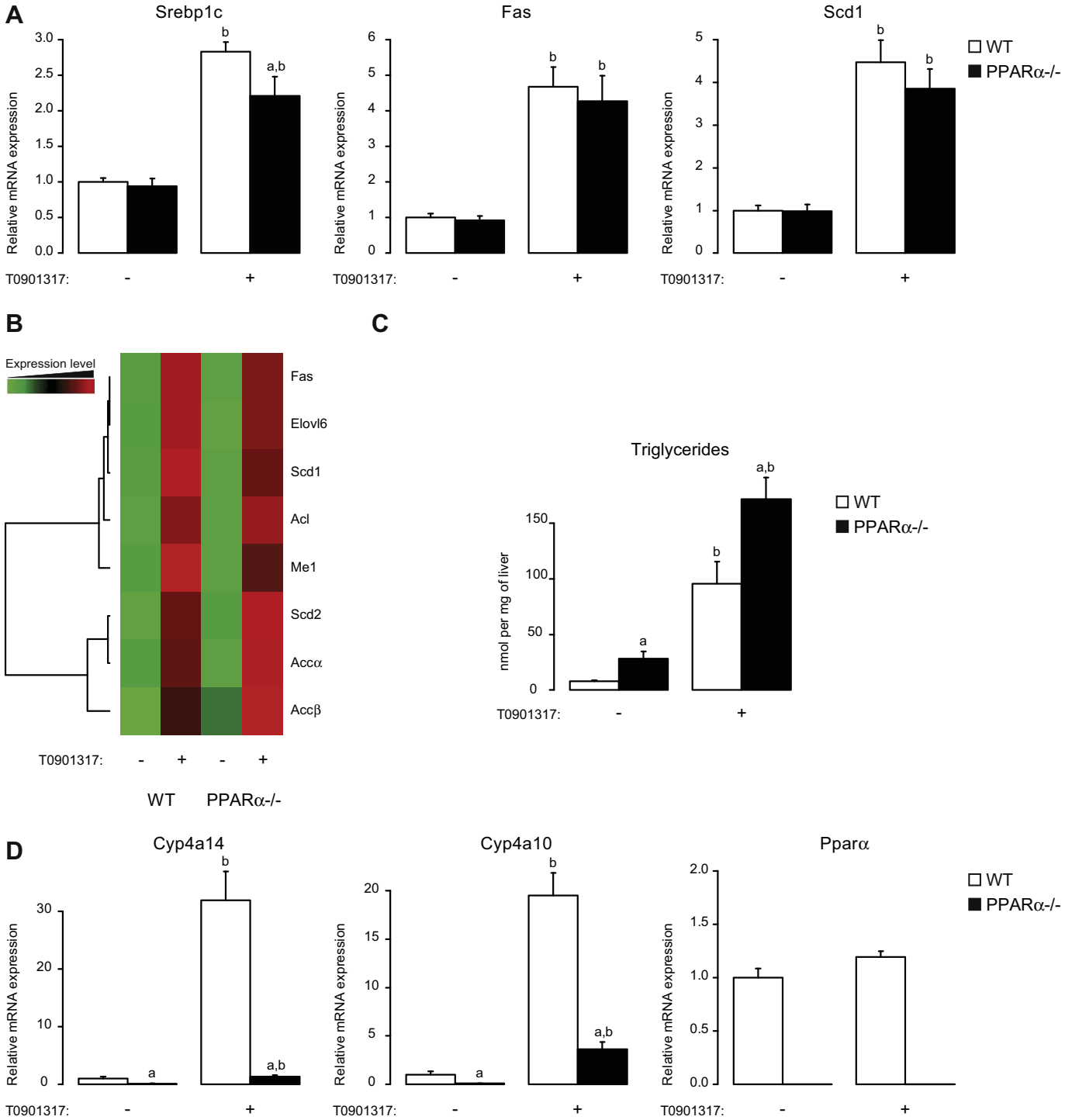

Fig. 5. PPARα deficiency does not impair lipogenic genes in response to T0 but alters some PPARα responsive genes. (A) Srebp1-c, Fas and Scd1 mRNA quantification was assayed by qPCR. Data are the mean ± SEM of values measured in WT and PPARα$^{-/-}$ mice treated or not with T0901317 ($n = 6$ animals/group). [a]Significant difference between genotypes. [b]Significant difference induced by T0901317 treatment. (B) Hierarchical classification of hepatic gene expression. mRNA level of genes involved in lipogenesis were measured by qPCR. (C) Liver triglycerides were analyzed by gas chromatography. The presence of internal standards enabled to quantify neutral lipids. Data are the mean ± SEM of values measured in WT and PPARα$^{-/-}$ mice treated or not with T0901317 ($n = 6$ animals/group). [a]Significant difference between genotypes. [b]Difference induced by T0901317 treatment. (D) Cyp4a14, Cyp4a10 and Acox1 mRNA quantification was assayed by qPCR. Data are the mean ± SEM of values measured in WT and PPARα$^{-/-}$ mice treated or not with T0901317 ($n = 6$ animals/group). [a]Significant difference between genotypes. [b]Difference induced by T0901317 treatment.

expression of genes involved in cholesterol synthesis is also modified in response to fenofibrate when LXRs are absent. This implies that the lack of LXRs may also impair the PPARα response through mechanisms that do not relate to the reduced expression of SREBP1-c. One possibility is that impaired lipogenesis due to the LXR deficiency reduces the abundance of PPARα ligands. Indeed, recent works have provided evidence that *de novo* synthesized lipids activate PPARα [5,21,22]. Another possibility is that LXR may influence the feed forward loop that promotes PPARα in response to its own activation [46]. In any case, these findings contrast with

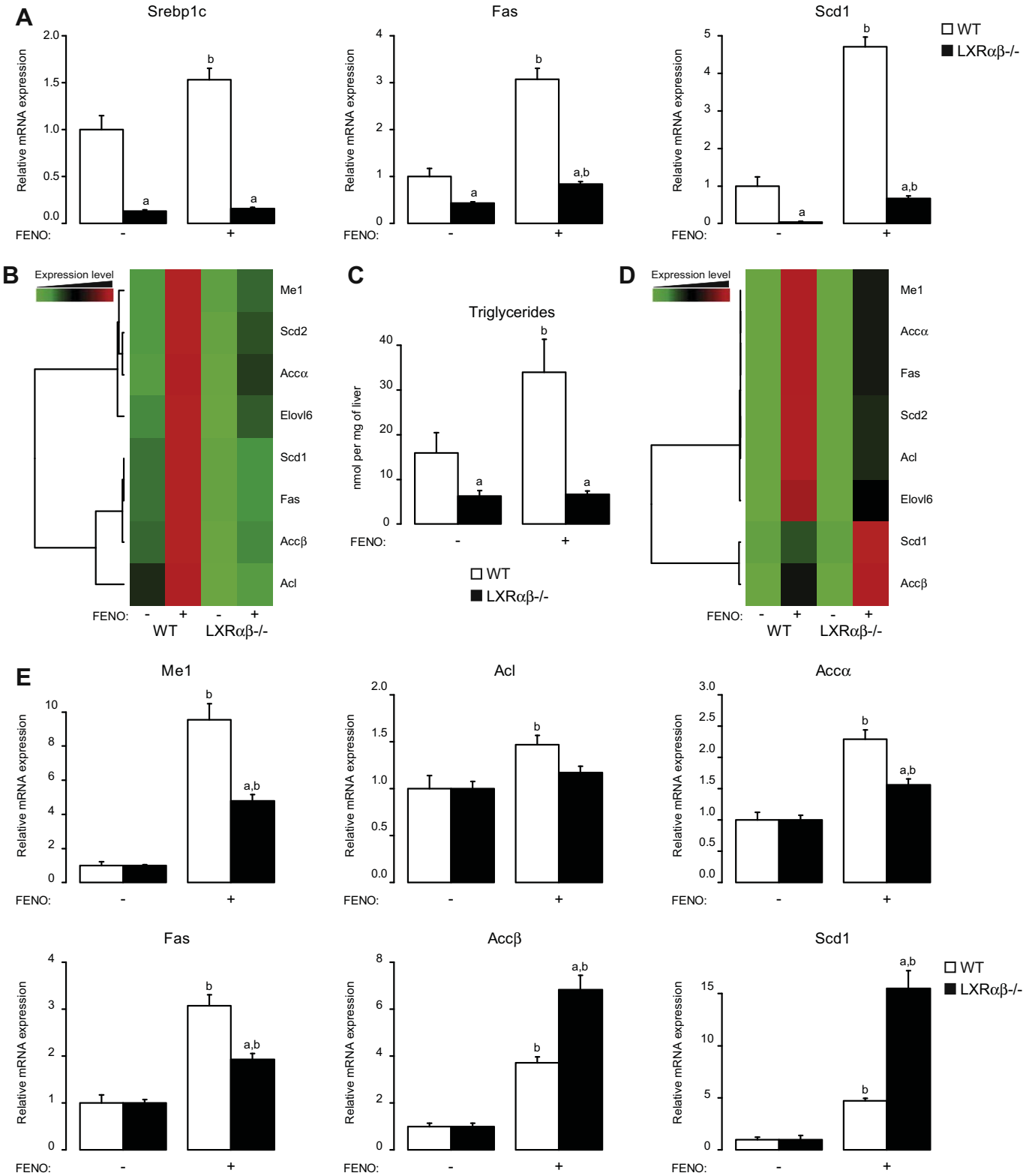

Fig. 6. LXR deficiency modifies lipogenic response to fenofibrate. (A) Srebp1-c, Fas and Scd1 mRNA quantification was assayed by qPCR. Data are the mean ± SEM of values measured in WT and LXRαβ$^{-/-}$ mice treated or not with fenofibrate ($n = 6$ animals/group). [a]Significant difference between genotypes. [b]Significant difference induced by fenofibrate treatment. (B) Hierarchical classification of hepatic gene expression. mRNA level of genes involved in lipogenesis were measured by qPCR. (C) Liver triglycerides were analyzed by gas chromatography. The presence of internal standards enabled to quantify neutral lipids. Data are the mean ± SEM of values measured in WT and LXRαβ$^{-/-}$ mice treated or not with fenofibrate ($n = 6$ animals/group). [a]Significant difference between genotypes. [b]Significant difference induced by fenofibrate treatment. (D) Hierarchical classification of hepatic gene expression measured by qPCR and normalized to be presented as fold-increase within genotype. (E) Me1, Acl, Accα, Fas, Accβ, Scd1 relative mRNA abundance when data have been normalized and presented as fold-increase within a genotype. Data are the mean ± SEM of values measured in WT and LXRαβ$^{-/-}$ mice treated or not with fenofibrate ($n = 6$ animals/group). [a]Significant difference between genotypes. [b]Significant difference induced by fenofibrate treatment.

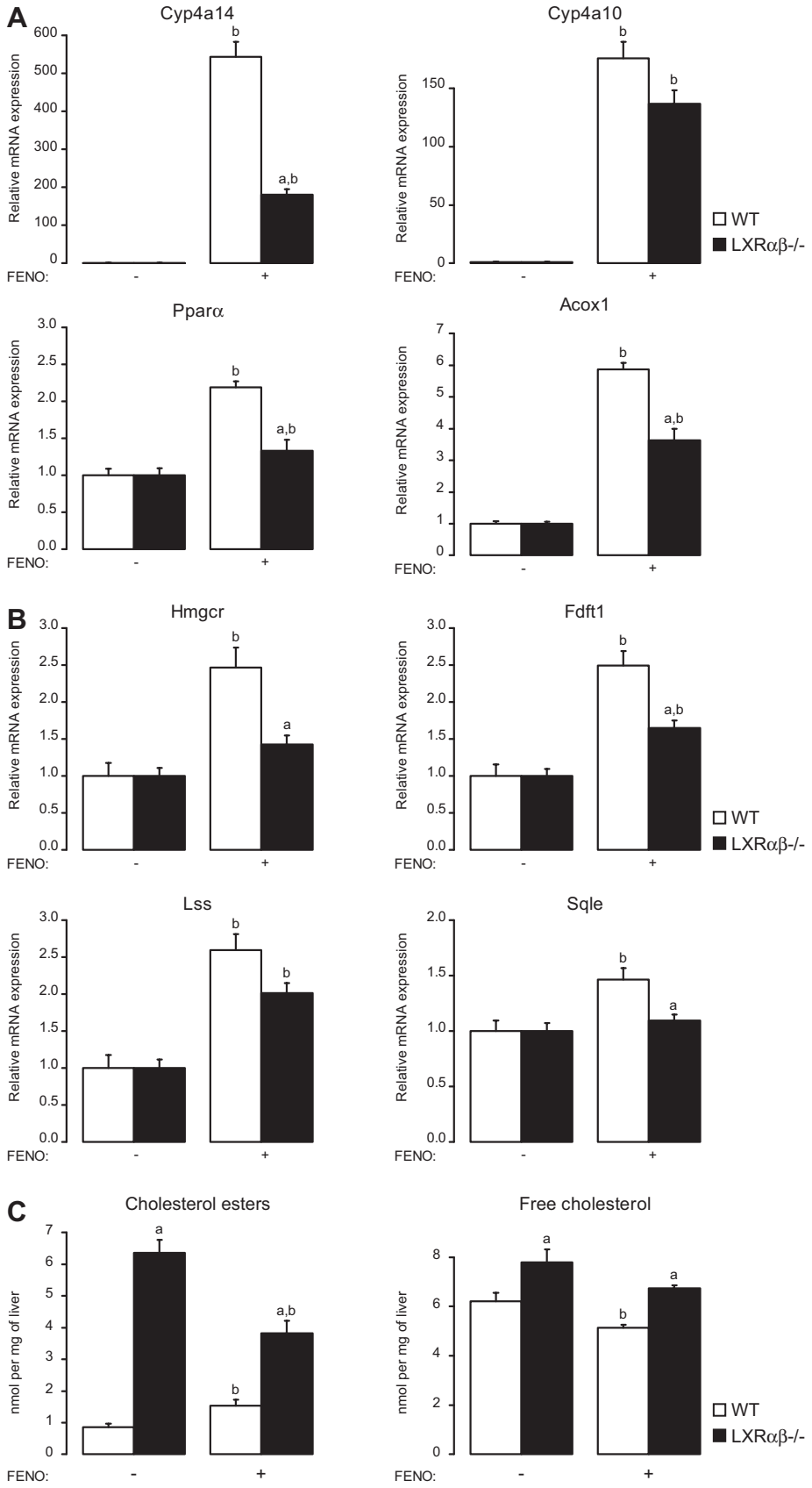

Fig. 7. LXR deficiency modifies PPARα-sensitive genes and the expression of cholesterogenic genes in response to fenofibrate. (A) Cyp4a14, Cyp4a10, Pparα and Acox1 mRNA quantification was assayed by qPCR. Data are the mean ± SEM of values measured in WT and LXRαβ$^{-/-}$ mice treated or not with fenofibrate. Data have been normalized and presented as fold-increase within a genotype ($n = 6$ animals/group). [a]Significant difference between genotypes. [b]Significant difference induced by fenofibrate treatment. (B) Hmgcr, Fdft1, Lss and Sqle mRNA quantification was assayed by qPCR. Data are the mean ± SEM of values measured in WT and LXRαβ$^{-/-}$ mice treated or not with fenofibrate. Data have been normalized and presented as fold-increase within a genotype ($n = 6$ animals/group). [a]Significant difference between genotypes. [b]Significant difference induced by fenofibrate treatment. (C) Free cholesterol and cholesterol esters were analyzed by gas chromatography. The presence of internal standards enabled to quantify neutral lipids. Data are the mean ± SEM of values measured in WT and LXRαβ$^{-/-}$ mice treated or not with fenofibrate ($n = 6$ animals/group). [a]Significant difference between genotypes. [b]Significant difference induced by fenofibrate treatment.

results obtained in cell culture that raised the possibility that LXR may inhibit PPARα activity [23].

Altogether, our data further points at LXR as a major player in liver lipid homeostasis. We also confirm mutual interaction between signalling by the oxysterol receptor LXR and the fatty acid sensor PPARα in the regulation of lipogenic genes and of other PPARα-sensitive genes. This is in agreement with a recent genome-wide mapping of LXR and PPARα binding sites that revealed extensive overlap and functional cross-talk [26]. In addition, our data do not support a unique model of reciprocal inhibition between these two nuclear receptors [23,24] in the liver. This cross-regulation might be important to consider since drugs targeting LXRs [47] and PPARs [48] might be of clinical relevance in NAFLD and other metabolic diseases.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.10.1016/j.biochi.2012.09.028

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Chapitre II

Comme nous l'avons vu dans le chapitre I, il est possible d'induire une augmentation de l'expression des gènes de la lipogenèse en utilisant un agoniste pharmacologique de LXR. De plus, nous avons vu dans l'introduction bibliographique et dans le chapitre I que LXR est important dans la limitation de l'inflammation.

Il a été montré dans un modèle expérimental de colite induite par du sulfate de dextran sodique (DSS) que l'inflammation intestinale pouvait perturber la lipogenèse hépatique, notamment la désaturation des acides gras en position n-9 catalysée par SCD1, et la production d'espèces lipidiques complexes aux propriétés bénéfiques sur l'inflammation (Chen, Shah et al. 2008). De plus, de nombreux travaux ont fait émerger l'hypothèse que la lipogenèse hépatique pouvait conduire à la synthèse de molécules lipidiques présentant des fonctions de signalisation et que certaines d'entre elles pouvaient jouer des rôles bénéfiques dont l'activation de PPARα (Chakravarthy, Pan et al. 2005) ou la dissociation de la stéatose et de la résistance à l'insuline (Benhamed, Denechaud et al. 2012; Sun and Lazar 2013).

L'activation de LXR pourrait permettre, dans ce modèle, en plus de ses propriétés anti-inflammatoires, la génération de lipides complexes jouant un rôle bénéfique sur l'inflammation intestinale. De plus, les souris exposées au DSS présentent un phénotype hépatique proche des souris invalidées pour *Lxr*, à savoir une augmentation de la quantité de cholestérol libre et une diminution de la quantité de triglycérides et de l'expression de l'expression de gènes impliqués dans la lipogenèse.

Dans cette étude, nous avons voulu déterminer si l'activation pharmacologique de LXR pouvait rétablir le phénotype hépatique, prévenir les effets délétères de l'inflammation colite induite expérimentalement et ainsi ouvrir de nouvelles perspectives pharmacologiques de traitement des maladies inflammatoires chroniques de l'intestin.

Chapitre -II- Implication de LXR dans un modèle de colite induite expérimentalement

INTRODUCTION

La prévalence des maladies inflammatoires chroniques de l'intestin (MICI) ou « inflammatory bowel disease » (IBD), incluant la maladie de Crohn et la recto-colite hémorragique est en augmentation dans le monde. Aux Etats Unis, les MICI affectent environ 1.4 millions de personnes (Loftus 2004). Les facteurs génétiques et environnementaux sont considérés comme les deux contributeurs majeurs au développement des MICI (Schreiber and Hampe 2000; Loftus 2004). La lumière intestinale contient nombre de composés comme des nutriments alimentaires, des composés bactériens et des polluants environnementaux qui pourraient contribuer au développement des MICI. Ces facteurs sont reconnus par les TLRs et peuvent induire un statut inflammatoire (Huang, Rutkowsky et al. 2012).

LXR est un récepteur nucléaire de classe II et est un senseur du cholestérol. LXR a également été décrit comme un répresseur de l'inflammation au niveau de plusieurs types cellulaires comme les macrophages (Zelcer and Tontonoz 2006), les lymphocytes (Walcher, Kummel et al. 2006), les neutrophiles (Hong, Kidani et al. 2011) mais également au niveau de cellules non immunitaires telles que les hépatocytes (Blaschke, Takata et al. 2006). Au niveau hépatique, LXR joue également un rôle important dans l'homéostasie des lipides en augmentant la transcription de gènes impliqués dans la synthèse endogène d'acides gras (Joseph, Laffitte et al. 2002; Chu, Miyazaki et al. 2006; Talukdar and Hillgartner 2006). Il a été montré qu'une colite induite par une exposition au DSS induisait une diminution de l'expression hépatique de *Scd1*, un gène cible de LXR impliqué dans la synthèse d'acides gras *de novo* (Chen, Shah et al. 2008). Dans cette étude, nous avons voulu savoir si une activation de LXR dans le modèle de colite induite par du DSS permettait de corriger des paramètres hépatiques et intestinaux associés à la colite. Nous montrons que l'activation de LXR parvient partiellement à améliorer les dommages associés à la colite.

1. MATERIEL ET METHODES

1. Animaux

Les études *in vivo* ont été réalisées sur des souris C57Bl/6J, mâles issues des animaleries JANVIER, et acclimatées dans l'animalerie de l'unité Toxalim de l'INRA (22 ±2°C) pendant une semaine avant le début des expériences. Les souris ont accès *ad libitum* à l'eau et à la nourriture (A04, SAFE, France). Les études *in vivo* ont été réalisées conformément aux directives européennes concernant l'expérimentation animale.

2. Traitement et suivi des souris

L'étude des effets au niveau hépatique d'une colite aigue induite par le DSS a été réalisée sur des souris de neuf semaines (n=10/groupe). Elles sont exposées pendant 6 jours au DSS (Sulfate de Dextran Sodique, 4% dans l'eau de boisson) (TDB, Suède). La veille de l'abattage, les souris sont abreuvées avec de l'eau sans DSS. L'eau de boisson ainsi que le DSS sont changés tous les 2 jours. La prise alimentaire et hydrique, ainsi que la masse corporelle sont mesurées pendant la durée de l'expérimentation.

L'étude des effets du gavage par *Citrobacter rodentium* a été réalisée sur des souris entre six et neuf semaines (n=5/groupe). Les souris sont gavées avec *C rodentium* (1.06×10^{10} U) et sont abattues 10 jours (maximum du pic inflammatoire) ou 30 jours après infection (inflammation résorbée).

L'étude du rôle de LXR dans le modèle de colite aigue induite par du DSS a été réalisée sur des souris de treize semaines (n=6/groupe). Trois jours avant le début de l'exposition au DSS et jusqu'à abattage, elles sont gavées avec du T0901317 (30 mg/kg/jour) (Cayman, France) ou le véhicule seul (0,5% m/v carboxyméthyl-cellulose, 0,5% v/v Tween80). Le protocole d'induction de la colite est le même que décrit précédemment.

3. Mesure in vivo de la perméabilité intestinale

Les souris sont gavées 4 heures avant abattage avec du dextran 4 kDa-FITC et du dextran 70 kDa-TRITC (400 mg/kg, TDB, Suède). Le sang est prélevé à l'abattage au niveau de la veine submandibulaire et le plasma est obtenu après centrifugation (15 min, 1500 g, 4°C). La concentration plasmatique de ces 2 composés est déterminée par mesure de la fluorescence (TECAN infinite M200, France). Le dextran4-FITC est dosé à $\lambda_{excitation}$ = 490 nm et $\lambda_{émission}$ = 525 nm, et le dextran70-TRITC, à $\lambda_{excitation}$ = 565 nm et $\lambda_{émission}$ = 594 nm.

4. Abattage

Les souris sont sacrifiées par dislocation cervicale. Le sang est prélevé au niveau de la veine submandibulaire. Le foie et le côlon sont prélevés, lavés, congelés dans de l'azote liquide puis conservés à -80°C.

Pour l'étude des effets du T0901317 sur la colite induite par le DSS, les souris sont d'abord anesthésiées (kétamine/xylazyne) afin de pouvoir prélever du sang au niveau de la veine porte. Elles sont ensuite euthanasiées par dislocation cervicale. Le foie et le côlon sont prélevés, lavés, congelés dans de l'azote liquide puis conservés à -80°C.

5. Translocation bactérienne

Soixante-cinq milligrammes de foie sont prélevés de façon stérile, puis broyés (Fast-Prep, 20 s, vitesse 4, Q-biogène, France) dans des milieux de culture liquides aérobie (Trypcase-Soja) (Biomérieux, France) et anaérobie (Schaedler + vitamines K3) (Biomérieux, France). Ces solutions mères obtenues sont diluées au 1/10 dans le milieu de culture correspondant. Cent microlitres de chaque solution (mère et diluée à 10^{-1}) sont ensemencés par étalement sur la gélose correspondante: aérobie (Plate Count Agar) (Biomérieux, France) ou anaérobie (Schaedler + 5% de sang de mouton) (Biomérieux, France). Les cultures sont placées en étuve (3 j, 37°C, 5% CO_2), dans des conditions en présence ou en absence d'O_2 respectivement, avant comptage des colonies.

6. Analyse de l'expression génétique

Les ARN totaux sont extraits à partir de 10 mg de foie après broyage au Fast-Prep (10 s, vitesse 4 ; Qbiogene, France) dans 1 mL de Trizol Reagent (Invitrogen, France). Après l'addition de 0.2 mL de chloroforme, agitation (15 s), décantation (5 min, température ambiante) et centrifugation (10 min, 16200 g, 4°C) la phase aqueuse est récupérée. L'ARN est précipité avec l'ajout de 0.5 mL d'isopropanol, incubé 5 min à température ambiante puis culotté par centrifugation (10 min, 16200 g, 4°C). Le surnageant est éliminé à la pompe à vide et le culot est lavé dans 1mL d'éthanol à 70%. Après une centrifugation (5 min, 16200 g, 4°C) le surnageant est éliminé puis l'ARN est repris dans 100µL d'eau milliQ. L'ARN (2µg) est rétro-transcrit (High-capacity reverse transcription kit, Applied Biosystems, France). Les ADNc issus de la reverse transcription sont dilués au vingtième. Les données d'expression sont normalisées par rapport à l'expression de la TATA-box binding protein (*Tbp*).

7. Extraction de la Myéloperoxydase du côlon et dosage de son activité

Le côlon est broyé dans 1,5 mL de tampon hexadécyltriméthylammonium bromide (HTAB) [0,5% p/v dans du tampon phosphate (pH 6, KH_2PO_4 0.2 M, K_2HPO_4 0.2 M, eau milliQ)] au Polytron (30 s, puissance 7) (Bioblock Scientific, France). Trois cycles de congélation (1 min, azote liquide à -195°C)-décongélation (10 min, bain-marie à 37°C) sont effectués à partir d'1 mL d'homogénat. Après centrifugation (15 min, 10600 g, 4°C), le culot est repris dans 500 µL de HTAB. Après sonication (10 s, puissance 70) (Soniprep 150, Fisher Scientific, France) et centrifugation (15 min, 10600 g, 4°C), 15 µL de surnageant sont récupérés, puis mélangés au tampon réactionnel (83 µL de solution d'H_2O_2 à 0.0005%, 56 µL de solution d'O-dianisidine DiHydroChloride à 1mg/mL, 61 µL de tampon phosphate). La cinétique de dégradation d'H_2O_2 est suivie au spectrophotomètre (TECAN infinite M200, France) à 450 nm. Ces valeurs sont normalisées par la quantité de protéines (dosage par la méthode de Bradford).

8. Dosage des lipides neutres

Environ 50 mg de foie sont broyés dans 1mL de solution EGTA/MeOH (EGTA 5 mM 50%, MeOH 50% v/v). Les lipides correspondant à 1 mg de tissu sont extraits selon la méthode de Blight & Dyer dans un mélange de chloroforme/éthanol/eau (2.5/2.5/2.1, v/v/v) en présence d'étalons internes : stigmastérol (3 µg), cholestérol-C17:0 (3 µg) et TG54 (6 µg) (Sigma, France). Les lipides neutres sont analysés en chromatographie en phase gazeuse (Focus, Thermo-Electron, France). La colonne utilisée est apolaire (5m x 0.32µm, Zebron 1). Le gaz vecteur est de l'hydrogène (3mL/min). La température dans la colonne est initialement de 200°C, et s'accroît de 5°C par min pour atteindre 350°C. Les températures de l'injecteur et du détecteur sont respectivement 315 et 345°C.

9. Analyses statistiques

Les données sont traitées *via* le logiciel R (http://www.r-project.org/). Les différents effets ont été analysés par une analyse de variance (ANOVA) des facteurs et des interactions appropriés. Quand un effet est significatif, un test de Student est réalisé en tenant compte de la variance résiduelle déterminée par l'ANOVA afin de comparer les différentes conditions. Le seuil de significativité retenu est de 5%.

2. RESULTATS

Effets intestinaux et hépatiques induits par le DSS.

Afin de nous assurer de la validité du modèle de colite induite chimiquement dans le laboratoire, nous avons d'abord exposé des souris de neuf semaines à 4% de sulfate de dextran sodique pendant six jours. Comme attendu, le DSS induit une augmentation du score inflammatoire macroscopique ainsi que l'infiltration de neutrophiles dans le côlon, mesurée par le dosage de l'activité myéloperoxydase dans ce tissu (Figure 1A). L'exposition des souris au DSS induit donc bien une inflammation marquée au niveau du côlon. La colite est accompagnée d'une augmentation de la perméabilité intestinale transcellulaire et paracellulaire (Figure 1B). La veine porte hépatique relie directement le tube digestif au foie. Afin d'évaluer si l'augmentation de la perméabilité intestinale pouvait conduire à la translocation de bactéries jusqu'au foie, nous avons mis en culture des échantillons de cet organe. Nous n'observons pas de présence de bactéries aérobies ou anaérobies dans le foie des souris contrôle ou exposées au DSS (Données non montrées).

Nous avons ensuite mesuré les quantités de triglycérides et de cholestérol libre dans le foie (Figure 1D). Le DSS induit une diminution de la quantité de triglycérides et une augmentation de la quantité de cholestérol libre.

Il a été préalablement montré qu'une exposition au DSS induisait une diminution de l'expression de *Scd1* dans le foie (Chen, Shah et al. 2008). Nous avons donc mesuré l'expression hépatique de *Scd1* mais également de deux autres gènes impliqués dans la lipogenèse : *Acc*α et *Fas* (Figure 1D). Conformément à cette étude, l'exposition au DSS induit une diminution de l'expression de *Scd1*. Le DSS induit aussi une diminution de l'expression de *Acc*α et de *Fas*.

Citrobacter rodentium ne modifie pas l'expression des gènes de la lipogenèse.

Chen *et al*. ont montré que de façon similaire au modèle de colite induite par le DSS, le gavage des souris avec *Citrobacter rodentium* conduisait au développement d'une colite et à la diminution de l'expression de *Scd1* dans le foie. Nous avons donc gavé des souris pendant 10 ou 30 jours avec cette même bactérie. Et contrairement à cette étude, nous n'observons pas de modulation significative de l'expression de Scd1 dans le foie ni d'autres gènes impliqués

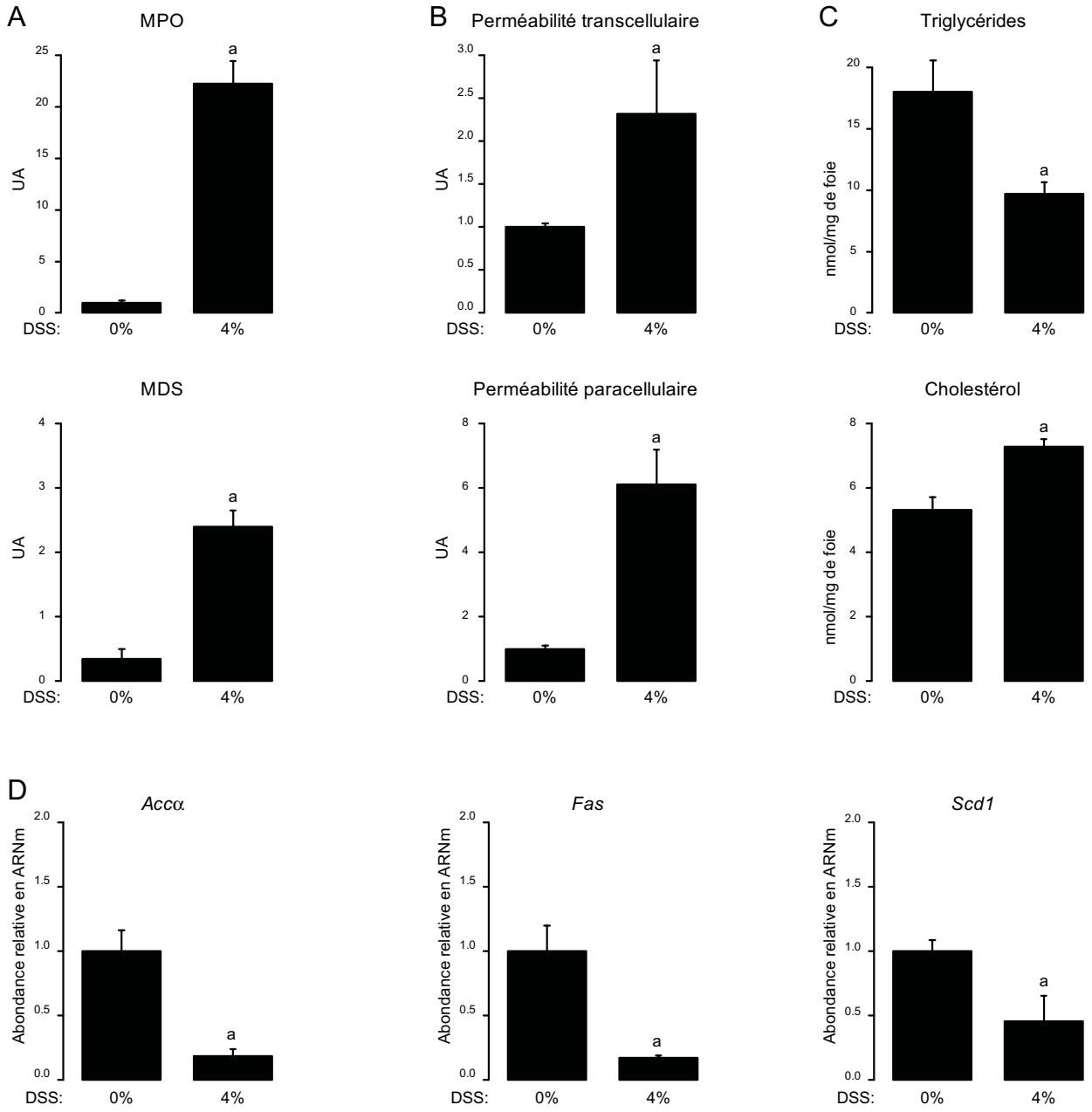

Figure 1. Effet du DSS sur les paramètres intestinaux et hépatiques. (A) Mesure du niveau d'inflammation dans le côlon par dosage de l'activité de la MyéloPerOxydase et score inflammatoire macroscopique dans le colon. (B) Mesure des perméabilités trans-et para-cellulaire. (C) Triglycérides et cholestérol libre hépatique. (D) Quantification des ARNm hépatiques des gènes de la lipogenèse (*Accα*, *Fas* et *Scd1*) par PCR quantitative en temps réel. Les données sont présentées sous forme de moyenne +/- SEM (n=10). « a » indique un effet significatif du DSS par rapport au contrôle.

dans a lipogenèse : *Acc*α, *Acc*β, *Fas* et *Elovl6* (Figure 2) en dépit d'une augmentation de l'inflammation dans le côlon (Données non montrées).

L'activation de LXR réduit partiellement la sévérité de la colite induite par le DSS.

La diminution de l'expression hépatique des gènes de la lipogenèse, ainsi que la diminution de la quantité de triglycérides et de l'augmentation de la quantité de cholestérol libre dans le foie induit par le DSS est un phénotype proche de celui des souris transgéniques invalidées pour les deux isoformes de *Lxr*. Le DSS pourrait donc conduire à une inhibition de l'activité de LXR. Etant donné ses fonctions connues dans la limitation de l'inflammation et dans l'induction de la lipogenèse (Zelcer and Tontonoz 2006), nous avons voulu savoir si l'activation pharmacologique de LXR pouvait atténuer les dommages associés à la colite.

De façon à déterminer si une activation de LXR pouvait atténuer les dommages de la colite, nous avons traité *per os* les souris exposées ou non à du DSS avec un agoniste de LXR, le T0901317. Le T0901317 réduit de façon significative l'augmentation de la perméabilité transcellulaire induite par le DSS. Le T0901317 tend aussi à réduire la perméabilité paracellulaire induite par le DSS. Cependant le T0901317 n'a pas d'effet sur l'activité MPO dans le côlon (Figure 3A). La colite est associée à une diminution de la masse corporelle et l'augmentation de ce facteur est corrélée à une amélioration de cette pathologie dans des modèles animaux et chez l'homme. Nous observons que le DSS diminue de façon significative la masse des souris et le traitement par le T0901317 tend à limiter cette baisse chez les souris exposées au DSS (Figure 3A). Cette expérience est exploratoire est a été réalisée sur un nombre restreint d'individus. Cependant nous avons réalisé une expérience similaire avec un nombre d'animaux plus conséquent (n=10) dans laquelle nous voyons que l'activation de LXR permet de limiter la perte de poids induite par le DSS (Données non montrées).

Le T0901317 induit une augmentation de l'expression hépatique de *Acc*α, *Fas* et de *Scd1* dont l'expression est diminuée par le DSS (Figure 3B). Cependant l'induction de l'expression de ces gènes n'est pas aussi importante chez les souris exposées au DSS que chez les souris contrôle.

Si les effets de l'exposition au DSS sur l'inflammation intestinale sont bien caractérisés, les conséquences au niveau hépatique n'ont pas été étudiées de façon approfondie. Afin d'appréhender un rôle pro-inflammatoire du DSS au niveau du foie, nous

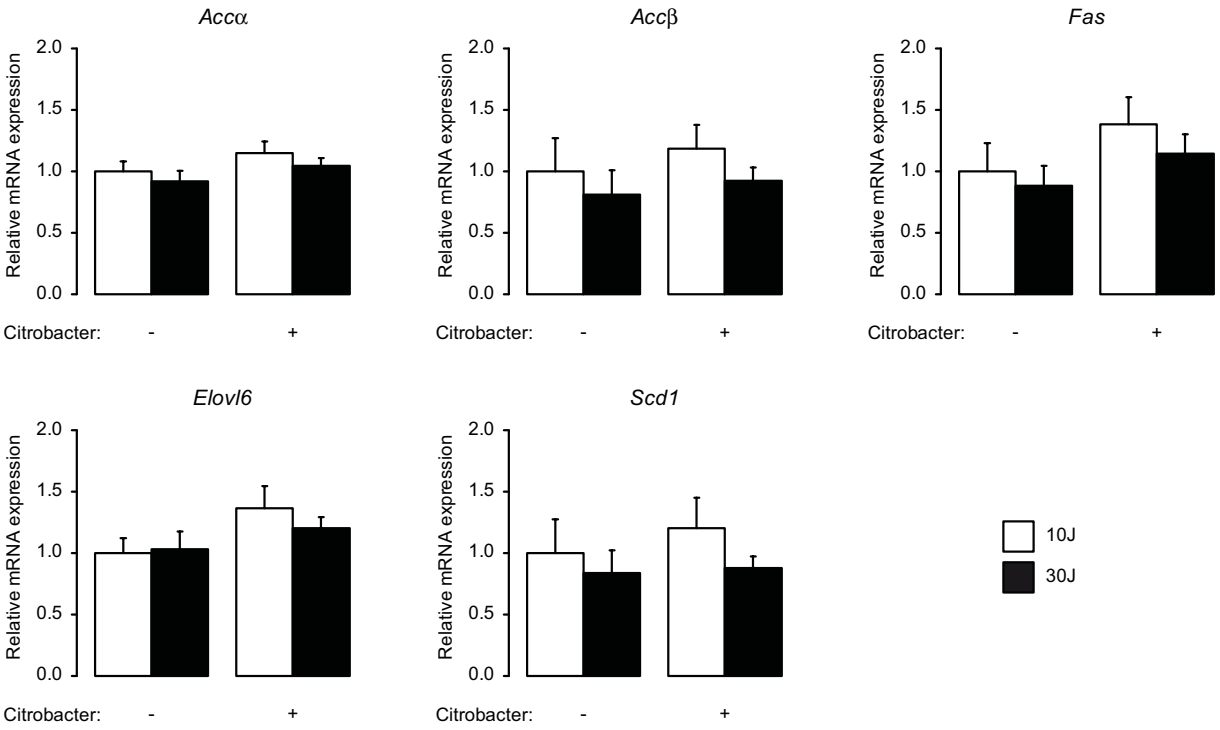

Figure 2. Effet de l'infection orale par *Citrobacter rodentium* sur l'expression hépatique des gènes de la lipogenèse. (A) Quantification des ARNm hépatiques des gènes de la lipogenèse (*Acc*α, *Acc*β, *Fas, Elovl6* et *Scd1*) par PCR quantitative en temps réel. Les données sont présentées sous forme de moyenne +/- SEM (n=5). « a » indique un effet significatif du gavage par *C rodentium* par rapport au contrôle.

avons mesuré l'expression des gènes *Hp*, *Apcs*, *Saa3*, *Saa1*, *Crp* et *Mbl1* codant pour des protéines de phase aigue (Figure 3C), ainsi que de *Il1β* et *Il18* codant pour les interleukines pro-inflammatoires 1β et 18 (Figure 3D). Le DSS induit une augmentation de l'expression de *Hp*, *Apcs*, *Saa3*, *Saa1* alors que ce n'est pas le cas de *Crp* et *Mbl1*. De façon intéressante, le T0901317 réduit de façon significative l'expression de *Hp*, *Apcs* et *Saa3* et est sans effet sur l'expression de *Saa1* (Figure 3C). Le DSS induit une diminution de l'expression de *Il1β* et de *Il18* (Figure 3D). Cependant cette diminution est en désaccord avec d'autres expériences similaires réalisées au laboratoire.

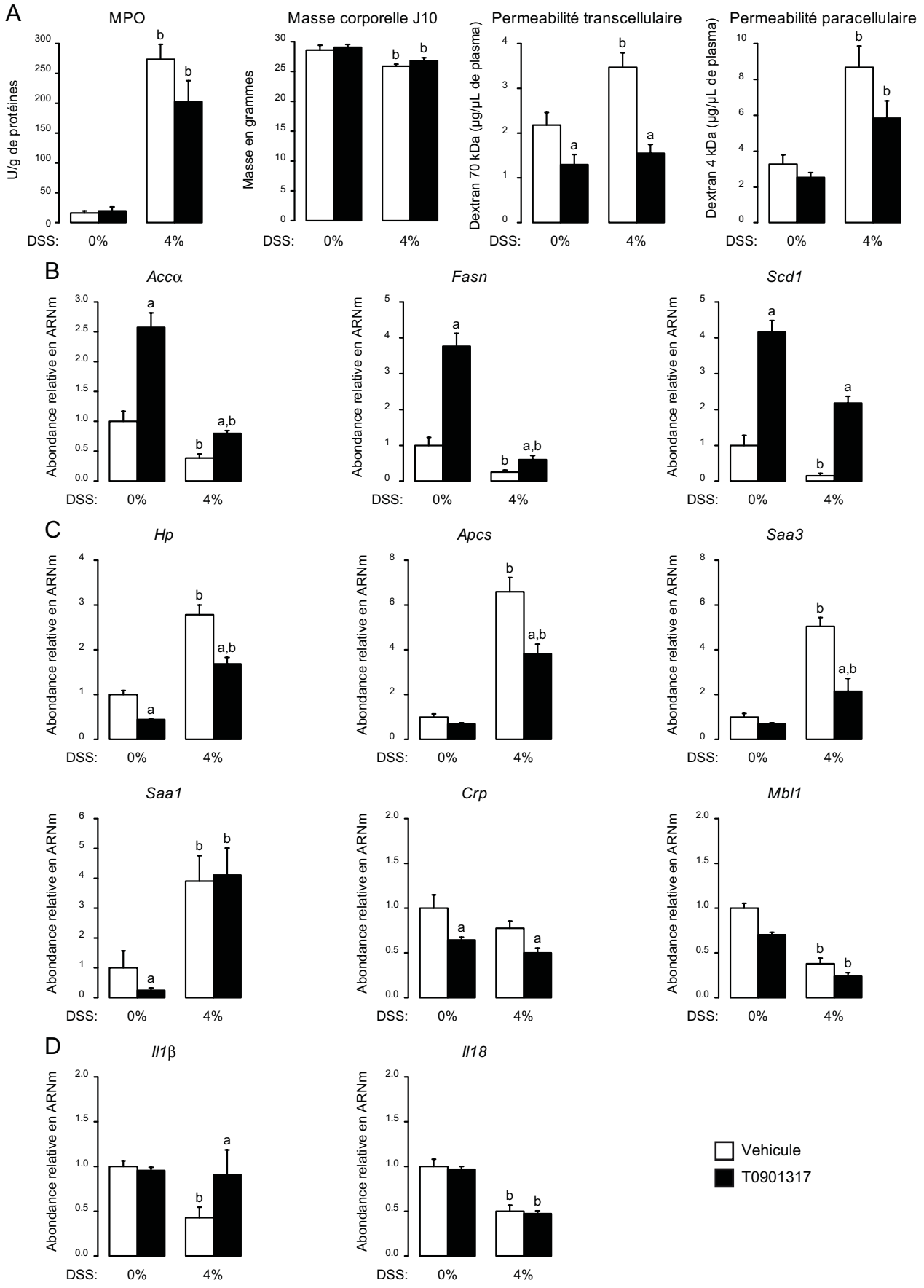

Figure 3. Effet de l'activation de LXR sur la colite induite par du DSS. (A) Mesure du niveau d'inflammation dans le côlon par dosage de l'activité de la myéloperoxydase, de la masse corporelle à J10, avant l'abatage, et mesure des perméabilités trans-et para-cellulaire. Quantification des ARNm hépatiques des gènes (B) de la lipogenèse (*Acc*α, *Fas* et *Scd1*), des protéines de phase aigüe (*Hp*, *Apcs*, *Saa3*, *Saa1*, *Crp*, et *Mbl1*), de cytokines pro-inflammatoires (*Il1*β et *Il18*) par PCR quantitative en temps réel. Les données sont présentées sous forme de moyenne +/- SEM (n=6). [a] indique un effet significatif du T0901317 par rapport au groupes non traité. [b] indique un effet significatif du DSS par rapport au groupe non exposé.

3. DISCUSSION

Dans ce chapitre nous nous sommes intéressés à l'implication de LXR dans un modèle de colite induite expérimentalement avec du sulfate de dextran sodique (DSS). Une exposition de six jours à du DSS induit chez les souris une colite. Ces souris présentent un phénotype hépato-métabolique proche des souris transgéniques invalidées pour les deux isoformes de *Lxr*, à savoir une diminution de l'expression des gènes de la lipogenèse et de la quantité de triglycérides et une augmentation de la quantité de cholestérol libre dans le foie. Cette diminution pourrait résulter de l'inhibition de l'activité LXR en réponse à des signaux pro-inflammatoires.

L'irrigation sanguine du foie fait qu'il est le premier organe, en dehors des organes en contact avec le milieu extérieur, à être exposé à des pathogènes ou des motifs bactériens issus de la flore intestinale. Nous avons donc appréhendé une translocation potentielle de bactéries de la lumière intestinale jusqu'au foie. Que ce soit chez les souris contrôle ou exposées au DSS nous n'observons pas de bactéries dans le foie. Nous avons également tenté de mesurer les taux de LPS circulant dans la veine porte chez les souris exposées au DSS et gavées avec l'agoniste de LXR. Malheureusement nous nous sommes heurtés à des problèmes d'ordre méthodologique.

Il a été montré qu'une inflammation induite par le gavage par *C rodentium* reproduisait les effets du DSS notamment sur la diminution de l'expression de *Scd1* dans le foie (Chen, Shah et al. 2008). Nous avons donc mesuré l'expression hépatique de *Scd1* de souris gavées avec la même bactérie. Contrairement à Chen *et al.* nous n'observons pas de modulation de l'expression de *Scd1* dans le foie de ces souris.

La similarité entre le phénotype des souris exposées au DSS et celui des souris invalidées pour *Lxr* peut révéler une inhibition de l'activité de LXR induite par l'exposition au DSS. Cette inhibition pourrait être due au passage de motifs bactériens au travers de la barrière intestinale et qui seraient transportés jusqu'au foie par la veine porte hépatique. En effet, il a été démontré que des signaux pro-inflammatoires peuvent diminuer l'activité de LXR (Lakomy, Rebe et al. 2009). Cette hypothèse est d'autant plus probable que le DSS induit une augmentation de la perméabilité intestinale. Le T0901317 n'a pas d'effet sur l'inflammation au niveau du côlon mais il tend à limiter la perte de poids associée à la colite

ce qui indiquerait un effet bénéfique de LXR dans ce modèle. Le T0901317 permet de diminuer l'expression des gènes codant pour des protéines de la phase aigüe qui est augmenté chez les souris exposées au DSS, révélant ici aussi les effets bénéfiques de l'activation de LXR dans ce modèle de colite. Il est également intéressant de noter que le T0901317 induit une augmentation de l'expression des gènes de la lipogenèse chez les souris non exposées au DSS mais également chez les souris exposées. Cependant, les niveaux d'expression chez les souris exposées restent plus faibles que les souris du groupe contrôle, confortant ainsi l'hypothèse d'une inhibition de l'activité de LXR par la colite induite par le DSS.

L'activation de LXR par le T0901917 permet aussi de restaurer la perméabilité trans-cellulaire à un niveau basal et tend à réduire la perméabilité para-cellulaire induite par le DSS. L'implication de LXR dans la régulation de la perméabilité intestinale n'a, à ce jour jamais été étudiée, et il est envisagé d'étudier les mécanismes plus en détail.

Chapitre III

La nature des acides gras alimentaires exerce des effets sur l'expression des gènes, et en particulier sur l'expression hépatique des gènes de la lipogenèse. La lipogenèse est donc sensible à la nature des acides gras alimentaires. En effet, il a été montré qu'une déficience en acides gras essentiels dans l'alimentation conduit à une induction de la lipogenèse (Sekiya, Yahagi et al. 2003; Alwayn, Javid et al. 2004; Pachikian, Essaghir et al. 2011), alors que les acides gras polyinsaturés alimentaires la diminuent (Jump and Clarke 1999).

Il a été montré que les AGPIs des familles n-6 et n-3 inhibent l'expression (Ou, Tu et al. 2001) et la maturation post-traductionnelle (Hannah, Ou et al. 2001) de SREBP-1c. De même, les régimes riches en AGPIs réduisent l'expression et la maturation hépatique de ChREBP (Dentin, Benhamed et al. 2005). Ainsi, l'effet inhibiteur qu'exercent les AGPIs sur la lipogenèse *via* SREBP-1c et ChREBP est bien établi. L'implication de LXR dans l'effet des AGPIs est controversée.

Il a été proposé, sur la base d'études menées sur des cultures d'hépatocytes de rat ainsi que dans la lignée de cellules rénales humaines HEK-293, que les AGPIs sont des antagonistes compétitifs de LXRs, limitant ainsi l'activation de LXR et ses actions sur l'expression des gènes qu'il contrôle (Ou, Tu et al. 2001). Cette observation en culture cellulaire (Pawar, Xu et al. 2002; Pawar, Botolin et al. 2003) et *in vivo* (Pawar, Botolin et al. 2003; Takeuchi, Yahagi et al. 2010) est controversée et l'effet antagoniste des AGPIs vis-à-vis de l'activité de LXR reste discutée.

Comme nous l'avons vu précédemment, LXR joue un rôle dans la régulation transcriptionnelle de la synthèse *de novo* d'acides gras. Dans ce chapitre, nous avons voulu tester la possibilité que LXR puisse être le médiateur de la lipogenèse induite par une déficience alimentaire en acides gras essentiels des familles n-6 et n-3.

Research Article

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Essential fatty acids deficiency promotes lipogenic gene expression and hepatic steatosis through the liver X receptor

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Background & Aims: Nutrients influence non-alcoholic fatty liver disease. Essential fatty acids deficiency promotes various syndromes, including hepatic steatosis, through increased *de novo* lipogenesis. The mechanisms underlying such increased lipogenic response remain unidentified.

Methods: We used wild type mice and mice lacking Liver X Receptors to perform a nutrigenomic study that aimed at examining the role of these transcription factors.

Results: We showed that, in the absence of Liver X Receptors, essential fatty acids deficiency does not promote steatosis. Consistent with this, Liver X Receptors are required for the elevated expression of genes involved in lipogenesis in response to essential fatty acids deficiency.

Conclusions: This work identifies, for the first time, the central role of Liver X Receptors in steatosis induced by essential fatty acids deficiency.

Keywords: Lipogenesis; Cholesterol; LXR; Polyunsaturated fatty acids; Steatosis.
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Abbreviations: PUFA, polyunsaturated fatty acids; FA, fatty acid; EFAD, essential fatty acids deficiency; PPAR, peroxisome proliferator-activated receptor; LXR, liver X receptor; HNF4α, hepatic nuclear factor 4α; TR, thyroid receptor; SREBP-1c, sterol responsive element binding protein 1c; ChREBP, carbohydrate response element binding protein; NAFLD, non-alcoholic fatty liver disease; TG, triglyceride; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; WAT, white adipose tissue.

Introduction

Essential fatty acids (FAs) of the n-6 and n-3 series, also called poly-unsaturated fatty acids (PUFAs) are critical nutrients that must be provided through the diet in mammals. They are involved in the regulation of many biological functions. They are also precursors of various FA-based molecules that act as regulators of cellular processes [1–3]. Therefore, essential fatty acids deficiency (EFAD) leads to deregulation of several functions, such as immunity, growth, reproduction, and metabolism [4–6].

Changes in dietary habits have modified PUFA daily intake in humans. The reduction of n-6 and more importantly n-3 FA intake over the last decades is considered to be a contributing factor to the current obesity epidemic [7]. Different mechanisms by which dietary PUFAs influence obesity have been evidenced [8]. One of them relies on the ability of PUFAs to influence the expression of genes involved in metabolic processes. PUFAs have been shown to regulate numerous transcription factors such as peroxisome proliferator activated receptors [9], liver X receptors (LXRs) [10], hepatic nuclear factor 4α [11], thyroid receptor [12], sterol regulatory element binding protein (SREBP) [10,13] and carbohydrate responsive element binding protein (ChREBP) [14] that are involved in controlling metabolism.

PUFAs have been shown to influence hepatic lipid metabolism. A high PUFA consumption prevents the development of steatosis [8,15], the first step of non-alcoholic fatty liver disease (NAFLD). Conversely, PUFA deficiency leads to NAFLD [8,16]. Recently, it was shown that n-3 PUFA deficiency is sufficient to induce NAFLD [17]. NAFLD itself is not considered a threat, but it can develop into further liver damages that may become irreversible [18]. Since the occurrence of NAFLD is highly correlated with obesity and its related metabolic syndrome, understanding the mechanisms by which nutritional signals influence the early

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Research Article

stages of the disease has become a major issue. NAFLD may occur as a consequence of increased FA synthesis and uptake or impaired secretion and degradation. The mechanisms by which high PUFA consumption participates in preventing steatosis involve various pathways. On the one hand, high PUFA consumption stimulates the expression of genes involved in FA catabolism, while repressing the expression of FA synthesis [15]. On the other hand, dietary PUFA deficiency is well-known to influence the expression of genes involved in FA synthesis to such an extent that it promotes hepatic steatosis [8]. Interestingly, little is known about the mechanisms responsible *in vivo* for increased *de novo* lipogenesis upon EFAD. It is known that SREBP1-c [19], ChREBP [20], and LXR [21] regulate the expression of genes involved in FA biosynthesis. In addition, the activity of these three distinct transcription factors is decreased by dietary PUFAs [8,10,14]. PUFAs inhibit both the expression and post-translational maturation of SREBP1-c [10,13]. In addition, the overexpression of SREBP1-c prevents the beneficial effects of dietary PUFAs on steatosis [8]. PUFAs also prevent the nuclear translocation and activity of ChREBP [14]. Finally, PUFAs antagonize the ligand-mediated activation of LXR and induce a trans-repressive effect on its activity [10]. Since LXR not only directly drives the expression of genes involved in FA biosynthesis but also regulates the expression of *Srebp1-c* [22] and *Chrebp* [23], it plays direct and indirect roles in the transcriptional control of lipogenesis.

In this study, we investigated the contribution of LXR to the development of steatosis induced by essential PUFA deficiency. We used a nutrigenomic approach in wild type mice and in transgenic mice lacking the two LXR isoforms. Altogether, the data presented in this study provide the first *in vivo* evidence for the critical role of LXR in the upregulation of lipogenic gene expression upon EFAD.

Materials and methods

Animals and diets

Details are given in Supplementary methods. The composition of the diets and oils are given in Supplementary Tables 1 and 2, respectively.

Blood and organ sampling

As described elsewhere [24].

Gene expression studies

RNA was extracted, reversed transcribed, analyzed as described elsewhere [24]. Primers are listed in Supplementary Table 3.

Immunoblot analysis

Cytoplasmic and nuclear proteins were determined as described elsewhere [24].

Biochemical assays

Lipid content and FA composition were determined as described elsewhere [24]. Plasma analyses were performed as described in Supplementary methods.

Histology

As described in Supplementary methods.

Statistical analysis

Data are mean ± SEM. Differential effects were analyzed by Anova followed by Student's *t*-tests. A p-value $\leqslant 0.05$ was considered significant.

Results

Dietary FAs influence hepatic triglyceride and cholesterol

We first assessed the effect of the different diets by measuring the relative abundance of hepatic FA (Supplementary Table 4). Major qualitative changes occurred as a consequence of specific diet consumption. We used the 7 following diets: COCO (essential FA deficient, 5% oil), High Fat (HF) COCO (essential FA deficient, 20% oil), REF (balanced diet providing PUFAs, 5% oil) HF REF (balanced diet providing PUFAs, 20% oil), FISH (n-3 PUFA-enriched diet, 5% oil), HF FISH (n-3 PUFA-enriched diet, 20% oil), and a fat free diet (FF). As expected, a significant amount of Mead acid (C20:3 n-9) was measured in the liver of mice fed essential FA deficient diets (FF, COCO, and HF COCO) but not in mice fed PUFA containing diets (REF, HF REF, FISH, HF FISH). We also observed an enrichment in the relative abundance of long-chain PUFAs, such as eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), in mice fed FISH or HF FISH compared with REF or HF REF, respectively. Finally, in mice fed essential FA deficient diets (Fat Free, COCO, HF COCO) a marked reduction in long-chain PUFAs was observed. These changes tended to occur both in male and female mice.

Next, we measured hepatic lipid content (Fig. 1A). We observed that hepatic free cholesterol was unchanged by dietary FA quantity or quality. Essential FA deficient diets led to higher levels of hepatic TGs compared with REF and HF REF diets while FISH diets tended to reduce hepatic TGs. Despite higher levels of hepatic TGs in females than in males, we observed similar trends in both genders. Interestingly, whatever the diet, we did not observe any marked effect of the quantity of dietary fat on TG levels. Parallel to TG levels, essential FA deficient diets induced an increase in cholesterol esters when compared to other diets. We also performed neutral lipid staining on liver sections (Fig. 1B) that confirmed the biochemical assays. Both male and female mice are susceptible to changes in hepatic lipid storage in response to dietary FA. Essential FA deficient diets increase neutral lipid storage while this is prevented by dietary PUFAs. Interestingly, our data emphasize that dietary FA quality rather than quantity determines the severity of hepatic steatosis in both male and female mice.

Role of LXR in steatosis induced by essential FA deficiency

In order to investigate the role of LXR in the effects of dietary FA on hepatic triglycerides, we performed a similar nutritional experiment in wild type mice and in transgenic mice lacking both LXR isoforms. We chose to use mice lacking both LXRα [25] and LXRβ [26] since both isoforms have been shown to be involved in liver steatosis. In addition, our data obtained through pharmacological challenges of LXR with T0901317, a potent LXR agonist [27], show that both LXR isoforms influence the expression of lipogenic enzymes (Supplementary Fig. 1). Since 5% of the total dietary fat was sufficient to address the effect of dietary essential FA on hepatic lipids, we used the three following diets: COCO, REF,

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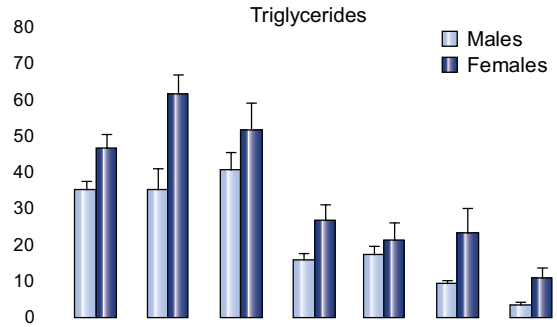
Triglycerides
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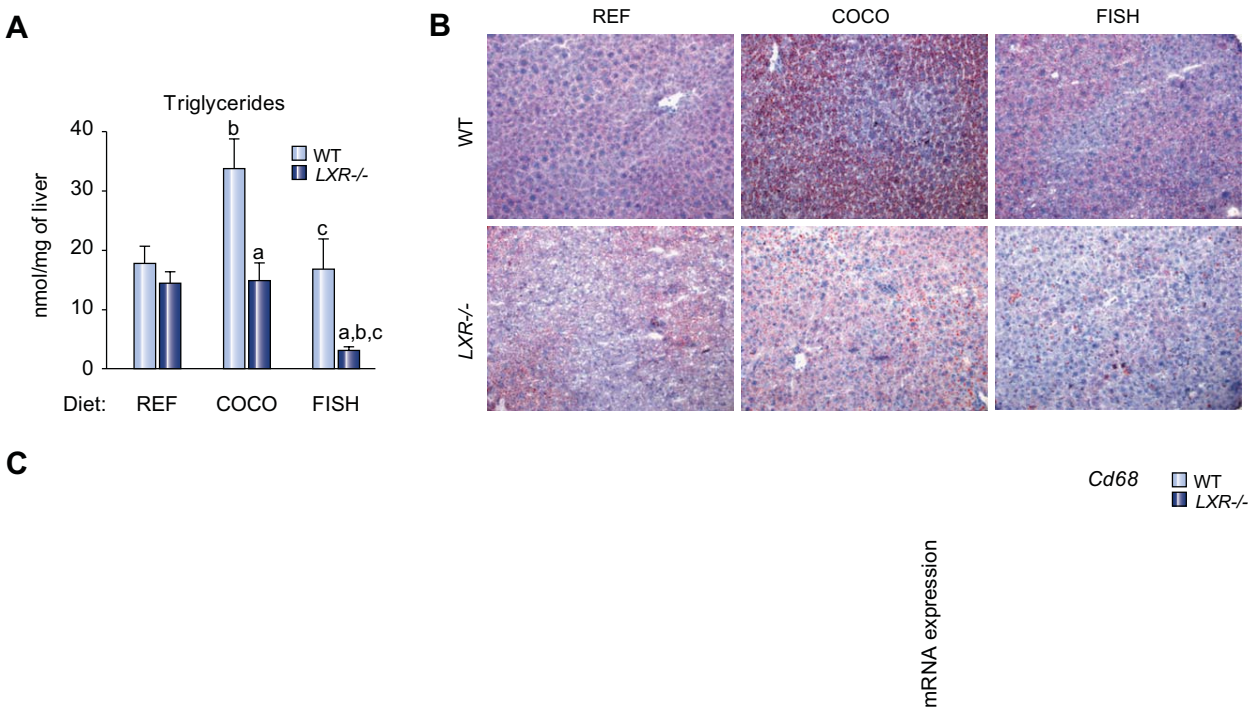


and FISH. We first measured hepatic FA to assess the impact of the different diets on hepatic FA profiles (Supplementary Table 5). As expected, the COCO diet led to significant accumulation of EFAD marker (C20:3n-9) and mono-unsaturated FA (such as oleic acid: C18:1n-9) while reducing PUFAs when compared to mice fed the REF diet. The FISH diet led to an increase in long-chain PUFAs when compared to the REF diet. Most observed changes were similar in both genotypes.

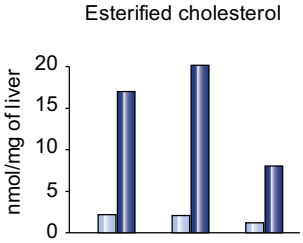
Moreover, neither the genotype nor the diet did significantly influence body weight, plasma insulin, and glucose concentration (Table 1). Wild type mice fed the COCO diet displayed a significantly higher liver mass, and a lower perigonadic WAT weight compared to the other groups. The COCO diet induced a decreased plasmatic triglycerides concentration in both genotypes (Table 1).

Hepatic triglycerides were significantly increased in wild type mice fed the COCO diet compared to wild type mice fed the REF diet. In wild type mice, the FISH diet had no effect on TG content compared to the REF group. LXR deficiency prevented the COCO diet-induced TG accumulation, and led to a decreased TG content in the FISH diet compared to the REF diet (Fig. 2A). Histological examination of neutral lipid staining was consistent with an LXR-dependent increase in hepatic TG content in response to EFAD (Fig. 2B). Moreover, the FISH diet cleared neutral lipid accumulation in LXR deficient mice but not in wild type mice.

Due to the important anti-inflammatory role of LXRs, we measured the expression and circulating levels of cytokines. The hepatic expression of *Il1b* was significantly increased in wild type mice fed the COCO diet, without any significant increase in *Tnfa*, *F4/80*, and *Cd68* (Fig. 2C). However, their expression was much more sensitive in LXR-deficient mice. Interestingly, they were found to be particularly elevated in LXR-deficient mice fed the COCO diet. Finally, amongst the ten cytokines we tested, no detectable circulating level was found with the exception of MCP1, whose level was significantly elevated in response to COCO in wild type mice (Supplementary Table 6).

LXR influences the regulation of gene expression in response to dietary FAs

To investigate the role of LXRs, we next measured by qPCR the expression of 142 genes whose function is either related to LXR primary functions in the liver (FA and cholesterol metabolism) or to other nuclear receptor signalings. Individual gene expression results are provided as Supplementary materials (Supplementary Table 7). Global data are first introduced as a heatmap coupled with hierarchical clustering (Supplementary Fig. 2). While some of the tested genes were neither regulated by LXRs nor by the diet, this heatmap highlights two main results. LXR are not critical for all the transcriptional regulations in response to dietary FA since, in some cases, similar changes occur in both wild type mice and transgenic mice. However, LXR deficiency influences the response to dietary FA, since major changes in the transcriptional profile appear to depend on the presence of the receptors.



Dietary PUFAs reduce hepatic and plasmatic cholesterol

Our hierarchical clustering reveals a cluster of genes involved in cholesterologenesis (Supplementary Fig. 2, cluster 1). We observed that all these genes involved in cholesterol biosynthesis were sensitive to dietary FA in both genotypes (Supplementary Fig. 3). The expression of these genes was higher in mice fed the COCO diet in both genotypes. It was lowered in LXR-deficient mice fed the FISH diet. To investigate the consequences of the transcriptional changes in cholesterol biosynthesis, we measured hepatic sterols (Fig. 3A). While free cholesterol was more abundant in mice lacking LXR than in wild type mice, we did not observe any effect of the diets on free cholesterol. Hepatic cholesterol esters, plasma cholesterol, and lathosterol also tended to be elevated in mice lacking LXRs. In contrast to what we previously observed (Fig. 1), no increase of cholesterol esters was detected in response to COCO. This could possibly be due to the mixed C57BL6/129SVJ genetic background that is different from the C57Bl6 used in the previous experiment.

However, the FISH diet induced a decrease in hepatic cholesterol esters, plasma cholesterol, and lathosterol in both genotypes. Consistent with an important role of FISH oils on cholesterol metabolism, the FISH diet tended to reduce the differences in gene expression linked to LXR deficiency (Fig. 3B). Interestingly, we found that the LXR-independent effects of the FISH diet not only influence the expression of genes involved in cholesterol synthesis (*Hmgcr*, *Pmvk*, *Fdps*, *Nsdhl*) but also in the mevalonate shunt of cholesterogenesis (*Sqle*, *Lss*) (Fig. 3B). These data suggest that dietary FAs may influence SREBP-2 activity [28]. In agreement with this possibility, two SREBP-2 targets involved in cholesterol uptake such as the *Ldl-r*, and *Pcsk9* (Fig. 3B) show the same pattern of expression.

LXR is required for the induction of genes involved in de novo lipogenesis in response to essential FA deficiency

The heatmap also highlighted a cluster of genes upregulated in wild type mice fed the COCO diet compared to the REF group, whose expression remained unchanged in *LXR−/−* mice (Supplementary Fig. 2 cluster 2). This cluster contains critical genes involved in *de novo* lipogenesis such as *Me*, *Acly*, *Acaca*, *Fas*, *Elovl6*, and *Scd1* (Fig. 4A). We also noticed that genes regulating synthesis of diacylglycerol, such as *Agpat1*, *Gpat1*, and *Lipin1*, were also upregulated in an LXR-dependent manner in mice fed the essential FA deficient diet (COCO, Supplementary Fig. 2 and Supplementary Table 7). The FISH diet had no significant effect or reduced the expression of lipogenic genes in both genotypes. In addition, *Spot 14*, and *Pnpla3*, whose expression is associated with lipogenesis [29,30], showed similar LXR-dependent induction in response to EFAD.

Proteins levels were consistent with mRNA changes (Fig. 4B and Supplementary Fig. 4). LXR regulates the expression of lipogenic genes directly and through two nuclear factors: SREBP1c [22] and ChREBP [23]. To determine if these two nuclear factors

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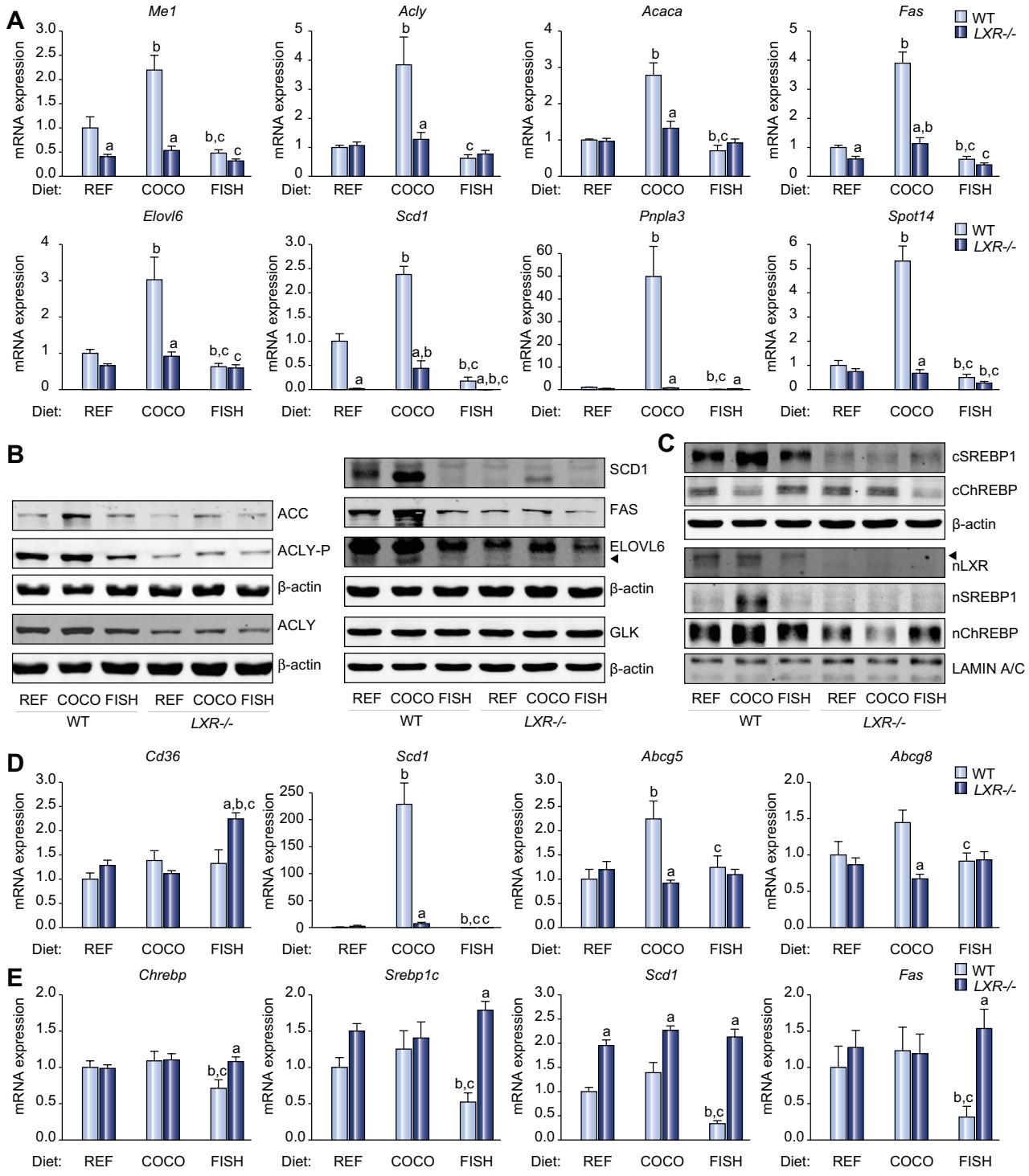

Fig. 4. LXR-dependent response to dietary FAs. (A) *Me1*, *Acly*, *Acaca*, *Fas*, *Elovl6*, *Scd1*, *Pnpla3*, and *Spot14* hepatic mRNA levels. (B) Hepatic proteins were probed using specific antibodies. A representative image is shown for each protein. (C) Cytoplasmic and nuclear proteins were normalised with β-actin and LAMIN A/C, respectively. (D) *Cd36*, *Scd1*, *Abcg5*, and *Abcg8* mRNA levels in jejunum. (E) *Chrebp*, *Srebp1c*, *Scd1*, and *Fas* mRNA levels in WAT. Values are mean ± SEM (n = 6). [a]Significant genotype effect. [b,c]Significant difference compared to REF and COCO, respectively.

might be involved downstream of LXR in EFAD, we measured both cytosolic and transcriptionally active nuclear fractions of these two proteins. Cytosolic forms of SREBP1c and ChREBP were not significantly influenced by the diet. Nuclear forms of SREBP1c

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and ChREBP were upregulated in the liver of wild type mice fed the COCO diet compared to wild type mice fed the REF diet. Such regulation did not occur in the liver of *LXR* knockout mice.

Interestingly, in mice fed diets containing either low or high PUFAs, the same differences in the expression of critical lipogenic genes were observed since the expression of lipogenic genes was higher in the absence of PUFA (Supplementary Fig. 5). In addition, treatment with T0901317 induced an additive LXR-dependent increase in lipogenic gene expression (Supplementary Fig. 5). Therefore, major changes in dietary FA intake influence the LXR-dependent expression of lipogenic enzymes when both LXRs are activated by T0901317.

We also tested whether dietary FAs may influence LXR-dependent expression of intestinal genes involved in FA uptake (*Cd36*), FA metabolism (*Scd1*) and cholesterol transport (*Abcg5*, *Abcg8*). The expression of *Scd1*, *Abcg5*, and *Abcg8* appeared sensitive to dietary FAs (Fig. 4D) since it tended to increase in response to COCO diet. This increase only occurred in wild type mice. The expression of lipogenic genes was also measured in white adipose tissue. In response to FISH, lipogenic genes such as *Chrebp*, *Srebp1-c*, *Scd1*, and *Fas* were downregulated in wild type mice but not in LXR-deficient mice (Fig. 4E).

Discussion

NAFLD has become a major public health issue because steatosis, the first step of this disease, can lead to further liver damage such as non-alcoholic steatohepatitis (NASH), cirrhosis, and cancer. Therefore, it is important to understand the dietary factors that influence the disease.

In this work, we highlighted that the quality and not the quantity of dietary FA strongly influences triglyceride and cholesterol esters accumulation in the liver of both male and female mice. Higher triglyceride accumulation was observed in mice fed fat free diets and essential FA deficient diets, emphasizing the role of essential dietary PUFAs in the prevention of steatosis. These results reveal that the severity of steatosis does not correlate with the total amount of dietary fat. In search for dietary models of experimental steatosis, the lack of FA (fat free diets) or the lack of dietary essential FA is a critical point to be considered, regardless of the total amount of dietary fat that is chosen. In addition, both males and females show similar changes in triglyceride accumulation in response to the diets.

Because liver X receptors are involved in the metabolism of FA and cholesterol in the liver, they are considered important players in hepatic lipid homeostasis. Interestingly, these nuclear receptors not only influence lipid metabolism, but also inflammation [31] and cholesterol homeostasis [21,32] that can strongly impact the progression of NAFLD to NASH [18,33].

The possibility that LXRs may be regulated by FA has been suggested through various studies [34,35]. However, work performed in cell cultures showed contradictory findings [36]. Moreover, many nuclear receptors such as PPARs, TR, HNF4α, RXR or transcription factors, such as SREBP-1c and ChREBP, are regulated by FA [9,10,12–14,37]. For the first time *in vivo*, the current study addresses the role of LXR in the response to dietary FAs. To address this issue, we used wild type mice and mice lacking both LXR isoforms. Indeed, while LXRα is an important regulator of hepatic lipogenesis [25], LXRβ might also be involved [26]. Consistent with this, we found that a pharmacological treatment with an LXR agonist results in an increase in the expression of lipogenic genes that is almost abolished in *LXRα−/−* mice and significantly reduced in *LXRβ−/−* mice. In the absence of both isoforms, the response of the lipogenic genes we tested was abolished (Supplementary Fig. 1). Our data are consistent with a major role of LXRα in the regulation of lipogenic gene expression [25]. We also provide pharmacological evidence that LXRβ may also influence hepatic lipogenesis.

To address the possible role of LXR in the regulation of lipogenesis in response to dietary FA, we used mice lacking both LXR isoforms. When challenged with different isocaloric diets, LXR-deficient and wild type mice display major changes in the hepatic FA composition. However, while triglyceride levels were elevated in the absence of dietary PUFAs in wild type mice, they were unchanged in LXR-deficient mice. Therefore, LXR deficiency protects from steatosis induced by the lack of dietary essential PUFAs. Interestingly, in the absence of LXR, the high PUFA diet results in lower TG and cholesterol accumulation in the liver, providing evidence for hypolipidemic effect of n-3 PUFAs that are also LXR independent. However, the steatosis that is induced by dietary EFAD is LXR-dependent.

By measuring the expression of 142 genes involved either in lipid metabolism or in nuclear receptor signalling, we were able to further evaluate the role of LXRs. First, we identified that LXRs are central for FA mediated regulation of gene expression as their absence markedly influenced gene expression in response to dietary FA. We identified *de novo* lipogenesis as a primary target. Most genes involved in *de novo* FA synthesis appeared to be upregulated in the absence of PUFAs. This upregulation requires the presence of LXRs. In addition, consistent with previous findings [38], we found that LXRs are required for the expression of SREBP1c but not ChREBP. Moreover, while SREBP1c nuclear translocation was markedly impacted by PUFA deficiency, the effect on ChREBP was more modest. In addition, the translocation of these two transcription factors in the nucleus, reflecting their activation level, was prevented in *LXR−/−* mice. Finally, we found that dietary PUFA deficiency also strongly influences cholesterogenesis in both wild type and *LXR−/−* mice. This work also provides evidence for FA sensitive pathways independent of LXRs that control cholesterol homeostasis in the liver. Interestingly, cholesterogenic genes show similar expression patterns to inflammatory markers, i.e., increased in response to EFAD, particularly in mice lacking LXR (Figs. 2C, 3B, Supplementary Fig. 2). Lastly, while the PUFA-rich diet represses LXR-sensitive lipogenic pathway, as well as cholesterogenic enzymes, it does not promote local or systemic inflammation (Fig. 2C). Therefore, despite the important anti-inflammatory role of LXRs, it seems possible to target steatosis through the PUFA-sensitive regulation of lipogenesis without promoting liver inflammation.

In summary, this work shows that LXRs play a central role in the upregulation of genes involved in *de novo* lipogenesis in response to dietary fat. While EFAD has long been known to promote lipogenesis and steatosis, our data show for the first time the requirement of LXRs for such regulation (Supplementary Fig. 6). Together with their established role in cholesterol homeostasis and immunity, our current study reveals that LXRs could also be a target for the prevention and treatment of NAFLD and/or NASH. Finally, our data further underline the importance of maintaining chronic dietary FA balance for hepatic metabolism.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2013.01.006.

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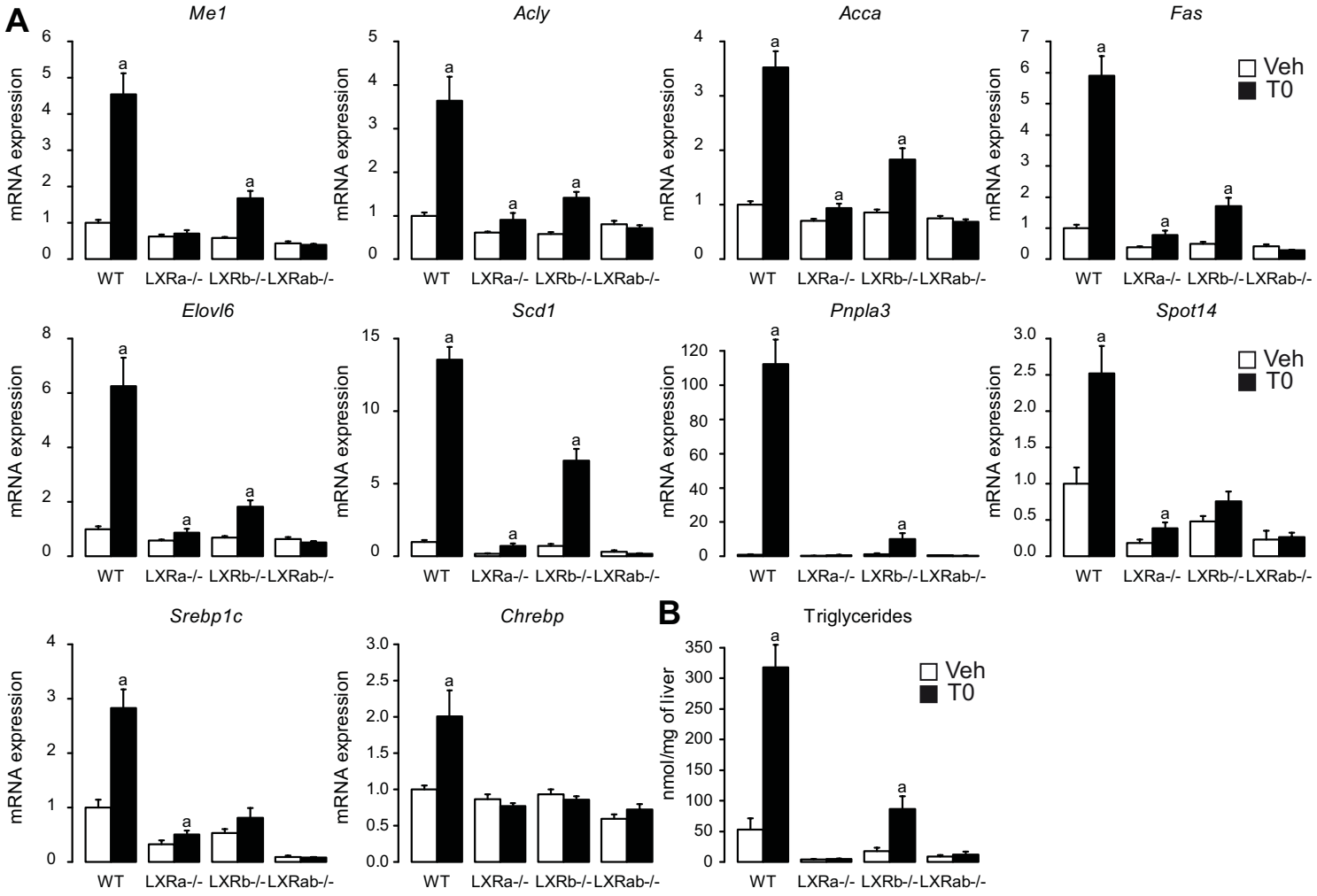
Figure S5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Acca
Fas
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Scd1
Pnpla3
Spot14
Srebp1c
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LXRab-/-
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Triglycerides
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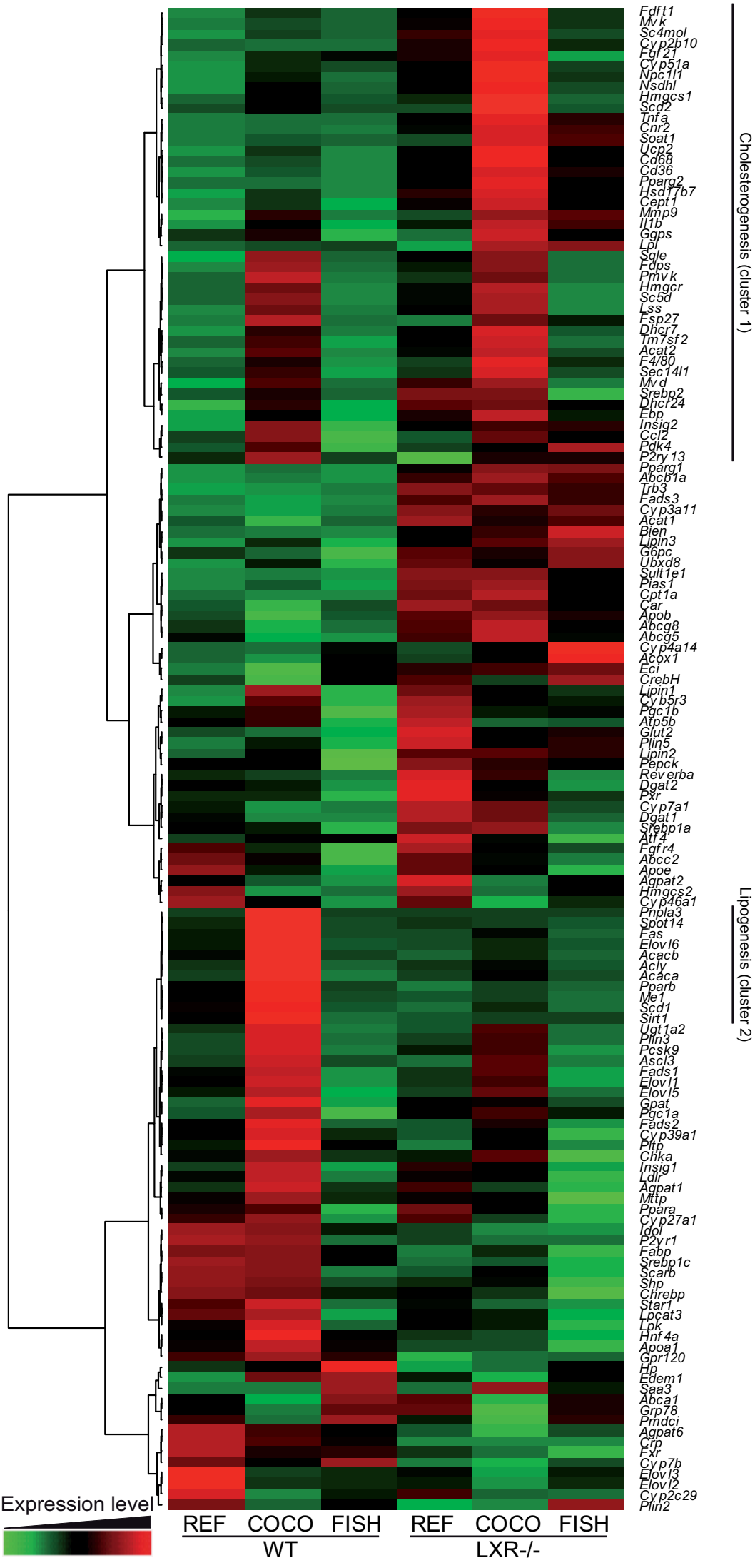
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Soat1
Ucp2
Cd68
Cd36
Pparg2
Hsd17b7
Cept1
Mmp9
Il1b
Ggps
Lpl
Sqle
Fdps
Pmvk
Hmgcr
Sc5d
Lss
Fsp27
Dhcr7
Tm7sf2
Acat2
F4/80
Sec14l1
Mvd
Srebp2
Dhcr24
Ebp
Insig2
Ccl2
Pdk4
P2ry13
Pparg1
Abcb1a
Trb3
Fads3
Cyp3a11
Acat1
Bien
Lipin3
G6pc
Ubxd8
Sult1e1
Pias1
Cpt1a
Car
Apob
Abcg8
Abcg5
Cyp4a14
Acox1
Eci
CrebH
Lipin1
Cyb5r3
Pgc1b
Atp5b
Glut2
Plin5
Lipin2
Pepck
Reverba
Dgat2
Pxr
Cyp7a1
Dgat1
Srebp1a
Atf4
Fgfr4
Abcc2
Apoe
Agpat2
Hmgcs2
Cyp46a1
Pnpla3
Spot14
Fas
Elovl6
Acacb
Acly
Acaca
Pparb
Me1
Scd1
Sirt1
Ugt1a2
Plin3
Pcsk9
Ascl3
Fads1
Elovl1
Elovl5
Gpat
Pgc1a
Fads2
Cyp39a1
Pltp
Chka
Insig1
Ldlr
Agpat1
Mttp
Ppara
Cyp27a1
Idol
P2yr1
Fabp
Srebp1c
Scarb
Shp
Chrebp
Star1
Lpcat3
Lpk
Hnf4a
Apoa1
Gpr120
Hp
Edem1
Saa3
Abca1
Grp78
Pmdci
Agpat6
Crp
Fxr
Cyp7b
Elovl3
Elovl2
Cyp2c29
Plin2
Cholesterogenesis (cluster 1)
Lipogenesis (cluster 2)
Expression level
REF COCO FISH REF COCO FISH
WT
LXR-/-

Figure S10

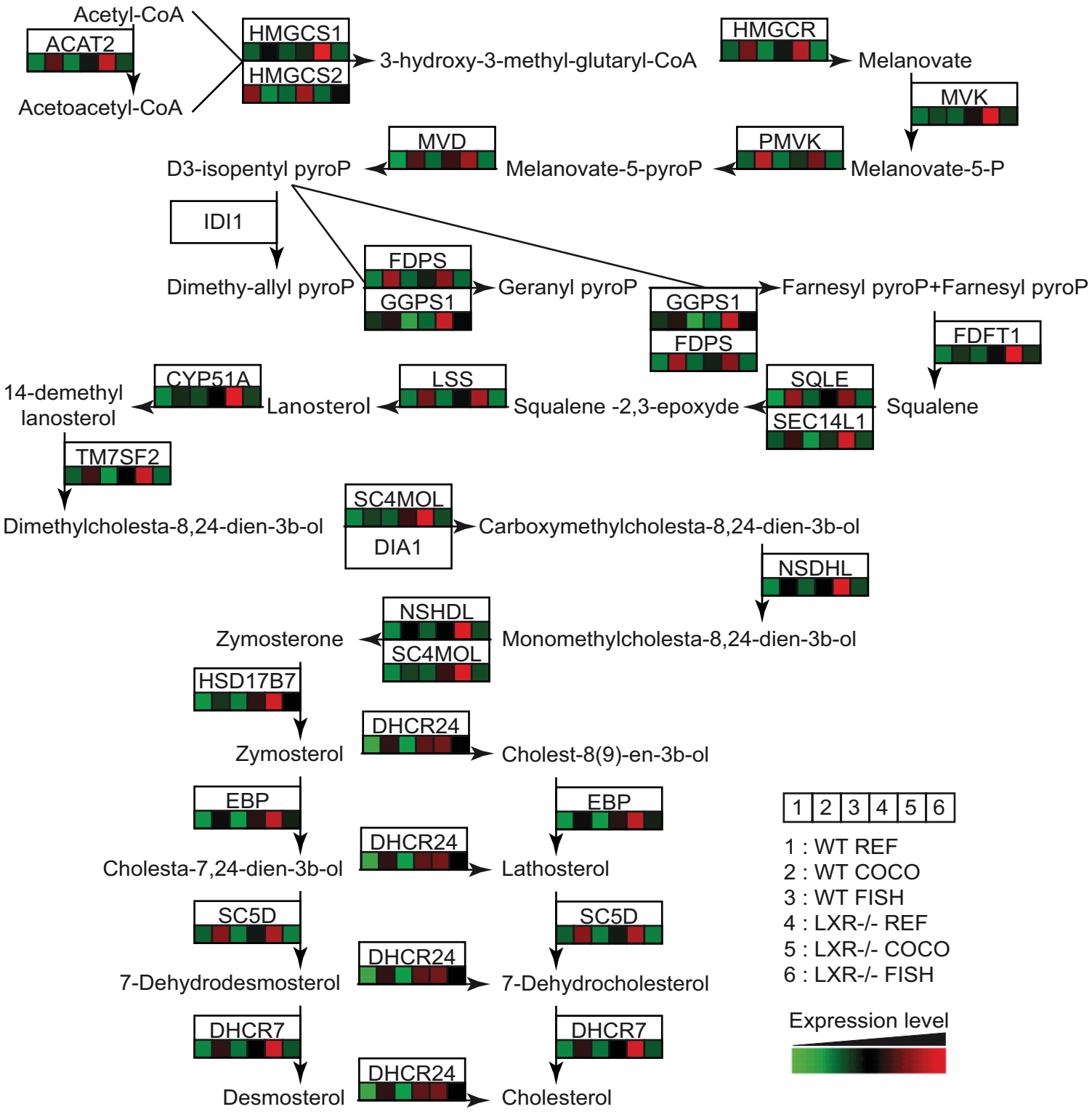
Acetyl-CoA
ACAT2
Acetoacetyl-CoA
HMGCS1
HMGCS2
3-hydroxy-3-methyl-glutaryl-CoA
HMGCR
Melanovate
MVK
Melanovate-5-P
PMVK
Melanovate-5-pyroP
MVD
D3-isopentyl pyroP
IDI1
Dimethy-allyl pyroP
FDPS
GGPS1
Geranyl pyroP
GGPS1
FDPS
Farnesyl pyroP+Farnesyl pyroP
FDFT1
Squalene
SQLE
SEC14L1
Squalene -2,3-epoxyde
LSS
Lanosterol
CYP51A
14-demethyl lanosterol
TM7SF2
Dimethylcholesta-8,24-dien-3b-ol
SC4MOL
DIA1
Carboxymethylcholesta-8,24-dien-3b-ol
NSDHL
Monomethylcholesta-8,24-dien-3b-ol
NSHDL
SC4MOL
Zymosterone
HSD17B7
Zymosterol
DHCR24
Cholest-8(9)-en-3b-ol
EBP
Cholesta-7,24-dien-3b-ol
DHCR24
EBP
Lathosterol
SC5D
7-Dehydrodesmosterol
DHCR24
SC5D
7-Dehydrocholesterol
DHCR7
Desmosterol
DHCR24
DHCR7
Cholesterol
1 2 3 4 5 6
1 : WT REF
2 : WT COCO
3 : WT FISH
4 : LXR-/- REF
5 : LXR-/- COCO
6 : LXR-/- FISH
Expression level

Figure S11

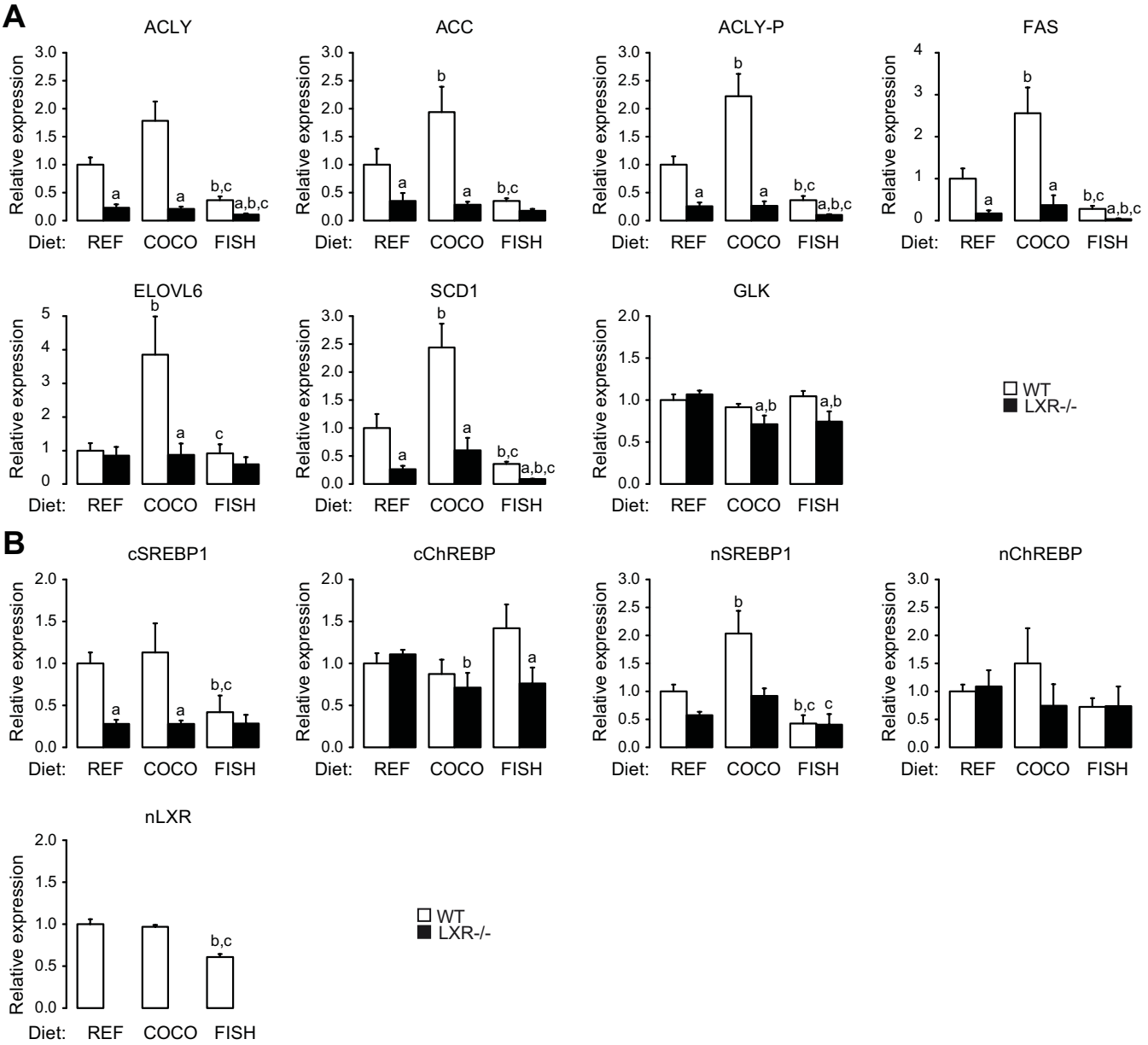
A
ACLY
ACC
ACLY-P
FAS
ELOVL6
SCD1
GLK
Relative expression
Diet: REF COCO FISH
WT
LXR-/-
B
cSREBP1
cChREBP
nSREBP1
nChREBP
nLXR

Figure S12

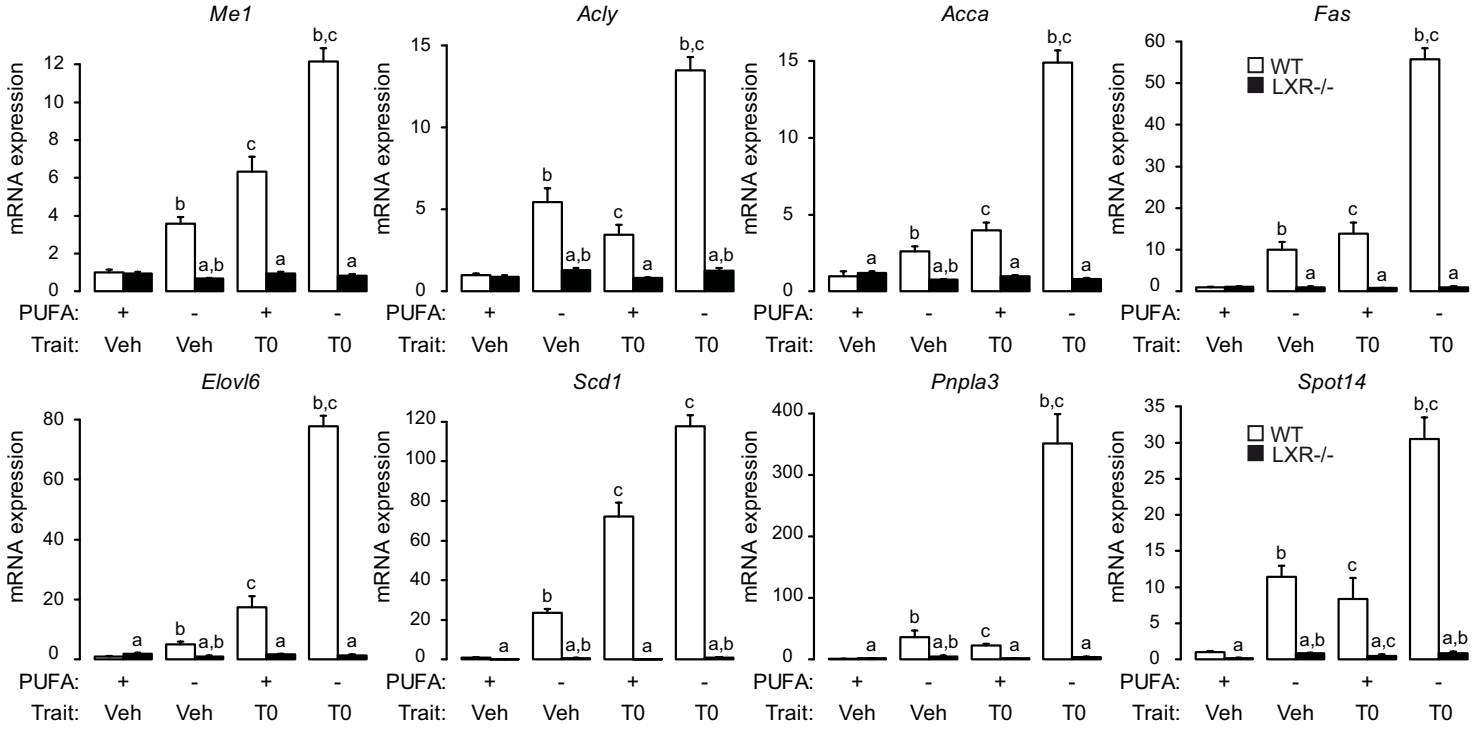
Me1
Acly
Acca
Fas
Elovl6
Scd1
Pnpla3
Spot14
mRNA expression
WT
LXR-/-
PUFA:
Trait:
Veh
T0

Figure S13

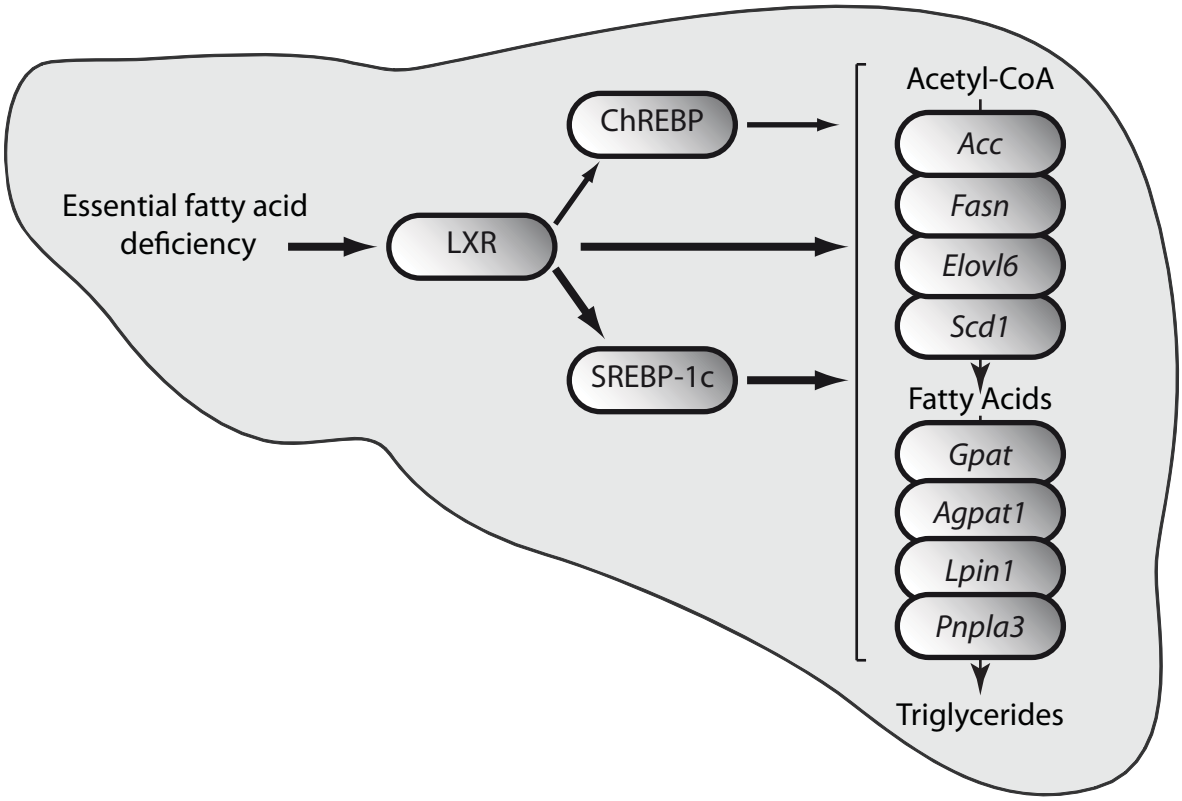
Acetyl-CoA
ChREBP
Acc
Fasn
Elovl6
Scd1
Essential fatty acid deficiency
LXR
SREBP-1c
Fatty Acids
Gpat
Agpat1
Lpin1
Pnpla3
Triglycerides

Table S1 : Diets composition

% (w/w)	Fat free diet	Low fat diets	High fat diets
Cellulose	2,0	2,0	2,0
Casein	22,0	22,0	22,0
Starch	46,2	43,6	33,6
Methionin	0,2	0,2	0,2
Sucrose	23,1	21,8	16,8
Minerals	4,5	4,5	4,5
Vitamins	1,0	1,0	1,0
Oil	0,0	5,0	20,0
Agar-Agar	1,0	0,0	0,0

Table S2 : Relative fatty acid composition of dietary oils

Diet	REF	COCO	FISH
12:00	0,0	47,7	0,0
14:00	0,0	23,0	0,0
16:00	5,9	13,1	4,4
18:00	2,8	15,3	2,1
18:1w9	39,7	0,9	30,5
18:2w6	47,0	0,0	34,7
18:3w3	4,6	0,0	3,4
20:5w3	0,0	0,0	5,3
22:6w3	0,0	0,0	19,5

Table S3: Oligonucleotide sequences for real time PCR

Gene	NCBI Refseq	Forward primer (5'-3')	Reverse primer (5'-3')
Abca1	NM_013454	GCGCTACAACATGGACATCCT	GCTGGGTCGGGAGATGAGA
Abcb1a	NM_011076	CATGACAGATAGCTTTGCAAGTGTAG	GGCAAACATGGCTCTTTTATCG
Abcc2	NM_013806	AAGCAGATTGACACCAACCAGAA	GGCCGAGCAGAAGACAATCA
Abcg5	NM_031884	TCGCCACGGTCATTTTCA	GCCAAAAGAGCAGCAGAGAAATA
Abcg8	NM_026180	ATCCATTGGCCACCCTTGT	GCGTCTGTCGATGCTGGTC
Acaca	NM_133360	TTACAGGATGGTTTGGCCTTTC	CAAATTCTGCTGGAGAAGCCAC
Acacb	NM_133904	CCTGAATCTCACGCGCCTA	CAGATGGAGTCCAGACATGCTG
Acat1	NM_144784	ATTACTCCCATCACCATCTCAGTG	CACGGTCTTGAGCTTTGGC
Acat2	NM_009338	GTTATGGGAGTAGGACCAATTCCAG	TCCAAGTTCTTTAGCTATTGCCG
Acly	NM_134037	AAAGCTTGGCCTCGTCGG	GGGACGAAGGGTTCAATGAGA
Acox1	NM_015729	CAGACCCTGAAGAAATCATGTGG	CAGGAACATGCCCAAGTGAAG
Agpat1	NM_018862	CATGAGGCTAGAAGGGAAGACG	CCGGGCCACAGCTCC
Agpat2	NM_026212	CGCACCGTGGATAACATGAG	CTCTGGTGATTAGAGATGATGACACA
Agpat6	NM_018743	AGCTTTGAAATTGGAGCCACTG	CAATGGCCCAACTGGTCATC
Apoa1	NM_009692	TGGGCCAACAGCTGAACC	TCCCAGAAGTCCCGAGTCAA
Apob	NM_009693	AATCTGTGGTTTCATCATGAGGAC	GGCCAGCTTGAGTTCGTACCT
Apoe	NM_009696	TTGGTCACATTGCTGACAGGAT	GAGTGGCAAAGCAACCAACC
Ascl3	NM_001033606	TGGACTGAATGAGACAGAGGTGA	CAGACGTGGGACCAAAGAGACTA
Atf4	NM_009716	ATGGGTTCTCCAGCGACAAG	CCGGAAAAGGCATCCTCC
Atp5b	NM_016774	AAGGATCACCACCACCAAGAAG	CAAATGGGCAAAGGTGGTTG
Bien	NM_023737	CGTCTCCTCGGTTGGTGTTC	ATTATCTTCTTTGCAGTATCTAGCTGCTT
Car	NM_009803	GCTGCAAGGGCTTCTTCAGA	CCTTCCAGCAAACGGACAGA
Ccl2	NM_011333	GGTGTCCCAAAGAAGCTGTAGTTTT	AGTTGTAGGTTCTGATCTCATTTGGTT
Cd36	NM_007643	GTTAAACAAAGAGGTCCTTACACATACAG	CAGTGAAGGCTCAAAGATGGC
Cd68	NM_009853	CTTCCCACAGGCAGCACAG	TGTAGCCTTAGAGAGAGCAGGTCA
Cept1	NM_133869	GAAACAGGCCAGAAGAACTAACAGTA	GCAATACAAGTTCCAAGAACCACA
Chka	NM_013490	AGGCCTTCGCGAGGACC	CAACGCTGGCTATGGAGTCTG
Chrebp	NM_021455	ACTCAGGGAATACACGCCTACAG	GAAGAAGGAATTCAGAGCTCAGAAA
Cnr2	NM_009924	CAAGGCTCCACAAGACCCTG	GTTGGTCACTTCTGTCTCCCG
Cpt1a	NM_013495	GAAGAAGAAGTTCATCCGATTCAAG	GATATCACACCCACCACCACG
Crebh	NM_145365	CATTGATGGTCTGGAGAACCG	TTGCTGGTTGACTGGACCAC
Crp	NM_007768	TCTCGGACTTTTGGTCATGAAGA	TGTAGAAATGGAGACACACAGTAAAGGT
Cyb5r3	NM_029787	GAGTAGCGGGTGTAGAACCGTG	TCATGAAGAGACTGTAGATGAACCAGA
Cyp27a1	NM_024264	CTGCACTTGCCCGACCTC	CACTAGCCAGATTCACATTGGTGT
Cyp2b10	NM_009999	TTTCTGCCCTTCTCAACAGGAA	ATGGACGTGAAGAAAAGGAACAAC
Cyp2c29	NM_007815	GCTCAAAGCCTACTGTCA	CATGAGTGTAAATCGTCTCA
Cyp39a1	NM_018887	ATGCAGTCTTCTGGACCCATTAC	CAAGCTCTTTGTTTATATTCAATCCG
Cyp3a11	NM_007818	TCACACACACAGTTGTAGGCAGAA	GTTTACGAGTCCCATATCGGTAGAG
Cyp46a1	NM_010010	AGTATGGTCCTGTTGTAAGAGTCAATGT	ACATCTTGGAGTCCTTGTTGTACTTG
Cyp4a14	NM_007822	TCAGTCTATTTCTGGTGCTGTTC	GAGCTCCTTGTCCTTCAGATGGT
Cyp51a	NM_020010	TGGCTGCCTCTGCCAAGT	GGTTCTTTTGACAGCCTGCG
Cyp7a1	NM_007824	AGAGCAACTAAACAACCTGCCAGTA	GCACTGGAGAGCCGCAGA
Cyp7b1	NM_007825	ACATGGTGACACTTTCACTGTCTTC	GAACTTCTGAAAGCTTAATTGTTTTGG

Dgat1	NM_010046	GGACCAGCGTGGGCG	AAGAATCTTGCAGACGATGGC
Dgat2	NM_026384	ACAGCTGCAGGTCATCTCAGTACTA	AGCACAGCTATCAGCCAGCA
Dhcr24	NM_053272	ATATCTACTACTACGTGCGCGCC	CTGCCCTGTTCCTTCCATTC
Dhcr7	NM_007856	GACCCTCATTAACCTGTCCTTCG	CCAGGTTTCATTCCAGAAGAAGTC
Ebp	NM_007898	TGATCGAGGGCTGGTTCTCT	ATATCGGCTATCTCCCTTGGAATA
Eci	NM_010023	GTTCACCATCAGCCTGGAGAAG	AGAAGATACCCGGGCATTCC
Edem1	NM_138677	AAGCCCTCTGGAACTTGCG	ATGGCCTGTCTGGATGTTCAC
Elovl1	NM_019422	GTACCTACACCTGGCGCTGTG	CAGGCCACTCGAACCATCC
Elovl2	NM_019423	CAGCTGGGAAGGAGGTTACAACT	AATCGTGTCCAGGAACTCCACTA
Elovl3	NM_007703	CGTAGTCAGATTCTGGTCCT	CCAGAAGAAGTGTTCCGTTG
Elovl5	NM_134255	TCGATGCGTCACTCGTACCTATT	ATTTTGGTCCCAGCCATACAAT
Elovl6	NM_130450	TCTGATGAACAAGCGAGCCA	TGGTCATCAGAATGTACAGCATGT
F4/80	NM_010130	ACACTTCCCACCCTGGGAC	CTGGACAGGAAGCCTCGTTTA
Fabp	NM_017399	GGCAAGTACCAATTGCAGAGC	AGGTCCTCGGGCAGACCTA
Fads1	NM_146094	TCAACATGCACCCCCTCTTC	GATGGTTGTATGGCATGTGCTT
Fads2	NM_019699	TCCAGTACCAGATCATCATGACAA	GGTGTAGAAGAAACGCATATAGTAGCTG
Fads3	NM_021890	TCATCGGCCACCACGG	TGCGCACAAAATGGAGATCTT
Fasn	NM_007988	AGTCAGCTATGAAGCAATTGTGGA	CACCCAGACGCCAGTGTTC
Fdft1	NM_010191	AGGAGTTCTATAACCTGCTGCGAT	GGTCTTCAAGCTGCTGCTGAGT
Fdps	NM_134469	TGCTATTGCCCGGCTCA	ATCCTGTTTCTTCGGCTCCA
Fgf21	NM_020013	AAAGCCTCTAGGTTTCTTTGCCA	CCTCAGGATCAAAGTGAGGCG
Fgfr4	NM_008011	GTATGGATCCCTCCCGGC	GGTCTGCCAAATCCTTGTCG
Fsp27	NM_178373	CATGAAGTCTCTCAGCCTCCTGTA	CAGCTGTTGGGTCACCACTG
Fxr	NM_009108	CCACCGGCTGTCAGGATT	CGCGTGTTCTGTTAGCATACCTT
G6pc	NM_008061	CTCACTTTCCCCACCAGGTC	GCTGAAAGTTTCAGCCACAGC
Ggps	NM_010282	AAGCTGAGAGGATTCTTCTAGAGCC	TGAAAGTTTGCTTCTCACCTGTTTA
Glut2	NM_031197	TTTGCAGTGGGCGGAATGG	GCCAACATTGCTTTGATCCTT
Gpat	NM_0081449	AGACGAAGCCTTCCGACGA	TGGACATGATAGCGCAGGACT
Gpr120	NM_181748	CCGGGACTGGTCATTGTGAT	CCAAGCTCAGCGTAAGCCTC
Grp78	NM_022310	GCCGAGGAGGAGGACAAGA	TCTTGAACACACCGACGCA
Hmgcr	NM_008255	CTTGTGGAATGCCTTGTGATTG	GAAGAATGTCATGAACACAAAGTAGTTG
Hmgcs1	NM_145942	CCTGGACCGCTGCTATTCTG	TGAAAGATCATGAAGCCAAAATCA
Hmgcs2	NM_008256	TGCAGGAAACTTCGCTCACA	AAATAGACCTCCAGGGCAAGGA
Hnf4a	NM_008261	CTTGGAGCCACCAAGAGGTC	CTAGCTCTGGACAGTGCCGAG
Hp	NM_017370	CAGCCCAGCCCTTCCAG	GGAGAGTGACAACAGCTCCCA
Hsd17b7	NM_010476	ACTTCGGTGCAGGGCGT	CACAGGTGGAGGTCATCGTCT
Idol	NM_153789	CCGAGCCATCACCGAAAC	TCATGACGGCACTGGTGACT
Il1b	NM_008361	AAAAAAGCCTCGTGCTGTCG	GTCGTTGCTTGGTTCTCCTTGT
Insig1	NM_153526	GAGGTGTCACAGTGGGAAACATAG	TCTTCATCACACCCAGGACCA
Insig2	NM_133748	TGTATATTTTTTGCTGGAGGCATAAC	TTCAGCAATAACTTTGCATTCATACAT
Ldlr	NM_010700	GCAAGGACATGAGCGACGA	CTCCCCACTGTGACACTTGAAC
Lipin1	NM_172950	ATGTTTCCCATAGAGATGAGCTCG	GAATGGTGGTACATCATTAGGAAGAG
Lipin2	NM_001164885	AGGACAATAGGAAGGAGGAGCAG	TTGTAGTCCTCTTCCTTTAAGGAAGC
Lipin3	NM_022883	CCCTCTGGGCATCCACAA	TGGCCCCCATCCCATACT
Lpcat3	NM_145130	TACAAGGACAGCTACCTCATCCATC	GAAGCACGACACATAGCAAGGA
Lpk	NM_013631	TCGACTCAGAGCCTGTGGC	AGTCGTGCAATGTTCATCCCT

Lpl	NM_008509	ATGGCAAGCAACACAACCAG	TGTGGAAACCTCGGGCAG
Lss	NM_146006	ATGAGTTGGGTCGGCAGAGAT	GCGCTTTTGGTAAGTCCGTG
Me1	NM_008615	CATTCGAGGCGTTTCGTTG	CAGGTAGGATCTGGTCATAATTAGTGC
Mmp9	NM_013599	CTCGAGGGCTTCCCTCTGA	GGCTGGAGGCCTTGGGT
Mttp	NM_008642	TCAGGAAGCTGTGTCAGAATGAAG	TTTCAAGTCCTCCCAGGATCA
Mvd	NM_138656	CGGTCAACATCGCAGTTATCAA	GTGCAGCGTGACGCTCAG
Mvk	NM_023556	GCTTCAGCGACTGGACACG	ACAGGTAGAGAAAGGCAAGCAGA
Npc1l1	NM_207242	ATCACCTTGCTGGGTCTGCTAC	GTACTGTGGGCAAGAAGGCTCT
Nsdhl	NM_010941	TGCTGGAGCGAGGCTATACTG	CAGTGGAAAACTGTGCTTACACCTT
P2ry13	NM_028808	CTGAGTCTCTTCCAAAACAAAGCTG	ACCGCTCAGACTTGTTGAAGC
P2yr1	NM_008772	GCACGAGATCCTAGCTCCTGA	GCACACACTGGTCTTTTGGTCA
Pcsk9	NM_153565	AGGAAGACCGCTCCCCTG	TGGTATCTAAGAGATACACCTCCACCT
Pdk4	NM_013743	ATCGCCAGAATTAAACCTCACAC	TGGATTGGTTGGCCTGGA
Pepck	NM_011044	GAACCCCAGCCTGCCC	GAGCAACTCCAAAAAACCCG
Pgc1a	NM_008904	CAATCGGAAATCATATCCAACCA	CTGTGAGGACCGCTAGCAAGT
Pgc1b	NM_133249	CTTTGCGGCACGGCAG	CTGGGCTGAGCTTGGTGTCT
Pias1	NM_019663	CCTCATCCAGGACTACAGGCAC	TGTTGTAATGCTGATTGTCTCCTGAT
Plin2	NM_007408	CCATTTCTCAGCTCCACTCCAC	GTGTCGTCGTAGCCGATGC
Plin3	NM_025836	GGCTGGACAGACTGCAGGA	TCTTGAGCCCCAGACACTGTAG
Plin5	NM_025874	CGCTCCATGAGTCAAGCCA	CTCAGCTGCCAGGACTGCTA
Pltp	NM_011125	GGATTAAAGTGTCCAATGTCTCCTG	GTGGAGAAAAAGTTATACATCCTCCTG
Pmdci	NM_016772	GGAAAGATGTTCACTTCAGGTATTGAC	CGGGCCGCATCATCTC
Pmvk	NM_026784	GGAAGGCGTGTCCCAGC	GCCCCATAGGCCTCCTGA
Pnpla3	NM_054088	ACGCGGTCACCTTCGTGT	AGCCCGTCTCTGATGCACTT
Ppara	NM_011144	CCCTGTTTGTGGCTGCTATAATTT	GGGAAGAGGAAGGTGTCATCTG
Pparb	NM_011145	AAGTGGCCATGGGTGACG	TGGTCCAGCAGGGAGGAAG
Pparg1	NM_011146	CCACCAACTTCGGAATCAGCT	TTTGTGGATCCGGCAGTTAAGA
Pparg2	NM_011146	ATGGGTGAAACTCTGGGAGATTCT	CTTGGAGCTTCAGGTCATATTTGTA
Pxr	NM_010936	AGAGATCATCCCTCTTCTGCCAC	GATCTGGTCCTCAATAGGCAGGT
Reverba	NM_145434	CAGCTGGTGAAGACATGACGAC	GGAGGAGCCACTAGAGCCAA
Saa3	NM_011315	CATTGCCATCATTCTTTGCATC	GTAGGCTCGCCACATGTCTCT
Sc4mol	NM_025436	AAGCCATCTATTTCTTGTTCTCTTTACCT	CAAACACTTCCACTGGCCTTC
Sc5d	NM_172769	CAGCATCCCCACCGTCTC	AAAAGAGGAAGGATACGACGCTAA
Scarb	NM_016741	TCCCTCATCAAGCAGCAGGT	ACCTCGTTTGGGTTGACCAC
Scd1	NM_009127	CAGTGCCGCGCATCTCTAT	CTGACTGGCAAATATAGCTGTATCCT
Scd2	NM_009128	CCCCTACGACAAGAACATTAGC	GGTAGTTGTGGAAGCCCTCG
Sec14l1	NM_028777	TCCTTGTCCCAGATGCTGCT	GATGTAGTCGGCATCTAATTTATCGT
Shp	NM_011850	CCCAAGGAGTATGCGTACCTGA	TGTGCGATGTGGCAGGAG
Sirt1	NM_019812	GCTGTGAAGTTACTGCAGGAGTGT	CCGCAAGGCGAGCATAGATA
Soat1	NM_009230	TGTTGGCAGCAGAGGCG	GGTCACAAAGTCATCGAAGTGG
Spot14	NM_009381	AACGGAGGAGGCCGAAGAAG	GTTGATGCACCTCGGGGTCT
Sqle	NM_009270	GGAGGCTACCGTGTTCTCCA	CTGCACTTGGTTGGTTTCTGAC
Srebp1a	NM_011480	CAGACACTGGCCGAGATGTG	CTTGGTTGTTGATGAGCTGGAG
Srebp1c	NM_011480	CAGACACTGGCCGAGATGTG	CTTGGTTGTTGATGAGCTGGAG
Srebp2	NM_033218	GTACTGCGCCCAGAGGAGC	GCCTGAGGTTTCACCAAGGAC
Star1	NM_011485	AAGGCCTTGGGCATACTCAAC	TGGCACCATCTTACTTAGCACTTC

Sult1e1	NM_023135	ATTTCACTTCTTCCACGGGAAC	CCAAAAACTTCATAATACTCAGGCATAG
Tbp	NM_013684	ACTTCGTGCAAGAAATGCTGAA	GCAGTTGTCCGTGGCTCTCT
Tm7sf2	NM_028454	AAGGCCTGGAACTGAAGGACA	ACCAGAGCCTGGAAGCCAT
Tnfa	NM_013693	TCCCCAAAGGGATGAGAAGTTC	TGCTCCTCCACTTGGTGGTT
Trb3	NM_175093	GGCCTTATATCCTTTTGGAACGA	CACCGCCTGGGCCTC
Ubxd8	NM_178397	CCAGCTCGGCCCCTACA	GTAATAACCCCATCCAAGCAGG
Ucp2	NM_011671	CATGGTAGCCACCGGCA	CTTCAATCGGCAAGACGAGAC
Ugt1a2	NM_201645	TCCTTCCTCTTATATTCCGAACCTAC	GAAAACAACGATGCCATGCTC

Table S4 : Relative abundance of hepatic fatty acids

	Fat Free		REF				COCO				FISH			
	0		5%		20%		5%		20%		5%		20%	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
C14:0	0,422^{c}	0,364^{c}	0,202	0,226	0,161	0,179	0,877^{b}	1,048^{b}	2,077^{q,b}	1,785^{q,b}	0,226^{c}	0,554^{c}	0,098^{c}	0,159^{c}
C16:0	19,756	17,251^{s,c}	20,111	18,338	18,270^{q}	16,167^{s,q}	21,11	19,159^{s}	23,583^{q,b}	21,400^{s,q,b}	25,187^{b,c}	20,969^{s,b,c}	19,154^{q,c}	18,536^{q,b,c}
C16:1 n-9	1,142^{b}	1,160^{b}	0,713	0,809	1,232^{q}	1,018	1,083^{b}	1,240^{b}	1,104	1,074	0,510^{c}	0,829^{s,c}	0,450^{b,c}	0,926^{s}
C16:1 n-7	7,627^{b}	5,703^{s,b}	3,426	2,479	1,420^{q}	1,981	8,026^{b}	6,384^{s,b}	7,211^{b}	5,451^{s,b}	2,714^{c}	2,938^{c}	0,521^{q,c}	0,878^{q,c}
C18:0	4,710^{b}	5,315^{b}	9,32	8,265	8,86	9,834	5,981^{b}	5,058^{b}	6,168^{b}	6,387^{b}	9,976^{c}	9,702^{c}	12,181^{b,c}	9,158^{s,c}
C18:1 n-9	44,521^{b}	50,960^{s,b}	22,778	33,474^{s}	22,732	26,883^{q}	38,660^{b}	48,719^{s,b}	35,932^{b}	38,661^{q,b}	16,692^{b,c}	25,830^{s,b,c}	12,010^{b,c}	16,709^{q,b,c}
C18:1 n-7	9,408^{b}	7,388^{s,b}	3,381	2,886	1,947	2,672	9,300^{b}	7,863^{b}	10,187^{b}	8,427^{b}	1,505^{c}	3,589^{c}	1,012^{c}	3,679^{s,c}
C18:2 n-6	1,802^{b}	1,708^{b}	16,526	14,563	22,919^{q}	21,149^{q}	2,928^{b}	1,465^{b}	2,303^{b}	3,573^{b}	13,642^{c}	11,416^{c}	21,165^{c,q}	18,618^{q,c}
C18:3 n-6	0,068	0,06	0,365	0,417	0,478	0,547	0,084	0,043	0,054	0,052	0,1	0,104	0,113	3,748^{s,q,b,c}
C18:3 n-3	0,067^{b}	0,057^{b}	0,361	0,339	0,665^{q}	0,654^{q}	0,036^{b}	0,048^{b}	0,029^{b}	0,089^{b}	0,334^{c}	0,279^{c}	0,542^{q,c}	0,608^{q,c}
C20:1 n-9	1,612^{b,c}	1,152^{s,b}	0,544	0,44	0,683	0,496	0,997^{b}	1,098^{b}	1,075^{b}	0,975^{b}	0,368^{c}	0,463^{c}	0,357^{b,c}	0,275^{c}
C20:3 n-9	3,411^{b}	3,370^{b}	0	0	0	0	3,056^{b}	2,933^{b}	3,175^{b}	2,825^{b}	0,000^{c}	0,000^{c}	0,000^{c}	0,028^{c}
C20:2 n-6	0	0	0,427	0,4	0,437	0,853	0	0	0	0,000^{b}	0,216	0,768^{s,c}	0,26	0,197^{q,b}
C20:3 n-6	0,467^{b,c}	0,483^{b}	1,402	0,832^{s}	1,735^{q}	0,852^{s}	0,703^{b}	0,363^{s,b}	0,591^{b}	0,500^{b}	1,086^{b,c}	0,882^{c}	1,124^{b,c}	0,888^{c}
C20:4 n-6	3,095^{b}	3,172^{b}	12,82	9,907^{s}	11,33	9,949	4,900^{b}	2,595^{s,b}	4,295^{b}	3,274^{b}	3,819^{c}	4,165^{b}	3,776^{b}	2,877^{b}
c20:3 n-3	0,019	0,008	0,42	0,049	0,027	0,021	0,005	0,015	0,016	0,014	0,017	0,014	0,025	0,645^{s,q,b,c}
C20:5 n-3	0,066	0,047	0,118	0,08	0,218	0,159	0,061	0,038	0,062	0,725	3,872^{b,c}	3,646^{b,c}	4,371^{b,c}	3,028^{s,b,c}
C22:4 n-6	0,049	0,048	0,402	0,304	0,427	0,293	0,07	0,05	0,063	0,042	0,027	0,036	0,015	0,570^{s,q,c}
C22:5 n-6	0,536^{b}	0,421	0,72	0,515^{s}	0,277^{q}	0,249^{q}	0,622	0,470^{s}	0,625^{b}	0,414^{s,b}	0,123^{b,c}	0,169^{c}	0,211^{c}	0,134^{c}
C22:5 n-3	0,018^{b}	0,026	0,287	0,161	0,416	0,246	0,028^{b}	0,009	0,020^{b}	0,159	1,214^{b,c}	0,834^{s,b,c}	1,106^{b,c}	0,981^{b,c}
C22:6 n-3	1,204^{b}	1,308^{b}	5,677	5,515	5,766	5,801	1,473^{b}	1,009^{b}	1,430^{b}	3,865	18,372^{b,c}	12,814^{s,b,c}	21,508^{b,c}	14,480^{s,b,c}

Table S6 : Relative abundance of hepatic fatty acids

Fatty acid	WT REF	WT COCO	WT FISH	KO REF	KO COCO	KO FISH
C14:0	0,263	0,986[b]	0,150[c]	0,237	0,845[b]	0,116[b,c]
C16:0	22,53	28,155	33,924[c]	18,516	21,017	19,367[a]
C16:1 n-9	0,791	0,706	0,793	0,767	0,937	0,778
C16:1 n-7	3,385	6,870[b]	2,172[c]	4,977	8,565[a,b]	3,180[b,c]
C18:0	12,057	8,823	14,067[c]	13,81	11,300[b]	15,294[c]
C18:1 n-9	16,605	28,269[b]	11,489[b,c]	14,215	21,953[a,b]	8,328[b,c]
C18:1 n-7	3,578	9,457[b]	1,811[b,c]	4,425	10,740[b]	2,240[b,c]
C18:2 n-6	15,968	3,200[b]	12,787[c]	20,862[a]	7,192[a,b]	14,606[b,c]
C18:3 n-6	0,383	0,122	0,39	0,352	0,257	0,362
C18:3 n-3	0,313	0,034[b]	0,291[c]	0,663[a]	0,077[b]	0,358[b,c]
C20:1 n-9	0,383	0,608[b]	0,237[c]	0,477	1,078[a,b]	0,319[b,c]
C20:2 n-6	0,393	0,000[b]	0,148[b,c]	0,426	0,000[b]	0,196[b,c]
C20:3 n-9	0,000	2,886[b]	0,000[c]	0,000	1,922[a,b]	0,000[b,c]
C20:3 n-6	1,516	0,878[b]	0,508[b,c]	1,569	1,358[a,b]	0,579[b,c]
C20:4 n-6	12,859	5,300[b]	2,043[b,c]	10,906	7,274[b]	2,812[b,c]
C20:3 n-3	0,133	0,035	0,009[b]	0,315[a]	0,099	0,011
C20:5 n-3	0,281	0,102	2,612[b,c]	0,396	0,220[b]	8,153[a,c]
C22:4 n-6	0,214	0,079[b]	0,027[b]	0,282	0,276[a]	0,173[a,b,c]
C22:5 n-6	0,51	1,180[b]	0,133[b,c]	0,234[a]	0,688[a,b]	0,258[b,c]
C22:5 n-3	0,263	0,147	0,774[b,c]	0,502[a]	0,160[b]	1,392[a,c]
C22:6 n-3	7,202	1,820[b]	15,391[b,c]	5,78	3,054[b]	21,213[a,b,c]
C24:1 n-9	0,373	0,342	0,244	0,291	0,989[a]	0,266[c]

Table S7 : Circulating cytokines

	WT REF	WT COCO	WT FISH	KO REF	KO COCO	KO FISH
IFN	nd	nd	nd	nd	nd	nd
IL1β	nd	nd	nd	nd	nd	nd
IL2	nd	nd	nd	nd	nd	nd
IL6	nd	nd	nd	nd	nd	nd
IL10	nd	nd	nd	nd	nd	nd
IL12	nd	nd	nd	nd	nd	nd
IL17	nd	nd	nd	nd	nd	nd
TNF	nd	nd	nd	nd	nd	nd
KC (pg/mL)	148,2	185.0	201,3	131,2	127,0	142,3
MCP1 (pg/mL)	107,3	171,6[b]	136,0	101,0	98,1[a]	111,0

Table S8: qPCR data of the expression of genes present in the heatmap.

	WT REF	WT COCO	WT FISH	KO REF	KO COCO	KO FISH
Lipid metabolism and traficking						
Acly	1,000	3,847[b]	0,621[c]	1,064	1,273[a]	0,77
Acaca	1,000	2,790[b]	0,707[b,c]	0,968	1,330[a]	0,92
Acacb	1,000	3,748[b]	0,528[b,c]	0,177[a]	0,744[a,b]	0,256[a,c]
Elovl1	1,000	1,194	0,889[c]	0,964	1,081	0,878[c]
Elovl2	1,000	0,448[b]	0,473[b]	0,481[a]	0,303[b]	0,474
Elovl3	1,000	0,497[b]	0,530[b]	0,573[a]	0,375	0,557
Elovl5	1,000	2,126[b]	0,380[b,c]	0,881	1,774[b]	0,639[a,b,c]
Elovl6	1,000	3,030[b]	0,639[b,c]	0,658	0,923[a]	0,596[c]
Spot14	1,000	5,308[b]	0,504[b,c]	0,747	0,678[a]	0,275[b,c]
Gpat	1,000	1,798[b]	0,883[c]	1,194	1,319	1,049
Agpat1	1,000	1,325[b]	0,988[c]	1,164	0,975[a]	0,841[b]
Agpat2	1,000	0,933	0,904	1,11	0,92	1,003
Agpat6	1,000	0,919	0,884	0,798	0,714	0,805
Dgat1	1,000	0,865	0,875	1,258	1,184[a]	0,906[b,c]
Dgat2	1,000	0,956	0,845	1,251	1,025	0,849[b]
Fads1	1,000	1,981[b]	0,494[b,c]	0,846	1,585[b]	0,459[b,c]
Fads2	1,000	2,389[b]	0,486[b,c]	0,562[a]	1,355[a,b]	0,274[a,b,c]
Fads3	1,000	0,903	0,954	1,686[a]	1,935[a]	1,605[a]
Fas	1,000	3,896[b]	0,586[b,c]	0,606[a]	1,136[a,b]	0,395[c]
Scd1	1,000	2,381[b]	0,178[b,c]	0,022[a]	0,442[a,b]	0,005[a,b,c]
Scd2	1,000	2,131[b]	0,920[c]	0,962	6,225[a,b]	0,807[c]
Pnpla3	1,000	49,880[b]	0,175[b,c]	0,475	0,787[a]	0,386[a]
Lipin1	1,000	2,464[b]	0,747[c]	2,251[a]	1,651	1,368
Lipin2	1,000	1,265	0,660[c]	1,469	1,477	1,392[a]
Lipin3	1,000	1,116	0,966	1,201[a]	1,292	1,369[a]
Cd36	1,000	1,574[b]	1,172	2,656[a]	4,677[a,b]	2,970[a,c]
Cept1	1,000	1,158	0,921[c]	1,389[a]	1,767[a,b]	1,365[a,c]
Lpcat3	1,000	1,077	0,664[b,c]	0,833	0,805[a]	0,651
Pltp	1,000	2,701[b]	1,109[c]	0,458[a]	1,167[a,b]	0,404[a,c]
Fabp	1,000	1,018	0,769	0,577[a]	0,699[a]	0,461[a,c]
Plin3	1,000	1,436[b]	0,955[c]	1,02	1,235	0,954[c]
Plin2	1,000	0,885	0,946	0,853	0,871	1,011
Plin5	1,000	1,08	0,937	1,348[a]	1,107	1,189
Chka	1,000	1,219	0,969	0,988	1,156	0,761[c]
Fsp27	1,000	2,756[b]	1,110[c]	1,031	2,419[b]	1,498
Sterol metabolism and traficking						

Acat1	1,000	0,717[b]	0,950[c]	1,637[a]	1,346[a,b]	1,465[a]
Acat2	1,000	2,651[b]	0,978[c]	1,712[a]	3,321[b]	1,355[c]
Hmgcs1	1,000	2,114	1,178	1,733	5,447[b]	1,068[c]
Hmgcs2	1,000	0,716[b]	0,750[b]	1,016	0,750[b]	0,845
Hmgcr	1,000	2,344[b]	0,846[c]	1,469	2,703[b]	0,815[b,c]
Mvk	1,000	1,371	1,167	2,445[a]	4,287[a]	1,611[c]
Pmvk	1,000	4,208[b]	0,776[c]	1,464	3,409[b]	0,872[c]
Mvd	1,000	4,826[b]	1,795[c]	4,493[a]	6,004	1,621[b,c]
Fdps	1,000	5,727[b]	1,254[c]	2,396	5,383[b]	1,216[c]
Ggps	1,000	1,071	0,918	0,966	1,178	1,05
Fdft1	1,000	4,925[b]	2,791	9,996[a]	23,194[a]	4,897[c]
Sec14l1	1,000	1,29	0,902[c]	1,068	1,547[b]	1,029[c]
Sqle	1,000	10,820[b]	3,159[c]	6,087[a]	10,659	2,679[c]
Lss	1,000	2,826[b]	1,089[c]	1,842	3,187	1,016[c]
Cyp51a	1,000	2,742[b]	2,161	3,498[a]	8,256[a,b]	2,260[c]
Tm7sf2	1,000	2,273[b]	0,691[c]	1,789	3,163	0,943[b,c]
Sc4mol	1,000	3,059[b]	2,164	6,592[a]	11,065[a]	2,786[b,c]
Cyb5r3	1,000	1,830[b]	0,830[c]	2,040[a]	1,485	1,354[a,b]
Nsdhl	1,000	4,029[b]	1,730[c]	3,910[a]	9,231[b]	2,324[c]
Hsd17b7	1,000	1,723[b]	1,122[c]	2,709[a]	3,967[a]	2,187[a]
Ebp	1,000	1,285	0,973	1,32	1,559	1,178
Sc5d	1,000	1,992[b]	0,855[c]	1,283	2,118	0,865[c]
Dhcr7	1,000	4,253[b]	1,357[c]	3,311[a]	6,804[b]	1,765[c]
Dhcr24	1,000	1,901[b]	1,101[c]	2,049[a]	2,106	1,658[a]
Soat1	1,000	1,351	1,159	1,391	4,522[a,b]	3,129[a,b]
Star1	1,000	1,151	0,738[b,c]	0,847	0,761[a]	0,709
Idol	1,000	0,982	0,712[b,c]	0,669[a]	0,568[a]	0,552
Scarb1	1,000	0,994	0,778[b,c]	0,802[a]	0,879	0,733[c]
Npc1l1	1,000	1,818[b]	1,256	2,128[a]	3,972[a,b]	1,665[c]
Glucose metabolism						
Glut2	1,000	0,905	0,779	1,835[a]	1,249[b]	1,362[a,b]
G6Pc	1,000	0,916	0,648	1,351	1,242	1,435[a]
Me1	1,000	2,195[b]	0,483[b,c]	0,406[a]	0,540[a]	0,319[c]
L.Pk	1,000	1,404	0,703[b,c]	0,909	0,851[a]	0,523[b,c]
Pepck	1,000	0,974	0,658[b,c]	1,179	1,063	0,990[a]
Pdk4	1,000	1,761	0,523[c]	1,09	1,491	2,097[a]
Transcriptional regulations						
Chrebp	1,000	0,976	0,770[b]	0,809	0,742[a]	0,511[a,b,c]
Cnr2	1,000	1,787	1,097	5,504[a]	17,031[a,b]	10,633[a]

Hnf4a	1,000	1,152	1,004	0,968	0,955	0,904
Srebp1a	1,000	0,974	0,768	1,213	1,237	0,846[b,c]
Srebp1c	1,000	0,954	0,544[b,c]	0,294[a]	0,347[a]	0,137[a,b,c]
Srebp2	1,000	1,296	0,965[c]	1,458[a]	1,461	0,770[b,c]
Ppara	1,000	1,052	0,732	1,084	0,97	0,736[b]
Pparb	1,000	1,784[b]	0,651[c]	0,477[a]	0,667[a]	0,540
Pparg1	1,000	1,344	0,938	3,410[a]	4,779[a]	4,717[a]
Pparg2	1,000	0,92	0,755	2,233	4,248[a]	2,244[a]
Pxr	1,000	0,994	0,763[b,c]	1,455[a]	1,137[b]	0,987[a,b]
Reverba	1,000	0,928	0,766	1,887	1,395	0,735
Shp	1,000	0,983	0,728	0,762	0,788	0,581
Pgc1a	1,000	1,681[b]	0,669[c]	1,258	1,422	1,120[a]
Pgc1b	1,000	1,148	0,743	1,289	1,001	1,01
Fxr	1,000	0,856	0,876	0,750[a]	0,692	0,606[a]
Car	1,000	0,534[b]	1,029[c]	2,077[a]	1,921[a]	1,405[b]
Sirt1	1,000	1,129	0,938	0,952	0,961	0,962
Insig1	1,000	2,196[b]	0,626[c]	1,543	1,439	0,612[b,c]
Insig2	1,000	1,583[b]	0,896[c]	1,253	1,451	1,412[a]
Crebh	1,000	0,884	1,074	1,132	1,001	1,195
Ubxd8	1,000	1,059	0,977	1,139	1,1	1,159
Ascl3	1,000	1,933[b]	0,962[c]	0,85	1,569	0,808[c]
Gpr120	1,000	1,183	0,972	0,463[a]	0,628[a]	0,650
Pias1	1,000	1,029	0,985	1,169[a]	1,194[a]	1,085
RE Stress						
Trb3	1,000	1,072	1,191	2,776[a]	2,560[a]	2,345
Grp78	1,000	0,867	1,128	1,127	0,746	1,063
Edem1	1,000	1,188	1,22	1,079	0,978	1,089
Atf4	1,000	1,034	1,041	1,123	1,018	0,939
Fatty acids oxidation						
Cpt1a	1,000	0,946	0,947	1,525[a]	1,681[a]	1,200[c]
Cyp4a14	1,000	0,345	6,683[b,c]	2,959[a]	8,505[a]	29,045[b]
Acox1	1,000	0,971	1,083	1,031	1,075	1,276
Pmdci	1,000	0,822	1,092	0,893	0,725	0,985[c]
Eci	1,000	0,712[b]	1,282[c]	1,444[a]	1,494[a]	1,6
Ucp2	1,000	1,915[b]	1,145[c]	2,630[a]	5,425[a]	2,823[a]
Bien	1,000	0,943	0,831	1,769[a]	2,208[a]	3,115[a,b]
Fgf21	1,000	1,695	1,824	2,490[a]	3,936[a]	0,830[b,c]
Lipoproteins metabolism						
Pcsk9	1,000	3,659[b]	0,647[c]	1,44	2,509	0,511[b,c]

Ldlr	1,000	1,518[b]	0,772[c]	1,156	1,130[a]	0,603[b,c]
ApoA1	1,000	1,317	0,995[c]	0,750[a]	0,758[a]	0,520[a,b,c]
ApoB	1,000	0,863	0,981	1,169	1,226a	1,124
ApoE	1,000	0,814[b]	0,702[b]	0,949	0,865	0,666[b,c]
Lpl	1,000	1,065	1,152	0,773	2,163[b]	2,045[b]
Mtp	1,000	1,151	0,899	1,002	0,944	0,665[a,b,c]
Mmp9	1,000	5,094[b]	2,044	2,693[a]	6,516	5,763[a]
Oxysterols Biosynthesis						
Cyp27a1	1,000	1,082	0,800[c]	1,029	0,875	0,759[b]
Cyp46a1	1,000	0,801	0,589[b]	0,921	0,551[b]	0,711
Detoxication						
Cyp2b10	1,000	0,714	0,456	18,716[a]	40,099[a]	7,182[a,c]
Cyp3a11	1,000	0,513[b]	1,189[c]	6,158[a]	4,658[a]	5,751[a]
Cyp2c29	1,000	0,667	0,759	0,867	0,699	0,684
Ugt1a2	1,000	4,319[b]	0,292[b,c]	0,716	2,752[b]	0,367[c]
Abcb1a	1,000	1,241	1,074	2,959[a]	3,841[a,b]	3,227[a]
Abca1	1,000	0,866	1,152[c]	1,108	0,841[b]	1,063[c]
Abcc2	1,000	0,934	0,730[b]	0,993	0,884	0,805
Sult1e1	1,000	2,179	0,680[c]	18,988[a]	19,421[a]	11,730[a]
Bile acid metabolism						
Cyp7a1	1,000	0,5	0,586	2,035	1,746[a]	0,776
Abcg5	1,000	0,77	0,801	1,189	1,411[a]	1,017
Abcg8	1,000	0,746	0,876	1,316	1,538[a]	1,095
Cyp7b	1,000	0,678	1,127[c]	0,293[a]	0,146[a,b]	0,421[a,c]
Cyp39a1	1,000	1,916[b]	0,769[c]	0,589[a]	1,012[a,b]	0,135[a,b,c]
Fgfr4	1,000	0,874	0,730[b]	1,084	0,92	0,849
Inflammation						
F4/80	1,000	1,755	0,810[c]	1,165	2,668[b]	1,286
Cd68	1,000	1,294	0,857	1,917[a]	4,292[a,b]	2,268[a]
Tnfa	1,000	1,501	1,314	8,555[a]	27,429[a,b]	14,315[a]
Il1b	1,000	2,095[b]	0,853[c]	1,806[a]	3,638[a,b]	2,706[a]
Crp	1,000	0,840	0,749[b]	0,448[a]	0,437[a]	0,444[a]
Hp	1,000	1,143	1,850[b,c]	0,733	0,859	1,175
Saa3	1,000	0,995	3,227	1,092	3,176	1,686
Ccl2	1,000	2,066	0,268[c]	0,88	1,901	1,475[a]
Others						
Atp5b	1,000	1,043	0,824[c]	1,152	0,887[b]	0,899[b]

P2ry13	1,000	1,484	0,939	0,561[a]	1,197[b]	1,216[b]
P2yr1	1,000	0,966	0,584[b,c]	0,654[a]	0,590[a]	0,593

Chapitre IV

Le fructose est un sucre dont la consommation dans les pays industrialisés est en constante augmentation. Sa consommation conduit au développement de NAFLDs, des pathologies hépatiques dont la caractéristique commune est une accumulation de triglycérides dans le foie (Nomura and Yamanouchi 2012; Stanhope 2012).

Le fructose semble posséder des effets stéatogéniques plus importants que le glucose, et ce, principalement pour deux raisons. Tout d'abord, quand le fructose entre dans l'hépatocyte, il est dégradé et produit du glycéraldéhyde-3-phosphate qui est ensuite oxydé par la glycolyse. Le catabolisme du fructose n'est pas soumis à la réaction catalysée par la phosphofructokinase, qui subit un rétrocontrôle négatif quand la glycolyse est fortement activée (Nomura and Yamanouchi 2012). De plus, le fructose influe sur l'expression des gènes hépatiques impliqués dans la lipogenèse (Nomura and Yamanouchi 2012; Stanhope 2012).

Il a été montré que PGC-1β est nécessaire à l'induction de l'expression des gènes de la lipogenèse en réponse au fructose (Nagai, Nishio et al. 2002). PGC-1β est un co-activateur de facteurs de transcription tels que SREBP-1c et LXR. Cependant les effets lipogéniques du fructose alimentaire persistent chez des souris invalidées pour *Srebp-1c* (Miyazaki, Dobrzyn et al. 2004). L'implication de LXR dans la réponse à un régime riche en fructose n'a pas été étudiée.

Il y a quelques années, il a été montré que le glucose pouvait se lier et activer LXR (Mitro, Mak et al. 2007). Cependant, cette étude a été fortement discutée et ses conclusions remises en question (Lazar and Willson 2007). En effet, les effets hépatiques du glucose sur l'expression des gènes de la glycolyse et de la lipogenèse dépendent essentiellement de ChREBP (Denechaud, Bossard et al. 2008).

Les souris invalidées pour *Scd1* sont résistantes à l'induction de l'expression des gènes de la lipogenèse par le fructose. Cependant, l'ajout d'acide oléique, un produit de synthèse de SCD1, dans l'alimentation, permet de rétablir cette induction (Miyazaki, Dobrzyn et al. 2004). Etant donné le rôle de LXR dans l'induction de la lipogenèse en réponse aux acides gras que nous avons étudiée dans le chapitre précédent, nous avons voulu savoir si LXR pouvait intervenir dans la réponse transcriptionnelle induite par le fructose et par l'acide oléique. Dans cette étude, menée en collaboration avec l'équipe du Dr Catherine POSTIC, nous avons donc étudié *in vivo* le rôle de LXR mais également de ChREBP dans la réponse à un challenge chronique ou aigu au fructose alimentaire.

Dual role of LXR and ChREBP in fructose-induced hepatic lipogenesis

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List of Abbreviations

FA: Fatty Acid; PGC-1β: Peroxisome Proliferator-Activated Receptor General Coactivator-1β; LXR: Liver X Receptor; SREBP-1c: Sterol Responsive Element Binding Protein-1c; ChREBP: Carbohydrate Response Element Binding Protein; NAFLD: Non Alcoholic Fatty Liver Disease; TG: Triglyceride.

Conflict of interest

The authors report no conflict of interest

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Abstract

Background & Aims. Fructose is a highly lipogenic carbohydrate whose excess consumption may promote such an intense *de novo* fatty acid synthesis in mammalian liver that it ultimately results in hepatic steatosis. This increased lipogenesis is known to depend, at least in part, on transcriptional control. Several transcription factors are involved in the transcriptional control of hepatic lipogenesis in response to hormones and nutrients. The Liver X Receptors (LXRs) are class II nuclear receptors that are abundant in the liver, where they act as cholesterol sensors. Importantly, LXRs also regulate the expression of both Carbohydrate Responsive Element Binding Protein (ChREBP) and Sterol Regulatory Element Binding Protein-1c (SREBP-1c). ChREBP and SREBP-1c play a central role in the transcriptional response to glucose and insulin, respectively.

Methods. Using wild-type and LXR-deficient mice, we investigated whether LXRs might be involved in the transcriptional control of hepatic lipogenesis in response to fructose *in vivo*.

Results. We provide evidence that LXRs are critical for the hepatic response to chronic and to acute fructose challenge.

Conclusions. LXRs play an important role at the crossroads between cholesterol, fatty acid and carbohydrate homeostasis. It is essential for the liver ability to cope with specific nutritional challenges.

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Introduction

In the liver, transcriptional regulation of lipogenic genes is under the control of various transcription factors including the Liver X Receptors (LXRs), class II nuclear receptors [1]. They are sensitive to oxidized cholesterol derivatives, the oxysterols, which bind to and activate both LXR isotypes (α, NR1H3 and β, NR1H2). A rise in oxysterol concentration triggers transcription of genes involved in the reverse cholesterol transport and degradation into biliary acids. LXRα and β also drive several other functions including hepatic lipogenesis [2]. LXRα binds to promoters of lipogenic genes such as Fatty acid synthase (Fasn) [3] and Stearoyl-CoA desaturase 1 (Scd1) [4], thereby directly regulating *de novo* fatty acid synthesis. It also impacts indirectly on fatty acid synthesis by regulating the expression of Carbohydrate Responsive Element Binding Protein (ChREBP) [5] and Sterol Regulatory Element Binding Protein-1c (SREBP-1c) [6], whose activity also controls the hepatic expression of lipogenic enzymes. ChREBP and SREBP-1c activity are highly regulated through post-translational control of their nuclear translocation in response to glucose [7] and insulin [8], respectively.

In addition to glucose, many other nutrients are known to influence lipogenesis and triglyceride deposit in liver. Because high sugar consumption is thought to be an important factor for the obesity epidemic, a lot of attention has been paid to carbohydrates and more specifically to fructose [9]. While the relationship between fructose consumption and obesity remains debated, it is well accepted that fructose can strongly promote the expression of genes involved in fatty acid biosynthesis. However, the transcriptional mechanisms mediating the effects of fructose are not fully understood. The Peroxisome Proliferator-Activated Receptor Gamma (PPARγ, NR1C3) Coactivator-1 β (PGC-1β) was shown to be essential for the lipogenic response to fructose [10]. PGC-1β co-activates a number of other transcription factors such as SREBP-1c and LXRs. Indeed, in response to a high fructose diet, LXRs and

SREBP-1c appear to be activated by PGC-1β [11]. However, whether LXRs are required for the lipogenic response to fructose is not known.

We first questioned whether LXRs could be involved in the up-regulation of lipogenesis that leads to steatosis in response to chronic high fructose consumption. We evidenced that, unlike wild-type mice, LXR-/- mice show reduced up-regulation of hepatic lipogenic genes and triglycerides in response to high fructose. However, we found that the Liver pyruvate kinase (*Lpk*) remained sensitive to fructose in the absence of LXR. Based on this observation we questioned the additional role of ChREBP in LXR-independent regulation of *Lpk* in mice fed fructose. Consistent with other reports suggesting a role for ChREBP in fructose-induced steatosis, we revealed that ChREBP activity is enhanced in response to high fructose feeding. This occurs through increased acetylation of nuclear ChREBP. We next hypothesized that oleic acid, another lipogenic signal independent of SREBP1-c and required for fructose-induced lipogenesis, may influence hepatic lipogenesis through LXRs. We demonstrate that LXRs are required for the lipogenic response to a diet providing a high content of oleic acid. Finally, we showed that in the absence of LXRs the response to an acute challenge of dietary fructose is also compromised. Altogether, our data suggest that LXRs are involved in fructose-induced up-regulation of lipogenic gene expression that leads to hepatic steatosis.

Materials and Methods are provided as supplementary materials

Results

LXRs mediate the lipogenic effects of fructose.

We first performed an *in vivo* experiment to investigate whether both LXR isotypes may be involved in the fructose-induced changes in hepatic lipid metabolism. We stained neutral lipids with red oil on frozen liver sections from wild-type and LXR-/- mice fed fructose or not for 35 days. Fructose rich diet leads to neutral lipid accumulation in the liver of wild-type mice (Fig. 1A). In LXR-/- mice, fructose rich diet does not increase neutral lipids as much as observed in wild-type mice. We next assayed hepatic levels of triglycerides (TG), cholesterol esters and free cholesterol (Fig. 1B). LXR-/- mice show higher free cholesterol levels compared to the wild-type. In both genotypes, fructose consumption significantly reduces hepatic free cholesterol, while increasing cholesterol ester level. Finally, fructose consumption also elevates liver TG in wild-type and LXR-/- mice. Altogether, total neutral lipid accumulation in response to fructose is primarily due to TG in wild-type mice while it is a combination of cholesterol esters and TGs in LXR-/- mice.

We next performed liver fatty acids profiling by gas chromatography (Table 1A). Essential fatty acids such as C18:2ω6, C18:3ω6, C18:3ω3, C20:4ω6, C22:4ω6 and C22:6ω3 were reduced when mice of both genotypes are fed the fructose-rich diet. Conversely, fructose rich diet increased saturated and mono unsaturated fatty acids such as C16:0, C16:1ω9, C16:1ω7, C18:1ω9, C18:1ω7 and C20:1ω9. These changes occurred in mice from both genotypes. Interestingly, the decrease of C18:0 and the increase C18:1ω9 in mice of both genotypes fed the fructose-rich diet is consistent with increased *de novo* lipogenesis and Δ9-desaturation.

LXRs are known to regulate transcription of genes involved in *de novo* fatty acids synthesis. Therefore, we investigated whether changes in hepatic triglycerides were associated with changes in lipogenic gene expression (Fig. 1C). Fructose induces an up-regulation of ATP Citrate lyase (*Acl*), Acetyl CoA Carboxylase α and β (*Accα/β*), Fatty Acid Synthase (*Fasn*), Elongase of very long chain 6 (*Elovl6*), Stearoyl CoA desaturase 1 (*Scd1*) and Patatin-like phospholipase domain containing 3 (*Pnpla3*) in the liver of wild-type mice. In LXR deficient mice, fructose consumption does not trigger any up-regulation (*Accα*, *Fasn*) or only results in a more modest induction of some of the genes tested (*Acl*, *Accβ*, *Elovl6*, *Scd1*, *Pnpla3*). In accordance with mRNA levels, the corresponding protein show the same accumulation pattern (Fig. 1D). Moreover, the active phosphorylated form of ACL (P-ACL) shows an up-regulation induced by the fructose rich diet that depends on LXR.

Finally, we measured the expression of two genes coding for two transcription factors that are regulated by LXRs and involved in the regulation of lipogenesis: Sterol response element binding protein 1c (*Srebp-1c*) and Carbohydrate response element binding protein (*Chrebp)* (Fig. 1C). *Srebp-1c* mRNA level was slightly down-regulated by fructose in the wild-type mice whereas no regulation was observed in the LXR-/- mice. *Chrebp* mRNA level was slightly up-regulated by the fructose in the LXR-/- mice, while its expression was insensitive to fructose in wild-type mice. Western blot analysis of these proteins on cytoplasmic and nuclear forms (Fig. 1E) reveals that cytoplasmic forms of SREBP-1 and ChREBP as well as the nuclear form of ChREBP were not sensitive to fructose in wild-type and LXR-/-mice. The nuclear form of SREBP-1 was increased in the liver of wild-type mice fed the fructose diet, whereas this regulation does not occur in the LXR-/- mice. *Lpk* expression is highly sensitive to ChREBP activity. While nuclear levels of ChREBP remain unchanged in response to fructose, we observed that fructose diet markedly up-regulates *Lpk* expression in mice in the absence of LXRs (Fig. 1C). This suggests that LXRs and ChREBP contribute to the regulation of gene expression in response to fructose.

Fructose increases ChREBP acetylation and activity.

Fructose feeding induced a LXR-independent increase in *Lpk* expression. Therefore we postulated that, in addition to LXRs, another transcription factor was involved in the response to fructose. Under standard nutritional conditions, ChREBP is equally expressed in wild-type and in LXR-/- mice. As it acts as a major regulator of *Lpk* expression in response to glucose [12, 13], we questioned its involvement in response to fructose. Since ChREBP level was not modified by fructose, we investigated whether its activity might be post-translationally regulated. To do so, we performed another *in vivo* experiment with chronic high fructose-induced steatosis (Fig.2A) and assayed the expression of hepatic genes to confirm the effect of fructose. Hepatic mRNA levels of *Lpk*, *Acc*, *Fasn* and *Scd1* (Fig. 2B) and ACC, FASN, SCD1 protein levels (Fig. 2C) were increased following fructose consumption. This occurred without significant change in nuclear ChREBP (Fig. 2F). We next measured cytoplasmic ChREBP acetylation level (Fig. 2D). The acetylated ChREBP *vs.* non acetylated ChREBP ratio was increased by fructose (Fig. 2E). Nuclear ChREBP acetylation increased in mice fed the fructose rich diet (Fig 2F). We next assayed the binding of ChREBP to the *Lpk* promoter (Fig 2G). We identified that fructose consumption increased the binding of ChREBP on the carbohydrate responsive element (ChoRE) located in the *Lpk* promoter. Altogether, these data suggest that while fructose consumption has little effect on ChREBP nuclear level, it increases its acetylation and its transcriptional activity, which contributes to glycolysis and lipogenesis regulation in response to fructose.

Oleate induces lipogenesis via LXR.

Oleate also promotes lipogenic genes in the liver. In addition, one study has reported that oleate is required for the effect of fructose on lipogenesis [14] and that oleate influences the expression of genes involved in lipogenesis [15]. Therefore, to investigate whether LXR may be involved in the lipogenic response to oleate, we also tested the effects of dietary oleic acid *in vivo*. A reference diet (REF, 40% oleic acid) and an oleate rich diet (OLIV; 82% oleic acid) were given for 9 weeks to wild-type and LXR-/- mice. The relative composition of hepatic fatty acids was measured (Table 1B). As expected we observed an enrichment of oleic acid in the liver of wild type and LXR-/- mice fed the OLIV diet compared with mice fed the REF diet. The OLIV diet leads to neutral lipids accumulation in wild-type mice, which was not observed in mice lacking both LXRs (Fig. 3A). We next assayed hepatic cholesterol, cholesterol esters and triglycerides (Fig. 3B). OLIV diet leads to higher levels of triglycerides in the liver compared with REF diet in wild-type mice but not in LXR-/- mice. LXR-/- mice fed the OLIV diet show an increase in cholesterol as well as cholesterol esters while this did not occur in wild-type mice.

To further investigate the role of LXRs, we next measured the hepatic expression of a set of 130 genes involved in hepatic metabolism (Fig. 3C). A heatmap coupled with hierarchical classification revealed a cluster of genes up-regulated in wild type mice fed OLIV diet compared to the REF group. This up-regulation did not occur in transgenic mice lacking LXR (Fig. 3C). This cluster contained genes involved in lipogenesis such as *Acl*, *Accα*, *Fasn*, *Elovl6*, *Scd1* and *Pnpla3* (Fig. 3D). In order to confirm this regulation, we measured protein accumulation of key lipogenic proteins (ACL, its active phosphorylated form P-ACL, ACC, FASN, ELOVL6 and SCD1) (Fig. 3E). Protein levels were consistent with the LXR-dependent changes in mRNA levels. To investigate the implication of SREBP-1c and ChREBP we next performed western blots analysis for both total and active fractions of these two proteins (Fig. 3F). Total SREBP-1 and ChREBP as well as the active form of ChREBP were not significantly influenced by the diets whereas SREBP-1 nuclear expression was

elevated in wild-type mice fed OLIV but not in LXR-/-. Therefore, high oleic diet led to a specific LXR-dependent regulation of hepatic metabolism.

LXR deficiency impairs the lipogenic response to an acute fructose challenge

We next tested whether an acute challenge, *i.e.* fasting-refeeding a high fructose diet may induce LXR-dependant response. We therefore fasted wild-type and LXR-/- mice for 24h prior to refeeding a high fructose diet. Fasting led to an expected hypoglycemia and hypoinsulinemia in both wild-type and LXR-/- mice (Fig. 4A). However, refeeding showed that LXR-/- mice were hypoglycemic compared to wild-type mice upon fructose diets. We next measured the expression level of genes involved in lipogenesis. We found that *Chrebp* and *Srebp-1c* expression were reduced in fasted mice (Fig. 4B). Their expression was induced in wild-type mice after refeeding. In LXR-/- mice, refeeding resulted in a modest increase in *ChREBP* mRNA, while the expression of *SREBP-1c* was blunted. Consistent with its regulation by ChREBP, *Lpk* expression increased both in wild-type and LXR-/- mice refed the fructose diet (Fig. 4B). Moreover, the expression of lipogenic enzymes was markedly induced after fructose refeeding in wild-type mice (Fig. 4C). In most cases, it was also slightly induced in LXR-/- mice refed fructose. Finally, gene expression levels were independently normalized to the expression in fed animals from wild-type and LXR-/- genotype (Fig. 4D). This presentation as a heatmap further highlights that the effect of fructose refeeding on lipogenic gene expression but not on *Lpk* shows a marked dependence of LXRs.

Discussion

Hepatic *de novo* lipogenesis is an essential process leading to the synthesis of fatty acids in mammalian [16]. These fatty acids can be further used for the synthesis of complex lipids

such as phospholipids, ceramides, cholesterol esters and triglycerides. Such lipids are then used locally or secreted for peripheral use and for storage in adipocytes. In the liver, increased lipogenesis may lead to excess triglyceride accumulation and steatosis [17]. Steatosis can then turn into more severe liver diseases such as fibrosis, steatohepatitis or hepatocarcinoma. However, under certain circumstances *de novo* fatty acid synthesis and lipid droplets protect the liver from lipotoxic damage [18, 19]. It is therefore very important to understand the impact of nutrients on the signaling pathways that control lipid deposition and may determine liver physiology.

LXRs are nuclear receptors that play a central part in liver physiology and participate in the transcriptional control of various functions, including hepatic lipogenesis [20]. It has been shown that LXRs can directly bind LXR response element located in the promoter of *Acc*, *Fasn* and *Scd1* that encodes rate-limiting enzymes in *de novo* fatty acid biosynthesis. However, LXRs also indirectly govern lipogenesis through the regulation of two other transcription factors SREBP-1c and ChREBP.

SREBP-1c is very important in the transcriptional regulation of lipogenesis by insulin. It is markedly decreased in the liver of LXR-/- mice [6]. Fructose was shown to induce lipogenic gene expression in the liver of SREBP-1c-/- mice [14]. Therefore, we used LXR-/- mice to investigate the specific role of LXRs in the effect of fructose on gene expression. Hepatic ChREBP, which is critical for the lipogenic response to high glucose, is expressed in mice lacking LXRs [13]. Therefore, while the role of LXRs in glucose signaling has been suggested [21], it has been made clear from experiments performed in LXR-/- mice that LXRs alone are not responsible for all the effects of dietary glucose on hepatic gene expression [13]. ChREBP is essential to the transcriptional control of gene expression in response to high glucose concentration in cultured hepatocytes and to high dietary glucose *in vivo* [13, 22].

In this work we investigated the transcriptional control of hepatic fatty acid synthesis in response to fructose. Fructose is a highly lipogenic carbohydrate and its excess consumption

might be influencing the obesity epidemic and its associated liver diseases such as NAFLD. We performed a nutritional study in wild-type and LXR-/- mice fed standard or fructose-rich diet [9]. In wild-type mice, fructose consumption elevates triglycerides, lipogenic genes as well as their protein products, in a LXR-dependent manner. We also investigate the expression of SREBP-1c and ChREBP. When inactive, SREBP-1c and ChREBP are retained in the ER membrane and in the cytosol, respectively. Once activated, they are released and translocate into the nucleus where they regulate lipogenic genes expression. Fructose consumption elevates the presence of the active form of SREBP-1c in the nucleus in an LXR-dependent manner. This is consistent with the important role of PGC-1β in this process [10], as PGC-1β can act as a co-activator for SREBP-1c and LXRs [11]. Since it has been shown that fructose induces lipogenic genes in the absence of SREBP-1c [14], we conclude that LXRs are important for both the regulation of SREBP-1c in response to fructose and for the direct regulation of fructose-induced gene expression.

We also observed that the *Lpk,* a ChREBP prototypic target gene [7], as well as some other lipogenic genes are sensitive to fructose consumption in mice of both genotypes. However, ChREBP nuclear accumulation seems insensitive to fructose. Therefore, we questioned whether post-translational regulation of ChREBP may be involved in the regulation of *Lpk* and other ChREBP sensitive targets independently of LXRs. We evidenced that ChREBP acetylation levels increases in response to fructose, which correlates with an enhanced binding of ChREBP on ChoRE present in the promoter of the *Lpk*. This finding is consistent with other previous works suggesting that ChREBP acetylation is increased in response to glucose [23] and that ChREBP regulates gene expression in response to fructose [24, 25].

When they observed that fructose up-regulates hepatic lipogenic genes in response to fructose in SREBP-1c-/- mice, Myiazaki *et al.* also showed that this transcriptional response required oleic acid [14]. We wanted to investigate whether LXR might be involved in this and fed both wild-type and LXR-/- mice an oleic acid rich diet (82% oleate) and a control diet (40%

oleate). In wild-type mice, high oleic acid elevates lipogenic genes expression, lipogenic proteins content and triglycerides accumulation in the liver. These regulations are lost in LXR-/- mice. The oleic rich diet has no effects on nuclear ChREBP accumulation but promotes SREBP-1c activation in an LXR-dependent way. Interestingly, in this second experiment, lipogenic genes and *Lpk* expression were not prone to changes in dietary oleate in LXR-/- mice. Unlike fructose that promotes both LXR- and ChREBP-sensitive regulations, oleate seems to be more specifically influencing LXR-sensitive regulation of metabolism. This is consistent with our recent finding that LXRs contributes to the regulation of hepatic lipogenesis in response to essential fatty acid deficiency [26]. Importantly, liver-specific disruption of *Scd1* impairs lipogenic gene expression and this pathway can be rescued by dietary oleic acid [15]. Our data support the involvement of LXR in this pathway. Altogether, these data evidence the importance of LXRs in the hepatic response to nutrients that were known to act independently of SREBP-1c on lipogenic response.

One recent report provided evidence for an insulin-independent effect of feeding on lipogenesis [27]. This pathway is responsive to an acute fructose challenge. Therefore, we questioned whether LXRs might be important for the lipogenic response to such high fructose challenge after one day of fasting. Our data reveal for the first time that fasting followed by refeeding high fructose induces a high expression of lipogenic enzymes that is largely dependent of LXRs.

Altogether, our data support the role of LXRs in the regulation of hepatic lipogenesis in response to a fructose-rich diet both upon both chronic and acute dietary challenges. These results further emphasize the importance of LXRs at crossroads for cholesterol, fatty acid and carbohydrate metabolism. It also highlights that deciphering the respective parts of LXRs, SREBP-1c and ChREBP in hepatic lipid homeostasis may be of particular importance to better understand NAFLD progression.

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Figure Legends

Figure 1: Chronic fructose challenge induces LXR-dependent changes in hepatic lipids. (**A**) Representative Oil Red O-stained frozen sections of liver from mice of LXR+/+ and LXR-/- mice fed Control or Fructose diet (original magnification X200). Neutral lipids appear in red. (**B**) Liver triglycerides, cholesterol and cholesterol esters analyzed by gas chromatography. (**C**) *Lpk*, *Acl*, *Acc*α, *Acc*β, *Fasn*, *Elovl6*, *Scd1*, *Pnpla3*, *Srebp-1c* and *Chrebp* mRNA quantification s assayed by qPCR. (**D**) Cytoplasmic proteins P-ACL, ACL, ACC, ELOVL6, SCD1, FASN, β-Actin, and (**E**) cytoplasmic and nuclear fractions of LXR,

SREBP-1 and ChREBP, protein levels assayed by Western Blotting (n=5). Representative image is shown for each protein.

Data are the mean +/- SEM of values measured in LXR+/+ and LXR-/- mice fed chow or fructose diet. [a] Significant genotype effect. [b] Significant effect of the diet (n = 5 mice per group).

Figure 2 : Chronic fructose challenge increases ChREBP acetylation and activity. (**A**) Liver triglycerides levels. (**B**) *Lpk*, *Acc*α, *Fasn*, *Scd1* mRNA quantification assayed by qPCR. (**C**) Cytoplasmic proteins ACC, FASN, SCD1, ChREBP and β-Actin, and (**D**) Acetylated proteins, total ChREBP and acetylated ChREBP levels assayed by Western Blotting. (E) ChREBP acetylation estimated by densitometry. (F) Acetylation of Nuclear ChREBP. (G) *Lpk* promoter occupancy by ChREBP measured by ChIP. Data are the mean +/- SEM of values measured in mice fed Control or Fructose diet. [a] Significant effect of the diet (n= 5 mice per group).

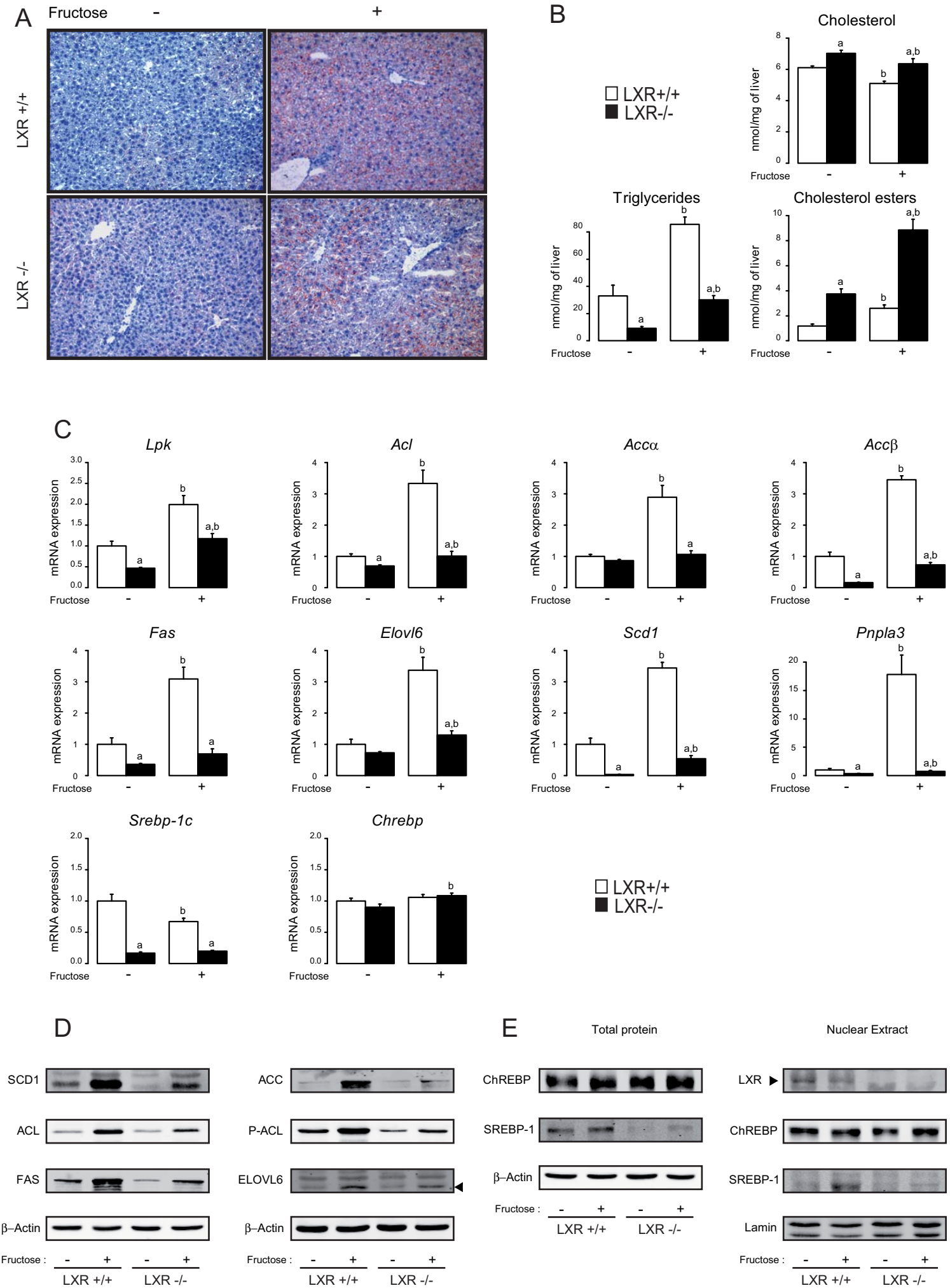
Figure 3: High oleic diet induces hepatic steatosis in LXR+/+ but not in LXR-/- mice. (**A**) Representative Oil Red O-stained frozen sections of liver from mice of both genotypes fed REF or OLIV diet (original magnification X200). Neutral lipids appear in red. (**B**) Liver triglycerides, cholesterol and cholesterol esters analyzed by gas chromatography. (**C**) Hierarchical classification coupled with a heatmap of hepatic gene expression. mRNA levels (130 genes related to lipid metabolism and nuclear receptor signaling) measured by qPCR. Gene expression from animals fed OLIV diets in both genotypes and normalized to the expression upon REF diet within the same genotype. (**D**) *Lpk*, *Acl*, *Acc*α, *Acc*β, *Fasn*, *Elovl6*, *Scd1*, *Pnpla3*, *Srebp-1c* and *Chrebp* mRNA quantification assayed by qPCR. (**E)** Cytoplasmic proteins P-ACL, ACL, ACC, ELOVL6, SCD1, FASN, β-actin, and cytoplasmic and (**F**)

nuclear accumulation of LXR, SREBP-1 and ChREBP assayed by Western Blotting. Representative image is shown for each protein.

Data are the mean +/- SEM of values measured in LXR+/+ and LXR-/- mice fed REF or OLIV diet. [a] Significant genotype effect. [b] Significant difference versus REF diet (n=6 mice per group).

Figure 4: Acute fructose challenge induces LXR-dependent changes in gene expression. (**A**) Circulating glucose and insulin levels. (B) *Srebp-1c, Chrebp*, *Lpk* mRNA quantification was assayed by qPCR (**C**) *Acl*, *Acc*α, *Acc*β, *Fasn, Elovl6*, *Scd1, Pnpla3* mRNA quantification was assayed by qPCR. Data are the mean +/- SEM of values measured in LXR+/+ and LXR-/- mice fed (Chow), fasted for 24h (fasted), fasted and refed fructose (FRfru). [a] Significant genotype effect. [b] Significant difference versus Chow. [c] Significant difference versus Fasted (n = 6 mice per group). (**D**) Hierarchical classification of hepatic gene expression. mRNA level (16 genes directly related to glycolysis and lipogenesis) was measured by qPCR. Gene expression has been normalized to the expression in the fed state (Chow) in each genotype.

Figure 1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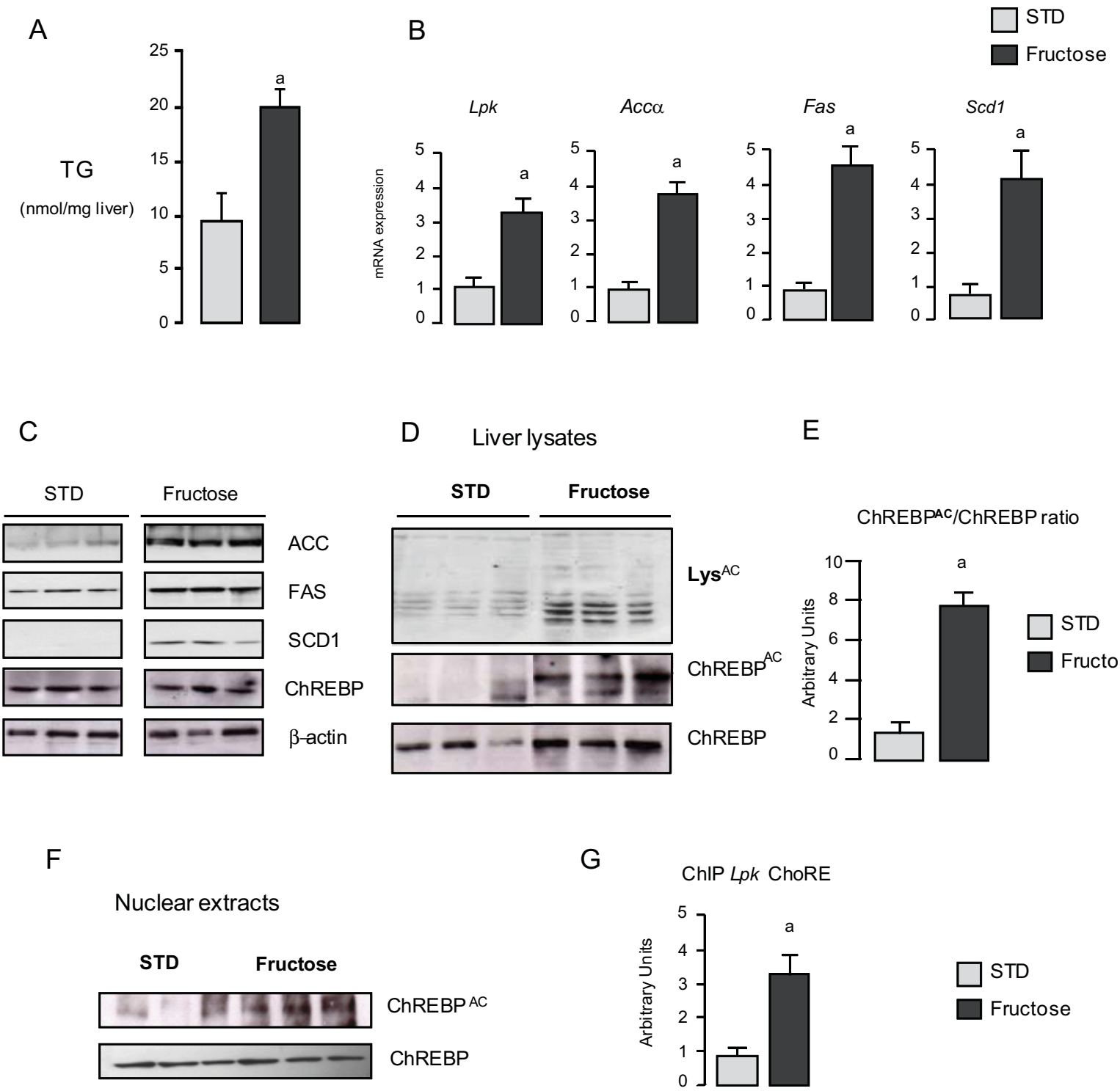
Fructose
-
+
LXR +/+
LXR -/-
B
LXR+/+
LXR-/-
Cholesterol
Triglycerides
Cholesterol esters
nmol/mg of liver
Fructose
C
Lpk
Acl
Accα
Accβ
Fas
Elovl6
Scd1
Pnpla3
Srebp-1c
Chrebp
mRNA expression
LXR+/+
LXR-/-
D
SCD1
ACL
FAS
β–Actin
ACC
P-ACL
ELOVL6
Fructose :
LXR +/+
LXR -/-
E
Total protein
Nuclear Extract
ChREBP
SREBP-1
β–Actin
LXR
Lamin

Figure 2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(nmol/mg liver)
25
20
15
10
5
0
a
B
STD
Fructose
Lpk
Accα
Fas
Scd1
mRNA expression
5
4
3
2
1
0
a
C
STD
Fructose
ACC
FAS
SCD1
ChREBP
β-actin
D
Liver lysates
STD
Fructose
Lys^AC
ChREBP^AC
ChREBP
E
ChREBP^AC/ChREBP ratio
Arbitrary Units
10
8
6
4
2
0
a
STD
Fructose
F
Nuclear extracts
STD
Fructose
ChREBP^AC
ChREBP
G
ChIP Lpk ChoRE
Arbitrary Units
5
4
3
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0
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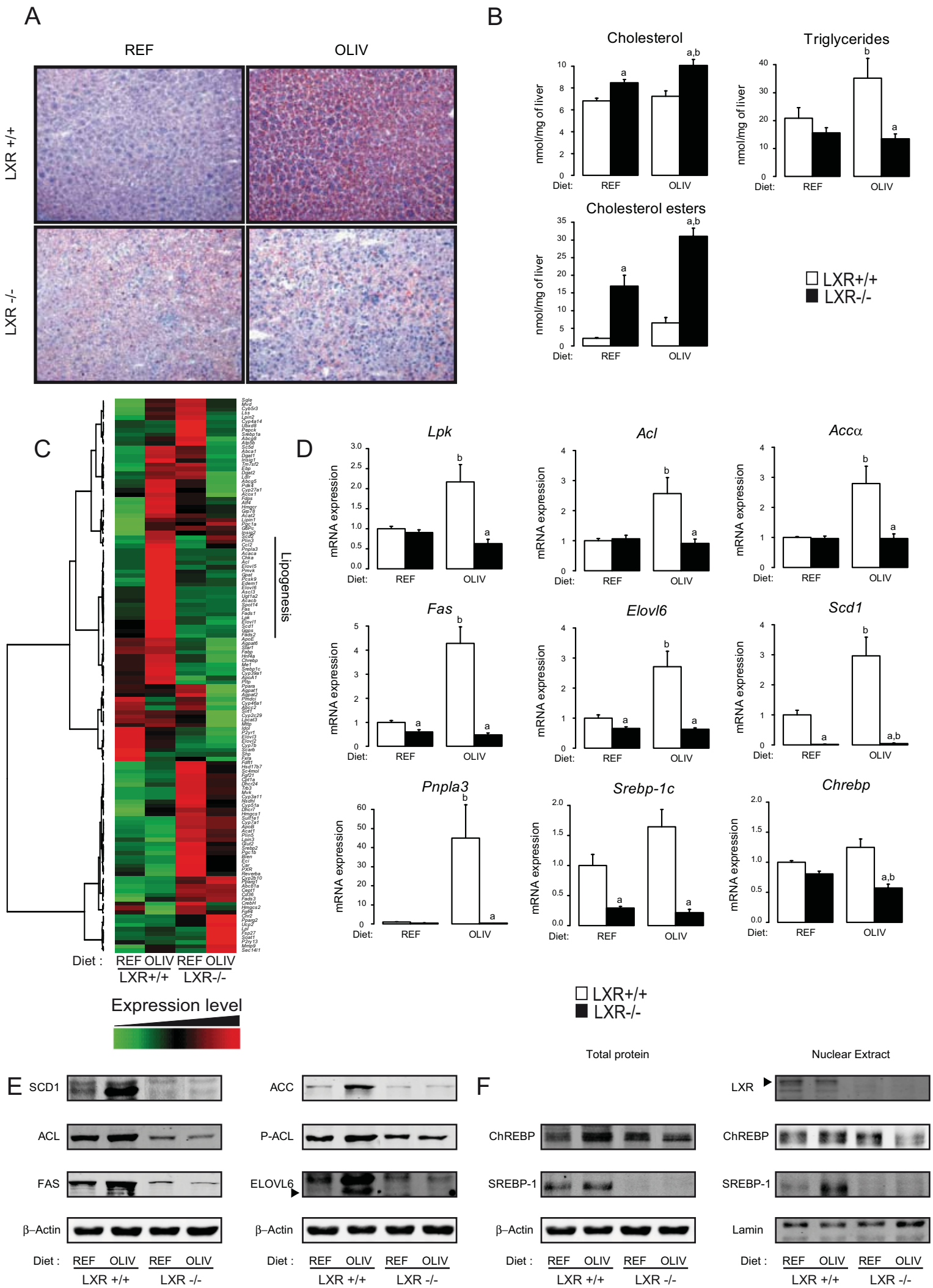
STD
Fructose

Figure 3


A
REF
OLIV
LXR +/+
LXR -/-
B
Cholesterol
Triglycerides
Cholesterol esters
nmol/mg of liver
Diet:
REF
OLIV
LXR+/+
LXR-/-
C
Lipogenesis
Diet : REF OLIV REF OLIV
LXR+/+ LXR-/-
Expression level
D
Lpk
Acl
Accα
Fas
Elovl6
Scd1
Pnpla3
Srebp-1c
Chrebp
mRNA expression
LXR+/+
LXR-/-
E
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ACC
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P-ACL
FAS
ELOVL6
β–Actin
Diet : REF OLIV REF OLIV
LXR +/+ LXR -/-
F
Total protein
Nuclear Extract
LXR
ChREBP
SREBP-1
β–Actin
Lamin
Diet : REF OLIV REF OLIV
LXR +/+ LXR -/-

Figure 4

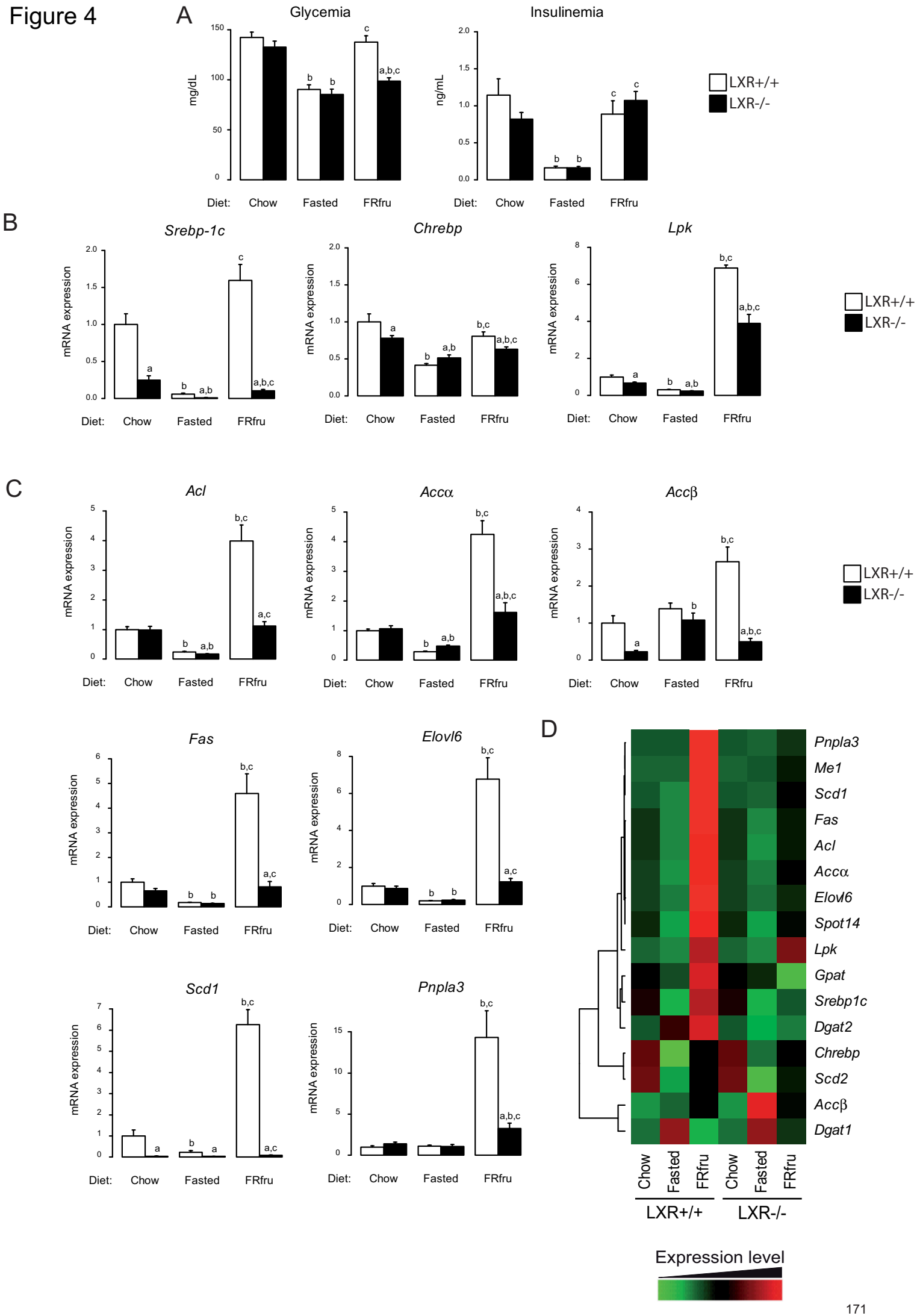
A
Glycemia
Insulinemia
mg/dL
ng/mL
LXR+/+
LXR-/-
Diet: Chow Fasted FRfru
B
Srebp-1c
Chrebp
Lpk
mRNA expression
C
Acl
Accα
Accβ
Fas
Elovl6
Scd1
Pnpla3
D
Pnpla3
Me1
Scd1
Fas
Acl
Accα
Elovl6
Spot14
Lpk
Gpat
Srebp1c
Dgat2
Chrebp
Scd2
Accβ
Dgat1
Chow Fasted FRfru Chow Fasted FRfru
LXR+/+ LXR-/-
Expression level

Table 1

A

	LXR+/+		LXR-/-	
	CTRL	FRU	CTRL	FRU
C14:0	0,237	0,276[a]	0,110	0,177[a,b]
C16:0	21,166	25,489[b]	21,296	25,310[b]
C16:1 ω9	0,731	1,262[b]	0,704	0,927[a,b]
C16:1 ω7	2,207	4,846[b]	2,378	5,016[b]
C18:0	9,417	4,807[b]	17,853[a]	11,799[a,b]
C18:1 ω9	15,642	37,385[b]	11,008[a]	26,900[a,b]
C18:1 ω7	3,087	7,695[b]	2,878	7,504[b]
C18:2 ω6	21,217	5,733[b]	21,650	9,110[a,b]
C18:3 ω6	0,220	0,060[b]	0,255	0,097[a,b]
C18:3 ω3	0,542	0,111[b]	0,389[a]	0,123[b]
C20:1 ω9	0,545	1,168[b]	0,455	0,632[a,b]
C20:2 ω6	0,603	0,723	0,541	0,868[b]
C20:3 ω6	1,452	1,339	1,298	1,538
C20:4 ω6	11,291	5,528[b]	11,743	6,393[b]
C20:3 ω3	0,062	0,030	0,088	0,039
C20:5 ω3	0,230	0,084	0,161	0,172
C22:4 ω6	0,543	0,178[b]	0,289[a]	0,150[b]
C22:5 ω6	0,589	0,422	0,372[a]	0,321
C22:5 ω3	0,588	0,107[b]	0,320[a]	0,213
C22:6 ω3	6,706	2,698[b]	5,933	2,611[b]
C24:1 ω9	0,556	0,061[b]	0,279	0,100[b]

B

	LXR+/+		LXR-/-	
	REF	OLIV	REF	OLIV
C14:0	0,263	0,488[b]	0,237	0,169[a]
C16:0	22,53	22,93	18,516[a]	16,280[a]
C16:1 ω9	0,791	1,056	0,767	1,306
C16:1 ω7	3,385	4,805	4,977[a]	5,342
C18:0	12,057	10,704	13,81	11,947
C18:1 ω9	16,605	32,629[b]	14,215	28,523[b]
C18:1 ω7	3,578	6,128[b]	4,425	6,787b
C18:2 ω6	15,968	5,450[b]	20,862[a]	10,809[a,b]
C18:3 ω6	0,383	0,355	0,352	0,416
C18:3 ω3	0,313	0,121[b]	0,663[a]	0,126[b]
C20:1 ω9	0,383	0,563[b]	0,477	0,792[a,b]
C20:2 ω6	0,393	0,000[b]	0,426	0,000[b]
C20:3 ω9	0	1,584[b]	0	1,627[b]
C20:3 ω6	1,516	1,616	1,569	1,45
C20:4 ω6	12,859	6,360[b]	10,906	8,438
C20:3 ω3	0,133	0,067	0,315[a]	0,185
C20:5 ω3	0,281	0,053	0,396	0,326
C22:4 ω6	0,214	0,196	0,282	0,380[a]
C22:5 ω6	0,51	0,812[b]	0,234[a]	0,507[a,b]
C22:5 ω3	0,263	0,794[b]	0,502	0,233[a]
C22:6 ω3	7,202	2,753[b]	5,78	2,966[b]
C24:1 ω9	0,373	0,537	0,291	1,393[b]

Table 1: Effects of fructose and oleic acid on hepatic fatty acid profile in LXR+/+ and LXR-/- mice. Fatty acids were analyzed by gas chromatography (n=5). (**A**) Data are the mean % of fatty acid masses measured in LXR+/+ and LXR-/- mice fed Control or Fructose diet. [a] Significant genotype effect. [b] Significant difference versus control diet. (**B**) Data are the mean % of fatty acid masses measured in LXR+/+ and LXR-/- mice fed REF or OLIV diet. [a] Significant genotype effect. [b] Significant difference versus REF diet.

SUPPLEMENTARY MATERIAL

MATERIAL AND METHODS

Animals and treatments.

For the effect of long term fructose study on LXR signaling, 7 week-old LXRαβ double deficient (LXR-/-) and wild-type mice with a mixed C57BL6J/129SVJ genetic background (Repa *et al.*, 2000) were fed *ad libitum* a control diet (Harlan, 2018, France) and a fructose rich diet (SAFE U8960A01R, Augy, France), with free access to water. For the long-term fructose study on ChREBP's activity, similar diets and 8 week-old mice from a C57BL6/J background were used. These fructose diets were given for 30 days.

In the nutritional study to investigate the effect of high oleic acid content, 8 week-old LXRαβ double deficient and wild-type mice with a mixed C57BL6/129SVJ genetic background were fed *ad libitum* for 9 weeks (pellets prepared by UPAE-INRA, Jouy-en-Josas, France, replaced twice a week) with free access to water. Diets were isocaloric and contain 5% fat (w/w). Oils used for experimental diet preparation were grape seed and colza oils (50/50) for the reference diet (REF) and olive oil for the OLIV diet.

For the acute fructose challenge, 12 week-old LXRαβ double deficient and wild-type mice with a mixed C57BL6/129SVJ genetic background were fed *ad libitum* a control diet (Harlan, 2018, France) and a fructose rich diet (D08040107, Research DIETS, New Brunswick), with free access to water. Mice were fasted for 24h and refed or not for 24h with the fructose diet.

Mice were sacrificed at ZT14. All mice were bred at INRA's transgenic rodent facility at 22 ± 2°C. *In vivo* studies were conducted under E.U. guidelines for the use and care of laboratory animals and were approved by an independent ethic committee.

Blood and organ sampling. Blood was collected at the submandibular vein in heparin-coated capillaries. Plasma was prepared by centrifugation (1500×g, 10 min) and kept at -80°C until use. Following euthanasia, tisues were removed, weighed, dissected, snap-frozen in liquid nitrogen and stored at -80°C until use.

Gene expression studies. Total RNA was extracted with TRIzol® reagent (Invitrogen, Cergy Pontoise, France). For real-time quantitative PCR (qPCR), total RNA samples (2 µg) were reverse-transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Courtaboeuf, France). Primers for SYBR Green assays are presented in Supplemental Table 1. Amplifications were performed on an ABI Prism 7300 Real Time PCR System (Applied Biosystems). QPCR data were normalized by TATA-box binding protein (TBP) mRNA levels and analyzed with LinRegPCR.

Immunoblot analysis. Protein extracts were prepared using the Proteo-Jet cytoplasmic and nuclear extraction kit (Fermentas, Saint-Rémy-lès-Chevreuses, France). Following separation by SDS-PAGE liver proteins were probed with primary antibodies from Cell Signaling (β-ACTIN: 4970 ; LAMIN A/C: 2032 ; ACLY: 4332 ; ACLY-P: 4331 ; ACC: 3662 ; FASN: 3189), Abcam (ELOVL6: 69857), Santa Cruz Biotechnology (SCD1: sc-14719; LXR: sc-13068), Lab Vision (SREBP-1: MS-1207-P1ABX), Novus Biological (CHREBP: nb400-135) and secondary antibodies from Biotium (CF680 or CF770-labeled). The images were analyzed on the Odyssey Infrared Imaging system (Li-Cor, Lincoln, NE). Band intensities were normalized to those of β-ACTIN or LAMIN A/C.

ChREBP acetylation. ChREBP was immunoprecipitated as previously described (Bricambert *et al.*, 2011) from 1 µg of liver proteins prior to immunoblotting. Acetylation level was assessed with anti-lysine antibody (Cell signaling).

ChREBP DNA binding. ChIP analysis of ChREBP binding to *Lpk* promoter was performed as described elsewhere (Dentin *et al.*, 2008).

Biochemical assays. Hepatic lipid content, FA composition and plasma biochemistry was performed as described earlier (Marmugi *et al.*, 2012).

Histology. Frozen liver samples were embedded in Neg 50 (Fisher Scientific, Courtaboeuf, France). Sections (5 µm, Leica RM2145 microtome, Nanterre, France) were stained with Oil-Red-O and visualized with a Leica DFC300 camera (Leica).

Statistical analysis. All data were analyzed using R (www.r-project.org). Data are expressed as the mean±SEM. Differential effects were analyzed by Anova followed by Student t-tests with a pooled variance estimate. A p-value ≤ 0.05 was considered significant.

Discussion et conclusion générale

Discussion et conclusion générale

Au cours de ces travaux, nous nous sommes intéressés à la régulation transcriptionnelle de la lipogenèse hépatique par le Liver X Receptor dans différents contextes.

Dans un premier temps, nous nous sommes intéressés au dialogue croisé entre les activités des récepteurs nucléaires PPARα et LXR par une approche pharmacologique. Nous avons montré que l'activation de PPARα par le fénofibrate induit l'expression de gènes impliqués dans la lipogenèse. L'activation de l'expression des gènes de la lipogenèse par un agoniste de LXR ne dépend pas de la présence de PPARα, cependant l'induction de l'expression de ces gènes par le fénofibrate est diminuée en l'absence de LXR, révélant donc l'importance de ce récepteur dans la régulation des effets de PPARα. L'activation de LXR induit aussi une augmentation de l'expression de gènes de l'oxydation des acides gras, qui est réduite chez les souris transgéniques n'exprimant pas *Pparα*. Cette étude met en évidence l'interrelation entre ces récepteurs nucléaires. Il a été montré que LXR et PPARα partageaient des éléments de réponse sur des promoteurs de gènes de la lipogenèse (Boergesen, Pedersen et al. 2012). L'interrelation entre ces deux récepteurs nucléaires est donc probable à un niveau génomique. Cependant cette régulation peut aussi être envisagée du point de vue du concept émergeant de lipoprotection ou de « lipoexpediency » (Lodhi, Wei et al. 2011). Le rôle des molécules lipidiques a pendant longtemps été envisagé dans les fonctions de stockage et de structures membranaires respectivement pour les triglycérides et les phospholipides. Mais il s'avère que les molécules lipidiques issues de la lipogenèse ont un rôle dans la signalisation cellulaire. Il a notamment été démontré que, au cours de la lipogenèse, les produits de synthèse de la FAS pouvaient être incorporés dans des lipides complexes et activer PPARα (Chakravarthy, Pan et al. 2005). Aussi il est possible que certains des effets dépendants de LXR et observés chez les souris PPARα-/- révèlent l'effet dépendant de PPARα des produits de la lipogenèse *de novo*.

L'approche utilisée dans notre étude est une approche pharmacologique. Cependant l'expression de LXR et de PPARα est soumise a une rythmicité circadienne (Yang, Downes et al. 2006). En effet LXR est plus exprimé pendant la nuit et est important pour réguler la transcription de gènes conduisant à l'anabolisme lipidique durant la phase d'alimentation des

souris. PPARα est plus exprimé pendant le jour, et joue donc un rôle important quand les souris sont à jeûn, pour réguler la transcription des gènes impliqués dans l'oxydation des acides gras. Ces interrelations que nous avons observées pourraient permettre de mieux diriger les flux de molécules lipidiques pendant les transitions nourriture/jeûne et inversement. Que cette interrelation se produise par le partage de site de liaison sur le génome ou *via* la production de molécules lipidiques signalisatrices, il nous paraît opportun d'étudier cette interrelation entre ces récepteurs nucléaires pendant les transitions du rythme circadien.

Nous nous sommes également intéressés au rôle de LXR dans un modèle de colite induite expérimentalement par du sulfate de dextran sodique (DSS). Il a été montré qu'une colite induite par le DSS est associée à une diminution de l'expression de *Scd1* dans le foie (Chen, Shah et al. 2008). Ces auteurs montrent aussi que les souris transgéniques invalidées pour *Scd1* sont plus sensibles à la colite induite par le DSS. Cependant, ces résultats ont été remis en question car les souris transgéniques invalidées pour *Scd1* boivent plus et sont donc plus exposées au DSS administré dans l'eau de boisson (Macdonald, Bissada et al. 2009). Cependant, dans notre modèle, nous confirmons une diminution de l'expression hépatique de *Scd1* mais également une diminution de l'expression de *Accα* et de *Fas* en réponse au DSS. Le DSS induit aussi une augmentation de la perméabilité paracellulaire et transcellulaire.

Dans ce modèle, l'activation pharmacologique de LXR est sans effet sur l'inflammation au niveau du colon. Cependant, l'agoniste de LXR tend à diminuer la perméabilité paracellulaire induite par le DSS et diminue significativement la perméabilité transcellulaire. La modulation de la perméabilité intestinale par LXR n'a, à ce jour, pas été l'objet d'études et est un point important à aborder.

L'activation pharmacologique de LXR inhibe l'augmentation de l'expression de gènes codant pour les protéines de la phase aigue hépatique en réponse au DSS. De plus, l'effet d'une activation pharmacologique de LXR ne restaure pas complètement l'expression des gènes de la lipogenèse en réponse à l'inflammation induite par le traitement au DSS. Cette étude préliminaire nécessite indéniablement de plus profondes investigations mais quelques pistes pourraient être intéressantes à explorer. La caractérisation du statut inflammatoire est à étudier plus en détail. S'il s'avérait que l'activation de LXR améliore bien l'inflammation dans le foie, il pourrait être intéressant de caractériser l'influence de l'inflammation sur la lipogenèse hépatique et réciproquement.

Une partie des travaux réalisés pendant cette thèse ont également porté sur l'implication de LXR dans la régulation de la lipogenèse par des facteurs nutritionnels tels que la déficience en acides gras essentiels ou la quantité de fructose dans l'alimentation.

La déficience en acides gras essentiels de la famille n-3 conduit à une augmentation de la lipogenèse et à une accumulation de triglycérides dans le foie (Sekiya, Yahagi et al. 2003; Alwayn, Javid et al. 2004; Pachikian, Essaghir et al. 2011). Les acides gras de la famille n-3 contribuent à la diminution de la lipogenèse en réprimant l'expression de *Srebp-1c* (Ou, Tu et al. 2001) ainsi que sa maturation post-traductionnelle (Hannah, Ou et al. 2001). Les acides gras polyinsaturés essentiels inhibent aussi la maturation de ChREBP (Dentin, Benhamed et al. 2005). Il a également été montré *ex vivo* que les AGPIs peuvent inhiber l'activation de LXR par ses ligands (Ou, Tu et al. 2001; Svensson, Ostberg et al. 2003). Cependant ces résultats sont contreversés (Pawar, Xu et al. 2002; Pawar, Botolin et al. 2003).

Nous avons donc étudié, *in vivo*, le rôle de LXR en fonction de la nature des acides gras alimentaires. Nous avons utilisé des régimes avec des concentrations variables en acides gras essentiels. La déficience en acides gras essentiels induite par un régime dont les acides gras composant les triglycérides sont exclusivement saturés, conduit à une augmentation de l'expression des gènes de la lipogenèse associée à une accumulation de triglycérides hépatiques dans le foie. Ces modifications se produisent uniquement chez les souris de type sauvage, révélant l'implication de LXR dans les effets lipogéniques des acides gras saturés. L'absence de LXR révèle cependant le potentiel inflammatoire des acides gras saturés et l'activation de LXR protège donc de cette inflammation. Cependant nos données ne permettent pas de déterminer si la protection observée chez les souris de type sauvage dépend des propriétés anti-inflammatoires propres à LXR ou de l'estérification de ces acides gras saturés dans des lipides neutres.

Nous avons également observé une diminution de la masse du tissu blanc péri-gonadique chez les souris de type sauvage nourries avec le régime contenant des acides gras saturés. Cette modulation est dépendante de la présence de LXR. Cette diminution se produit aussi chez les souris nourries avec le régime riche en oléate et également de façon LXR dépendante. Ces observations sont à mettre en parallèle avec une étude dans laquelle des souris *ob*/*ob* sont traitées avec un agoniste de LXR (Archer, Stolarczyk et al. 2013). Dans cette étude l'activation de LXR conduit à la dimminution de la masse du tissu adipeux viscéral, à l'augmentation de la masse du tissu adipeux sous-cutané, à une dimminution du

statut inflammatoire dans les tissus adipeux ainsi qu'à une augmentation de la sensibilité à l'insuline (Archer, Stolarczyk et al. 2013). Cette étude est la première à rapporter l'utilisation à long terme d'un agoniste de LXR. Ce schéma expérimental pourrait être utilisé afin de caractériser le rôle de LXR dans la transition stéatose/NASH. En effet, l'hypothèse que la stéatose hépatique n'est pas forcément associée à des effets délétères, notamment au niveau de la signalisation par l'insuline, émerge (Sun and Lazar 2013). Dans leur étude, Archer *et al.* observent une augmentation de la quantité de triglycérides dans le foie des souris traitées avec l'agoniste de LXR mais ne détaillent pas le statut hépatique. Il serait donc intéressant d'étudier ce paramètre dans le cadre d'une activation à long terme de LXR (Archer, Stolarczyk et al. 2013). De plus, si la stéatose résultante n'était pas la cause d'effets hépatiques délétères, l'étude de l'activation de LXR dans des modèles de NASH permettrait de caractériser les bénéfices et les risques associés à la modulation de l'activité de ce récepteur nucléaire impliqué dans le développement de la stéatose, dans la réponse inflammatoire et dans la fibrose.

Dans notre étude de nutrition, nous avons également mis en évidence que la déficience en acides gras essentiels induit l'augmentation de l'expression de gènes responsables de la synthèse de cholestérol, notamment de deux gènes, *Lss* et *Sqle,* qui codent pour deux enzymes dont la modulation est importante dans la génération du 24-25-epoxycholestérol, un ligand naturel de LXR (Wong, Quinn et al. 2007). De plus, la modulation de l'expression de ces gènes se produit dans le foie des souris des deux génotypes. Nous avons tenté, par deux approches différentes, de voir si l'induction de l'expression des gènes de la voie de synthèse du cholestérol pouvait générer un signal conduisant à l'activation de LXR. De façon directe, nous avons d'abord tenté de doser les oxystérols hépatiques. Cependant, ces composés sont présents en quantité très faible dans la cellule et sont extrêmement labiles. Les résultats que nous avons obtenus en chromatographie en phase gazeuse couplée à un spectromètre de masse présentent une variabilité trop importante pour donner un résultat convainquant. De façon indirecte, nous avons ensuite tenté d'inhiber la voie de synthèse du cholestérol en utilisant des statines sur des souris nourries avec un régime déficient en acides gras essentiels. Une fois encore, les résultats obtenus ne nous ont pas permis de confirmer ou d'infirmer notre hypothèse, la durée de régime retenue étant trop courte. Une autre approche envisagée consisterait à surexprimer dans le foie des souris de type sauvage et invalidées pour les deux isoformes de *Lxr* une forme tronquée constitutivement active de SREBP-2 ce qui aurait pour

effet d'induire la synthèse de cholestérol (Horton, Shimomura et al. 1998; Horton, Shah et al. 2003).

Dans cette même étude de nutrition utilisant différents types d'acides gras chez des souris de type sauvage et invalidées pour *Lxr*, nous avons mis en évidence une augmentation importante (d'un facteur supérieur à deux cent fois) de l'expression de *Scd1* dans la muqueuse du jéjunum. Cette régulation est dépendante de LXR et se produit aussi dans le duodénum mais pas dans l'iléon. La question se pose alors de savoir quel est le rôle de l'activation de *Scd1* dans la muqueuse intestinale. Il existe peu de données quand à l'existence et la régulation transcriptionnelle de la lipogenèse intestinale. Aussi, il serait intéressant de vérifier que l'augmentation de *Scd1* contribue à l'enrichissement de la muqueuse intestinale en acide oléique et/ou palmitoléique. Ensuite, il serait très intéressant de comprendre le rôle physiologique de cette régulation intestinale. Pour cela, nous envisageons l'utilisation de souris transgéniques avec une invalidation spécifiquement intestinale de *Scd1*. Le phénotype de ces souris pourra être étudié dans différentes conditions nutritionnelles (carences en lipides insaturés, régimes obésogènes) et pharmacologiques (activation de LXR, manipulation du microbiote par les antibiotiques), afin de mesurer l'importance physiologique de l'expression intestinale de *Scd1*.

Au cours d'une autre étude de nutrition, nous nous sommes intéressés à l'implication de LXR dans la stéatose induite par un régime riche en fructose. Nous avons montré que le fructose induit, chez les souris de type sauvage, une augmentation des gènes de la lipogenèse ainsi qu'une accumulation de triglycérides dans le foie. Cette régulation se produit aussi chez les souris transgéniques invalidées pour *Lxr* mais dans une ampleur moindre. Nous en avons déduit qu'un autre mécanisme pouvait être impliqué dans cette régulation chez les souris invalidées pour *Lxr*. Le régime riche en fructose n'induit pas d'augmentation de la forme active de ChREBP dans le noyau des cellules hépatiques des souris des deux génotypes. En collaboration avec l'équipe de Catherine Postic (INSERM U1016, Paris) nous avons montré que la consommation de fructose induisait une acétylation de ChREBP conduisant à l'augmentation de la fixation de ce facteur de transcription sur le promoteur d'un de ses gène cible, *Lpk*. Il a été montré que la consommation de fructose nécessitait l'ajout d'acide oléique dans l'alimentation pour induire une stéatose hépatique chez les souris transgéniques invalidées pour *Scd1* (Miyazaki, Dobrzyn et al. 2004). Nous avons donc nourris des souris de type sauvage et invalidées pour *Lxr* avec un régime contrôle et un régime dont les acides gras composant les triglycérides sont majoritairement de l'acide oléique. Nous avons montré que le

régime contenant de l'acide oléique induit une augmentation de l'expression des gènes de la lipogenèse et une accumulation de triglycérides dans le foie. Cette régulation est strictement dépendante de la présence de LXR.

ChREBP contrôle l'expression de *Scd1* (Benhamed, Denechaud et al. 2012). Dans notre modèle de lipogenèse induite par le fructose l'expression de *Scd1* est induite dans les deux génotypes. Les données de Miyazaki *et al.* ainsi que les nôtres font émerger l'hypothèse que SCD1 pourrait être le médiateur de l'activation de LXR en réponse à un régime riche en fructose. Nous envisageons donc de sur-exprimer *Scd1* dans le foie de souris de type sauvage et invalidées pour *Lxr* afin de voir si *Scd1* induit l'activité de LXR en produisant de l'acide oléique endogène. A partir de cette hypothèse, un parallèle peut être fait avec l'étude précédente. Etant donné que l'acide oléique alimentaire induit l'activation de LXR, il est envisageable de penser que l'induction de *Scd1* observée dans la muqueuse intestinale par un régime déficient en acides gras essentiels (contenant des acides gras saturés) pourrait induire l'activation de LXR dans le foie. Dans ce sens, une surexpression de *Scd1* dans l'intestin serait intéressante à étudier pour mesurer les conséquences *in vivo* y compris au niveau hépatique.

Dans l'ensemble, ce travail montre que le Liver X Receptor est un déterminant majeur de l'activité lipogénique hépatique. Cela est en accord avec les effets récemment décrits d'un premier agoniste inverse hépato-spécifique capable de prévenir la stéatose induite par un régime hyper lipidique. Nos résultats montrent que LXR est au cœur des régulations pharmacologiques, inflammatoires et nutritionnelles de la lipogenèse. Il nous reste à établir comment ces interactions s'inscrivent dans les réseaux de régulation impliquant d'autres facteurs de transcription importants (ChREBP, SREBP-1c, SREBP-2) et récepteurs nucléaires (PPARα, PPARβ, PPARγ). Les effets observés chez les souris LXR-/- soumises à un challenge aigue au fructose nous paraissent rejoindre celles faites récemment par Haas et al. (Haas, Miao et al. 2012). Il nous semble qu'il est important d'étudier le rôle de LXR dans la signalisation mTOR et l'homéostasie hépatique en réponse au fructose. Enfin, le rôle hépatique mais surtout intestinal de la régulation transcriptionnelle de Scd1 nous paraît également une perspective de recherche intéressante.

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Annexes

Les travaux présentés dans ce mémoire ont donné lieu aux publications et communications suivantes :

PUBLICATIONS

Ducheix S, Montagner A, Polizzi A, Lasserre F, Marmugi A, Bertrand-Michel J, et al. Essential fatty acids deficiency promotes lipogenic gene expression and hepatic steatosis through the Liver X Receptor. J Hepatol 2013. In Press. *IF= 9.3*

Ducheix S, Podechard N, Lasserre F, Polizzi A, Pommier A, Murzilli S, et al. A systems biology approach to the hepatic role of the oxysterol receptor LXR in the regulation of lipogenesis highlights a cross-talk with PPARalpha. Biochimie 2013;95:556-567. *IF = 3*

Ducheix S, Lobaccaro JM, Martin PG, Guillou H. Liver X Receptor: an oxysterol sensor and a major player in the control of lipogenesis. Chem Phys Lipids 2011;164:500-514. *IF = 2.2*

Marmugi A, **Ducheix S**, Lasserre F, Polizzi A, Paris A, Priymenko N, et al. Low doses of bisphenol A induce gene expression related to lipid synthesis and trigger triglyceride accumulation in adult mouse liver. Hepatology 2012;55:395-407. *IF = 11,6*

Demeure O, Lecerf F, Duby C, Desert C, **Ducheix S**, Guillou H, et al. Regulation of LPCAT3 by LXR. Gene 2011;470:7-11. *IF = 2.3*

COMMUNICATIONS ORALES

Ducheix S., Podechard N., Bertrand-Michel J., Polizzi A., Lasserre F., Marmugi A., Baron S., Lobaccaro J.M., Martin P., Guillou H. A nutrigenomic approach reveals that LXR is required for hepatic steatosis induced by essential fatty acid deficiency. 9th Conference of the International Society for the Study of Fatty Acids and Lipoproteins (ISSFAL), Maastricht (Pays-Bas), 29 Mai- 2 Juin 2010.

Podechard N., **Ducheix S.**, Bertrand-Michel J., Marmugi A., Lasserre F., Polizzi A., Pommier A., Lobaccaro J.-M., Martin P., Guillou H. Dual extraction of both lipid and mRNA from a single sample. 9th 7th Congrès de Lipidomique (GERLI), Anglet-Biarritz (France), 3 - 6 Octobre 2010.

Ducheix S., Podechard N., Bertrand-Michel J., Lasserre F., Polizzi A., Lobaccaro J.-M., Pineau T., Martin P., Guillou H. Oxysterol signaling in fatty acid metabolism. LipidomicNet-ENOR Joint Workshop, Munich (Allemagne), 19-20 November 2010.

Ducheix S., Guillou H. LXR: An oxysterol sensor and a master regulator of lipogenesis ENOR meeting, 22-24 Septembre 2011, Rome

Ducheix S., Benhamed F., Montagner A., Polizzi A., Lasserre F., Bertrand-Michel J., Lobaccaro J.M., Moschetta A., Postic C., Guillou H. Régulation du Liver X Receptor par le fructose et développement de la stéatose hépatique. Journées francophones de nutrition, 7-9 Décembre 2011, Reims, France

Ducheix S., Benhamed F., Montagner A., Polizzi A., Lasserre F., Bertrand-Michel J., Lobaccaro J.-M., Moschetta A., Postic C., Guillou H. Role of the Liver X Receptor in the transcriptional control of lipogenesis: insights from nutrigenomic studies. 3ème colloque de l'ITMO CMN, 29 Mars 2012, Paris, France.

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Ducheix S., Benhamed F., Montagner A., Polizzi A., Lasserre F., Bertrand-Michel J., Martin P, Lobaccaro J.-M., Ferrier L., Moschetta A., Postic C., Guillou H. LXR deficiency protects from steatosis induced by dietary fructose. 4th Health Food Symposium, SAS, 18-19 July 2012, Toulouse, France

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COMMUNICATIONS AFFICHEES

Ducheix S., Bertrand-Michel J., Lobaccaro J.-M., Martin P., Guillou H.. A role for Liver X Receptor in steatosis induced by dietary fatty acids. 3ème Colloque de Génomique Fonctionnelle du Foie, Rennes, 11-12 Mars 2010.

Marmugi A., Mselli-Lakhal L., **Ducheix S.**, Guillou H., Martin P. A potential role for PPARalpha in the regulation of thyroid hormone levels. 3ème Colloque de Génomique Fonctionnelle du Foie, Rennes, 11-12 Mars 2010.

Podechard N., **Ducheix S.**, Bertrand-Michel J., Marmugi A., Lasserre F., Polizzi A., Pommier A., Lobaccaro J.-M., Martin P., Guillou H. Analysis of LXR-dependent biological signatures after a dual extraction of both mRNAs and lipids from a single liver sample. 9th Conference of the International Society for the Study of Fatty Acids and Lipoproteins (ISSFAL), Maastricht (Pays-Bas), 29 Mai- 2 Juin 2010.

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Review

The liver X receptor: A master regulator of the gut–liver axis and a target for non alcoholic fatty liver disease

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ABSTRACT

Since it is associated to the obesity epidemic, non alcoholic fatty liver disease (NAFLD) has become a major public health issue. NAFLD ranges from benign hepatic steatosis, i.e. abnormally elevated triglyceride accumulation, to non alcoholic steatohepatitis (NASH) that can lead to irreversible liver damages. The search for pharmacological and dietary approaches to treat or prevent NAFLD has pointed at nuclear receptors as sensible targets. Indeed, nuclear receptors are ligand-sensitive transcription factors that play a central role in hepatic lipid metabolism. Among nuclear receptors, the liver X receptor has been identified as an oxysterol receptor. It is involved in the control of various aspects of lipid metabolism that are reviewed in this manuscript. We highlight the role of LXR in the gut–liver axis and the studies that have provided a rationale for strategies specifically targeting the hepatic activity of LXR in NAFLD.

Introduction

Non alcoholic fatty liver disease (NAFLD) ranges from steatosis to non alcoholic steatohepatitis (NASH) that can cause irreversible liver damage in the absence of excessive alcohol consumption [1–4]. The hallmark of steatosis is the accumulation of lipid droplets within hepatocytes [5]. However, following traditional model of NAFLD evolution, steatosis represents a first hit to the disease that may further develop into NASH. Unlike early steatosis that can be benign, NASH has been shown to promote fibrosis and may ultimately results in liver cirrhosis and hepatocellular carcinoma [1,3,4]. Since NAFLD is highly associated with the progression of the obesity epidemic and the metabolic syndrome, it has become a major public health issue worldwide [6,7].

NAFLD is a lipotoxic disease where lipid molecules accumulate in the liver and may thereby alter the organ function [8]. Since nuclear receptors (NRs) are involved in hepatic lipid metabolism, they represent a sensible drug target for obesity related diseases such as NAFLD [9,10]. NRs translate endocrine and metabolic signals into transcriptional control of gene expression. They regulate many crucial processes such as development, metabolism and circadian control. Importantly, several NRs have been shown to act as metabolite receptors critical in many lipid signalling pathways. Amongst NRs, the liver X receptor (LXR) is involved in fatty acid and cholesterol metabolism, in the gut–liver axis, and in the control of inflammation and immunity [11].

In this review, we introduce the possible roles of NRs in NAFLD with a focus on LXR. We briefly present LXR structure and function in cholesterol metabolism, including the regulation of the gut–liver axis. We also review the role of LXR in the regulation of hepatic fatty acid synthesis. Finally, we discuss recent pre-clinical evidences suggesting that LXR can be targeted in experimental models of NAFLD.

Abbreviations: ABC, ATP-binding cassette; ACC, acetyl-coA carboxylase; AF, activation function; ASC-2, activating signal cointegrator-2; CAR, constitutive androstane receptor; CH25H, cholesterol 25 hydroxylase; ChREBP, carbohydrate responsive element binding protein; CYP, cytochrome P450; DBD, DNA binding domain; DNA, deoxyribonucleic acid; FA, fatty acid; FASN, fatty acid synthase; FXR, farnesoid X receptor; HDAC, histone deacetylase; HDL, high density lipoprotein; HMGCR, 3-hydroxy-3-methylglutaryl CoA reductase; LBD, ligand binding domain; LPK, liver pyruvate kinase; LXR, liver X receptor; LXRE, LXR responsive element; NAFLD, non alcoholic fatty liver disease; NASH, non alcoholic steatohepatitis; NCoR, nuclear receptor corepressor; NR, nuclear receptor; OSC, oxydosqualene cyclase; PPARα, peroxysome proliferator activated receptor alpha; PXR, pregnane X receptor; RCT, reverse cholesterol transport; RNA, ribonucleic acid; RXR, retinoid X receptor; SCD, stearoyl-CoA desaturase; SE, squalene epoxydase; SHP, short heterodimer partner; Sin3, stress-activated MAP kinase interacting protein 3; SMRT, silencing mediator of retinoid and thyroid receptors; SREBP, sterol responsive element binding protein; STAT, signal transducer and activator of transcription; SUMO, small ubiquitin-like modifier.

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1. Nuclear receptors in diseases related to hepatic lipid metabolism

The nuclear receptor superfamily consist of 48 members in humans and 49 in mice [12]. While they are conserved from human to *C. elegans*, they are not present in plants and yeast, suggesting their essential function in animal cells. They all share the same structural organization as they are composed of a N-terminal activating domain (AF1), a DNA-binding domain (DBD), a large and variable ligand-binding domain (LBD), and a C-terminal activating function (AF2). The only exceptions are DAX1 and SHP that lack a functional DBD. The LBD is the hallmark of this family of transcriptional regulators. LBD generally forms a hydrophobic pocket, which is able to bind small molecules. The classical view of NRs activation involves a conformational change in NRs upon specific ligand binding to the LBD, which then allow the switch from a quiescent corepressor-bound transcription complex to an active one. In this scenario, corepressors associated to the NR are released prior to the recruitment of both the coactivators and the machinery (including RNA polymerase II) required for transcription. Nevertheless, the variety of their different domains, the high number of identified potential coregulators (estimated between 200 and 300 proteins), the increasing possibility of post-translational modifications, associated to the fine-tuned production of specific ligands for a given NR bring complexity and selectivity to initial model of NR-induced RNA transcription of their target genes [13–15]. Moreover, whether the NRs are already associated or not to their specific response elements in their target gene promoter region and the fact that they are able to act unliganded or through non-genomic ways increase their functional abilities [12]. NRs classification is based on phylogenetic similarities and led to the definition of 6 subfamilies of receptors that can be subdivided into 28 groups, each of which clusters several paralogous genes [12]. Moreover, they form 4 different classes (Fig. 1) based on their ability to function as a momoner or homo/hetero-dimer with RXR and on the type of response element they bind to [16,17].

Among the 49 members of NRs identified in mice (Fig. 1), more than twenty are highly expressed in the liver [18]. Importantly, most of them show marked circadian expression [18,19] suggesting a role of NRs in coupling central clock to hepatic function. In addition, they are involved in many aspects of liver metabolism including xenobiotic clearance, entero-hepatic cycle of bile acids and lipid homeostasis. Therefore, drugs targeting NRs may represent therapeutic options in many hepatic pathologies including drug-induced hepatotoxicity, cholestasis, gallstone disease and NAFLD [10,20].

The extensive use of transgenic mice and pharmacological agonists has allowed better definition of the role of NRs in lipid synthesis, clearance and catabolism (Fig. 1) [10,20]. In addition to their function in controlling the accumulation of potentially lipotoxic molecules in the early steps of NAFLD, a number of NRs also regulate systemic and local inflammation that may be influential during the transition from steatosis to NASH (Fig. 1). Therefore, the potential for NR sensitive pathways as treatment options to target NAFLD has been considerably explored in mouse models of NAFLD [2].

Up to date, preclinical studies in mice have shown promising results. However, most of them were disappointing when translated to human. This is, for instance, the case of peroxisome proliferator activated receptor α (PPARα) whose synthetic agonists (fibrates) reduced steatosis in rodents but seems to be ineffective in human NAFLD [21]. Similarly, the γ isoform of PPAR, whose expression increases in steatotic rodent liver and shows anti-inflammatory effects, is a pharmacological target in treatment of type II diabetes. PPARγ agonists have been used for decades. Long-term clinical trials conducted in patients with NAFLD show beneficial effects on insulin resistance and metabolic parameters such as circulating triglycerides but disappointing effects on fibrosis, hampering its use for NAFLD [22–24]. In contrast, other NRs emerge as promising targets to prevent and/or treat this pathology. The constitutive androstane receptor (CAR) [25], the pregnan X receptor (PXR) [26] and the farnesoid X receptor (FXR) [27] might be clinically relevant targets in the treatment of NAFLD. Recent reports have also suggested that LXR might be a sensitive target for NAFLD [28,29].

2. LXR: structure and function

The liver X receptor belongs to class II nuclear receptors. Both LXR isoforms, LXRα (also known as NR1H3) and LXRβ (also known as NR1H2) have been discovered in 1995 [30,31]. LXRα is highly expressed in the liver, the intestine, the kidney and the adipose tissues whereas LXRβ shows an ubiquitous expression. They form obligate heterodimers with the retinoic X receptor (RXR), the 9-cis retinoic acid receptor [32]. The heterodimer LXR/RXR binds DNA on sequences called LXR response elements (LXRE). These LXRE consist of two direct consensus sequence repeats (AGGTCA) separated by four nucleotides (DR4 motif).

LXR has first been considered an orphan receptor until it was also reported that oxygenated derivatives of cholesterol, the oxysterols, were able to induce LXRα activity in a gene reporter study [33]. These studies led to LXR "adoption". The use of transgenic mice lacking LXRα then revealed a role for this receptor in cholesterol homeostasis [34].

The oxysterols were discovered by Lifschutz in 1913. They were first described as modulators of cholesterol metabolism. Indeed, they are able to sequestrate the sterol responsive element binding protein-2 (SREBP-2) in the endoplasmic reticulum [35] and to induce the degradation of the rate-limiting enzyme of cholesterol synthesis the 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR) [35]. There are numerous oxysterol species which all share a cholesterol structure with an oxygen containing-fonctional group such as hydroxyl, keto or epoxy group [36]. Oxysterols can be derived from non-enzymatic as well as enzymatic oxidation of cholesterol. The most documented and the most relevant ligands for LXR are thought to be produced by enzymatic pathways [35]. These oxysterols are 20(S)-hydroxycholesterol [33], 22(R)-hydroxycholesterol [33], 24(S)-hydroxycholesterol [33,37], 25-hydroxycholesterol [33] and 27-hydroxycholesterol [33,38]. They are synthesized through the action of several enzymes: CYP11A1, CY11A1, CYP46A1, CH25H and CYP3A4 [39], CYP27A1 [40]. The 24(S),25-epoxycholesterol is another activating ligand described for LXR [37,41]. Unlike other oxysterols, this compound is not synthesized from cholesterol. Its biosynthesis occurs through a shunt in the cholesterogenesis pathway [42]. Two enzymes are involved in this "mevalonate shunt": the squalene epoxydase (SE) and the oxydosqualene cyclase (OSC).

When inactive, LXR/RXR heterodimer binds to LXRE in the promoter of target genes (Fig. 2A). The heterodimer interacts with corepressors such as nuclear receptor corepressor (NCoR) or silencing mediator for retinoic acid and thyroid hormone receptor (SMRT) [43], which block transcription by recruiting proteins with histone deacetylase activity (HDAC). HDACs and corepressors interact with the stress activated MAP kinase interacting protein 3A (Sin3A) [44]. Upon ligand binding, LXR undergoes a conformational change [45] which leads to the release of corepressor [43] and the recruitment of specific coactivators such as activating signal cointegrator-2 (ASC2) [46] or receptor-integrating protein 140 (RIP140) [47] on the helix 12 of the LBD [48]. The histones are then acetylated thereby switching the chromatin in a permissive state and enhancing the recruitment of the machinery required for transcription. This conventional model of LXR activation has been

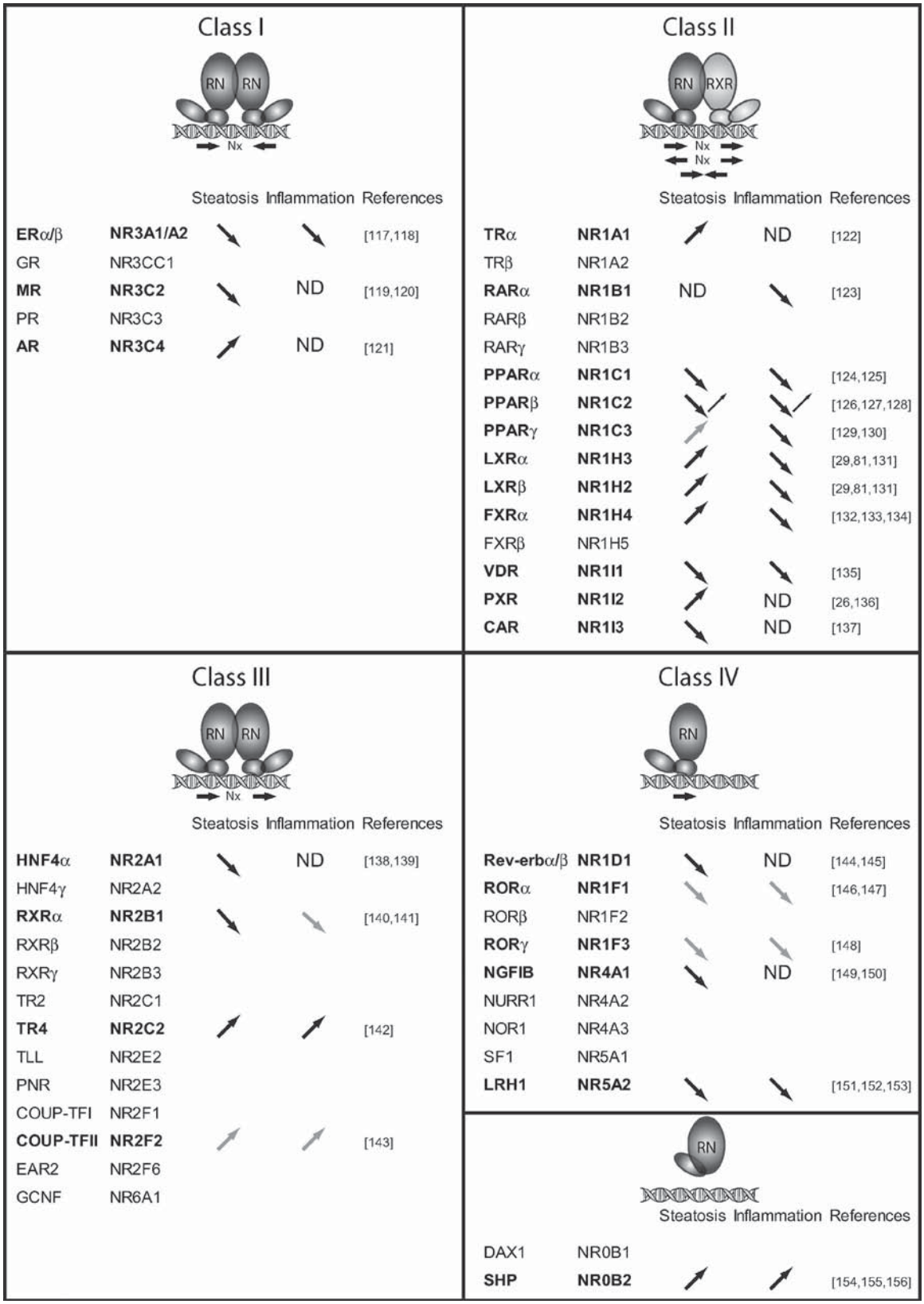

Fig. 1. Implication of nuclear receptors in steatosis and inflammation. Nuclear receptors are classified according to their ability to function as monomer or homo/heterodimer and way of binding to their response element. The receptors implicated [117–160] either in steatosis or in hepatic inflammation *in vivo* are highlighted in bold font. Their positive or negative role is indicated with black arrows. When gene expression is the only biomarker measured for these pathologies, the changes are noticed with grey arrows. ND: not documented.

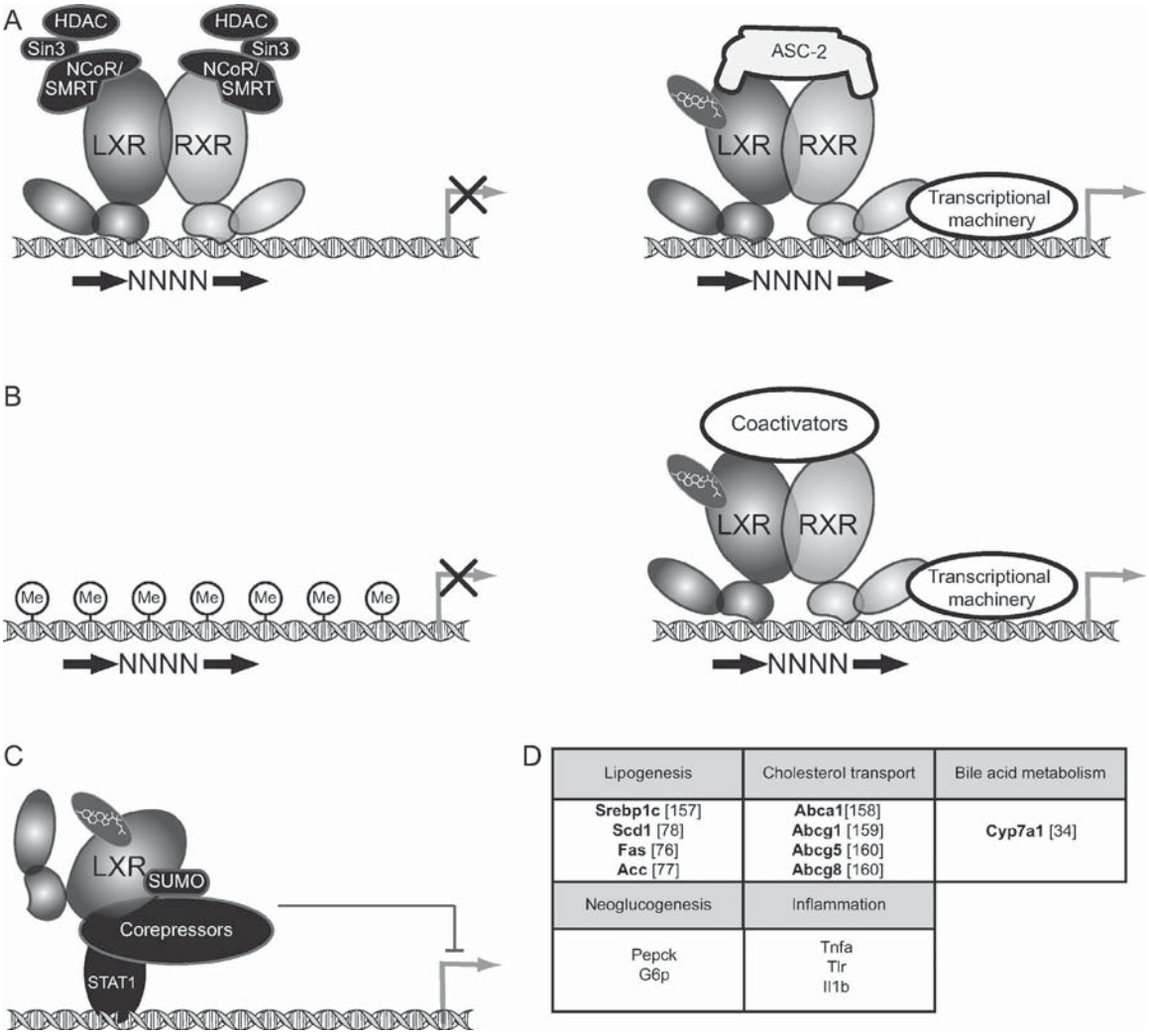

Fig. 2. Mechanisms of action of the liver X receptor and its physiological functions. (A) Classical LXR activation. At the basal state LXR interacts with corepressors such as nuclear receptor corepressor (NCoR) or silencing mediator for retinoic acid and thyroid hormone receptor (SMRT). Proteins with histone deacetylase activity (HDACs) interact with the corepressors through stress activated MAP kinase interacting protein 3A (Sin3A). Upon ligand binding to the LBD, LXR undergoes conformational changes which lead to the release of corepressors and the recruitment of co-activators such as activating signal cointegrator-2 (ASC2). (B) Alternative LXR activation. Epigenetic modulation, for instance histones de-methylation, leads to reveal LXR/RXR binding sites where the activated heterodimer can then interact and exert its transcriptional role. (C) Transrepression of inflammatory pathway by LXR. After being SUMOylated, LXR can interact with the corepressor docked to transcription factors such as STAT1. This consolidates the links between the transcription factor and the corepressor complex and thereby inhibits the pro-inflammatory pathways. (D) Metabolic functions governed by the LXR. For each depicted function, several genes whose expression is modulated by activated LXR are given. Direct LXR target genes appear in bold font.

challenged through various observations [49,50] providing evidence that a ligand-dependant mechanism of recruitment to promoter/enhancer occurs subsequently to changes in epigenetic marks (Fig. 2B).

LXR also contributes to transcriptional regulation of gene expression through transrepression (Fig. 2C), a mechanism by which LXR inhibits inflammatory pathways [51]. For instance, LXR is able to switch off the expression of proinflammatory genes induced by the transcription factor STAT1 [52]. This transrepressive mechanism requires LXR sumoylation. Sumoylated LXR binds STAT1 through corepressors and prevents the dissociation of corepressors from STAT1 and thereby inflammation.

3. Role of LXR in cholesterol homeostasis: a focus on a new path in the gut–liver axis

LXR is one of the transcription factors involved in cholesterol metabolism [53] (Figs. 2D and Fig. 3). It is essential for the clearance of cholesterol from the body by sensing cholesterol-derived oxysterols. Other NRs, including the FXR, participate to whole body sterol homeostasis. FXR senses bile acids [54]. Importantly, while cholesterol concentration can be toxic to the cells and must be tightly controlled, cholesterol is also an essential component for cell membrane integrity and for many signalling molecules. Therefore, upon cholesterol deprivation, it must be synthesised through the mevalonate pathway that is under the acute control of SREBP-2 [55,56].

A lot of evidence for the role of LXR in cholesterol metabolism has come from observations made in the transgenic mice lacking either one [34] or both [57] LXR isoforms. Mice lacking LXRα accumulate cholesterol esters in the liver [57,58]. In addition, further cholesterol accumulation occurs in these mice when fed a high cholesterol diet [59]. These data highlight the important role of LXR in clearing cholesterol that originates both from *de novo* synthesis controlled by SREBP-2 or from dietary sources. In mouse, this defective cholesterol clearance has been shown to relate to the reduced expression of *Cyp7a1* [34] which encodes a rate-limiting enzyme in cholesterol clearance.

In addition to its role in regulating hepatic *Cyp7a1*, LXR contributes to reduce the body load of cholesterol through its

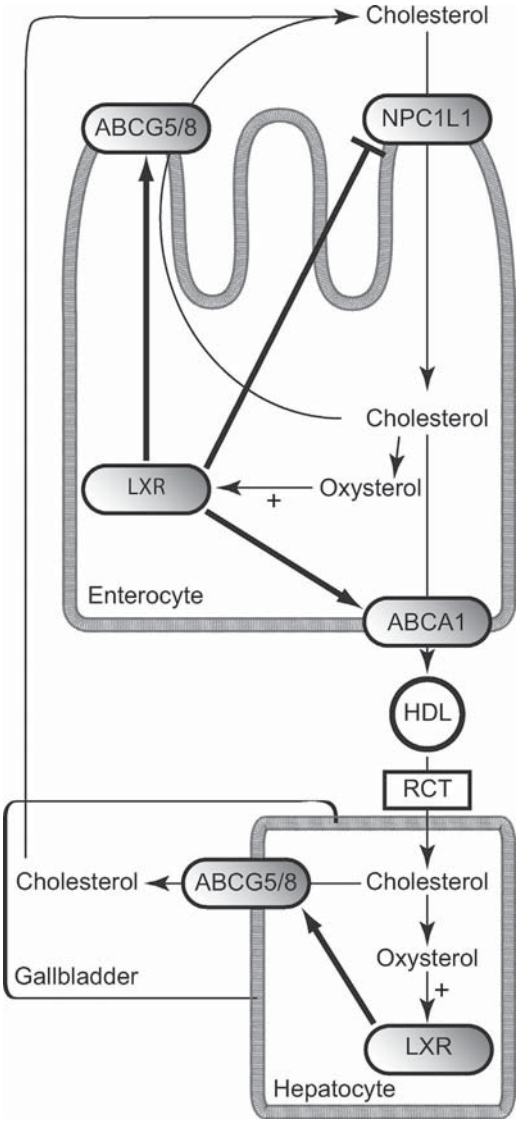

Fig. 3. The liver X receptor in the gut–liver axis. In the intestine cholesterol is absorbed by the transporter NPC1L1 whose gene expression is repressed by LXR. LXR activation leads to the up-regulation of genes coding for ABCG5, ABCG8 and ABCA1. ABCG5 and ABCG8 are located at the apical plasma membrane of enterocytes and are involved in the excretion of cholesterol in intestinal lumen. ABCA1 is located at the basal plasma membrane and promotes the efflux of cholesterol into nascent HDL. In the liver LXR increases the expression of genes coding for ABCG5 and ABCG8 which leads to the efflux of cholesterol into the gallbladder and into the lumen of the intestine.

important role in the regulation of genes involved in reverse cholesterol transport (Figs. 2D and Fig. 3). At the molecular level, LXR controls cholesterol efflux from peripheral cells such as macrophages by regulating the ABC transporters *Abca1* and *Abcg1* [60,61]. In turn, this process modulates transfer of intracellular cholesterol to HDL particles that are transported back to the liver for subsequent elimination. Finally, LXR regulates the expression of two half transporters called *Abcg5* and *Abcg8* that act as a dimer in cholesterol transport [62,63]. They are primarily expressed in the liver and in the intestine (Fig. 3) where they promote excretion and limit absorption of cholesterol respectively.

Interestingly, intestinal inflammation may also contribute to altered expression of LXR and subsequent enzymes involved in lipid metabolism. Indeed, it has been reported that the decrease in fatty acid synthase expression in ulcerative colitis patients may be at least partly attributed to the loss of LXR expression in the presence of proinflammatory cytokines [64]. A common LXR polymorphism contributing to risk of inflammatory bowel diseases described recently [65] also highlights the importance of LXR in the gut homeostasis. In addition, a new work emphasizes the implication of LXR in limiting the growth of intestinal tumors [66].

A study with liver-specific deletion of LXRα has provided evidence that it is essential for reverse cholesterol transport (RCT), cholesterol degradation and excretion [67]. However, pharmacological treatment of mice lacking hepatic LXRα was shown to be a beneficial anti-atherogenic strategy. One part of this benefit may relate to the contribution of LXRβ [68] and from the extra hepatic activity of LXRα. Several studies had reported the contribution of intestine (Fig. 3) in the regulation of RCT and the crucial role of this organ for plasma HDL homeostasis [69,70]. Interestingly, it was recently reported that the intestine could be a key player in the LXR-driven protection against cholesterol damages. The activity of a constitutively active version of LXRα in the intestinal epithelium decreases cholesterol absorption and induces RCT while hepatic activity of LXR does not contribute to elevate RCT [71]. Importantly, this work provides evidence that the overexpression of LXR in the intestine not only improves atherogenic markers but also hepatic lipid content. Indeed, mice overexpressing the active version of LXRα in the intestinal epithelium show reduced level of hepatic triglyceride and cholesterol accumulation upon high fat diet. These results show that the specific activity of LXR in the intestine may therefore influence NAFLD by modulating hepatic triglyceride and cholesterol levels.

4. Role of LXR in *de novo* hepatic fatty acid synthesis and NAFLD

Because LXR is such a key player in cholesterol metabolism, the potential use of LXR agonists as an option for the treatment of atherosclerosis, and other diseases, has been widely explored [72,73]. However, the enthusiasm for the possible value of LXR agonists has been limited by the activation of key genes critical for hepatic *de novo* lipogenesis [57] which is one of the sources of triglycerides that may accumulate in NAFLD [74]. The mechanism by which this occurs have been well described. LXR is direct regulator of the expression of critical genes involved in hepatic lipogenesis (Figs. 2D and Fig. 4). However, recent reports also highlight tissue-specific effect of LXR on lipogenesis in the adipose tissue [75] and the intestine [29].

LXREs have been described on the promoter of *Fasn* [76], *Acc* [77] and *Scd1* [78]. LXRβ seems to play a weaker role in hepatic lipogenesis than LXRα since transgenic mice lacking LXRα but not LXRβ show reduced lipogenic gene expression pattern when compared to the wild-type mice fed a high cholesterol diet [57]. However, a recent finding showed that LXRβ deficiency reduces the effect of T0901317 on lipogenic gene expression in mice fed a standard diet [29]. In addition, it has been shown that LXRβ is involved in the development of steatosis in response to glucocorticoids [79] and in the regulation of the acute phase response [80].

It has also been shown that LXR controls the expression of two transcription factors involved in lipogenesis: SREBP-1c [57] and the carbohydrate responsive element binding protein (ChREBP) [81]. Therefore, LXR plays both a direct and an indirect role in the regulation of lipogenesis. LXR together with ChREBP [82,83] and SREBP-1c [57,84] belong to a network of nutrient sensing factors involved in the control of hepatic fatty acid synthesis and thereby triglyceride accumulation. In a recent study we showed that the LXR-SREBP-1c axis plays a central role in the up-regulation of genes involved in *de novo* lipogenesis in response to dietary fat. While essential fatty acid deficiency was known to promote lipogenesis and steatosis [85], our data showed for the first time

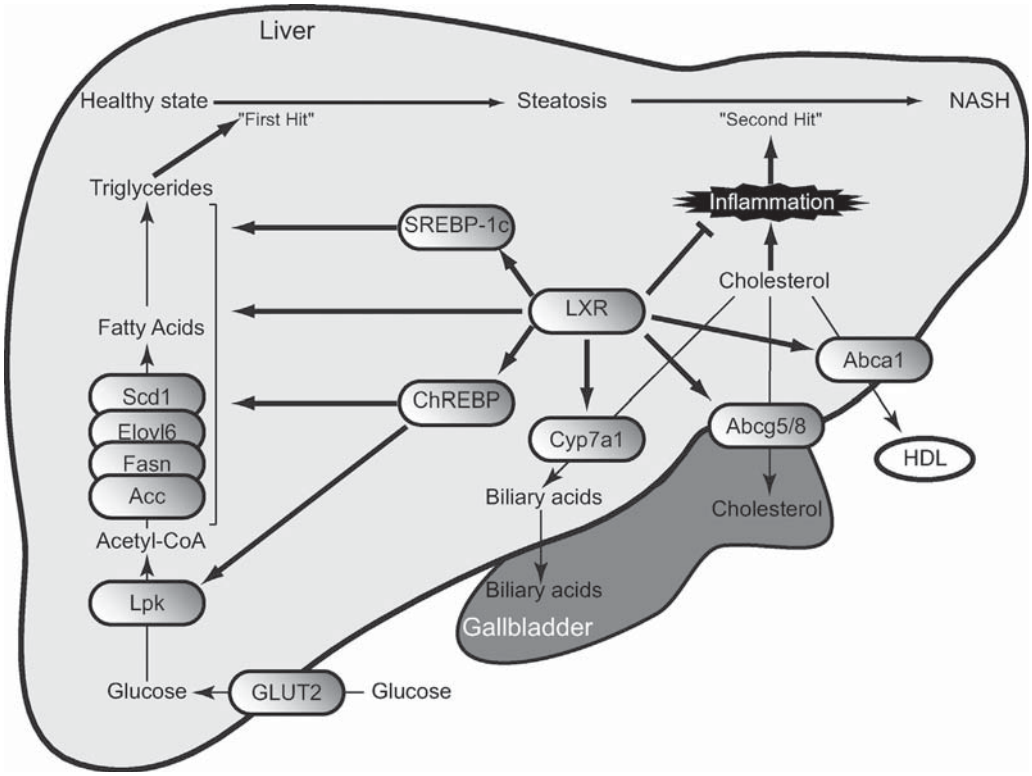

Fig. 4. Implication of the liver X receptor in non alcoholic fatty liver disease. NAFLD is a complex pathology whose development is thought to be triggered by two hits. The "first hit" is characterized by hepatic triglycerides accumulation or steatosis. LXR, a master regulator of hepatic lipogenesis, is strongly involved in steatosis development. Indeed, LXR controls the expression of lipogenic genes such *Scd1*, *Acc*, *Fas* as well as two transcription factors *Srebp-1c* and *Chrebp* also involved in lipogenesis. Upon fed state, glucose enters hepatocyte through the glucose transporter GLUT2 and is catalyzed into Acetyl-CoA *via* glycolysis and citrate cycle. LPK, a key enzyme in glycolysis, is under the transcriptional control of ChREBP. *In vivo* administration of T0901317, a LXR synthetic ligand leads to massive hepatic steatosis confirming its implication in the "first hit" of NAFLD. Steatosis is a benign and reversible state. However, it can lead to severe complication such as non alcoholic steatohepatitis (NASH). The steatosis/NASH transition is not fully understood, however it seems that inflammation is an important determinant of the "second hit". Free cholesterol in the liver is known to trigger inflammation and LXR could play a benefic role as a sterol sensor important in the degradation and the excretion of cholesterol. LXR controls the expression of *Abcg5/g8* genes as well as *Cyp7a1* gene involved in cholesterol excretion and degradation into bile acids, respectively. Moreover, it is reported that in hepatocytes LXR is able to excrete free cholesterol into HDL particles. The ability of LXR to maintain low levels of free cholesterol in the liver as well as its transrepressional effects on inflammatory processes make it a good target to limit NASH development.

the requirement of LXR for such regulation by dietary fatty acids [29]. This work underlined the importance of maintaining chronic dietary FA balance for hepatic metabolism. However, the most common cause of NAFLD is not imbalanced composition of fatty acids but excessive fat intake.

The development of the first selective synthetic LXR inverse agonist (SR9238) was recently described and tested in mice fed a high fat diet [28]. SR9238 displays high potency for both LXRα and β. It has been designed to specifically target the liver and avoid potential side effects due to suppression of LXR in extra hepatic tissues. The authors demonstrated that this compound effectively suppresses hepatic lipogenesis and hepatic lipid accumulation in a mouse model of NAFLD induced by high fat intake. Nevertheless, it would be interesting to investigate how the use of this molecule influence the cross-regulation with other nuclear receptors such as CAR [86,87], PXR [26], TR [88], PPARs [58,89–91] and FXR [54,92] involved in hepatic fatty acid metabolism. The significance of the cross-talk between PPARα and LXR seems to be particularly relevant to fatty acid metabolism and NAFLD. Indeed, PPARα is essential to fatty acid homeostasis, especially during prolonged fasting [93,94], high fat feeding [95] and diabetes [93]. Importantly, PPARα has been shown to be influenced by *de novo* lipogenesis [96,97] and therefore, it is likely to be indirectly influenced by LXR activity. In addition, both PPARα and LXR have been shown to influence each other [58,98,99] and to share a large number of genomic binding sites [50]. LXR and FXR [54] are both central to cholesterol metabolism and FXR cross-talk with LXR could also be considered in the early steps of NAFLD. FXR is activated by bile acids [100]. Upon activation, it induces the expression of the Short Heterodimer Partner (SHP) [101], an atypical orphan nuclear receptor lacking a DNA-binding domain [102]. It acts as a corepressor for many nuclear receptors, including LXR [103].

Unexpectedly, treatment of diet-induced obese mice with the LXR inverse agonist (SR9238) showed no signs of liver damage and reduction of plasma cholesterol levels [28]. This suggests that prevention of steatosis was not associated with inflammation or with impaired LXR-dependent clearance of hepatic cholesterol. These data reveal that liver-selective LXR inverse agonists may be extremely relevant for the early steps of NAFLD (Fig. 4). Increasing evidence shows that LXR has anti-inflammatory properties [104] and that free hepatic cholesterol acts as a lipotoxic molecule promoting the transition from steatosis into NASH [105,106]. LXR activity could be important to prevent direct toxicity of cholesterol but also to prevent activation of Kupffer [107] and hepatic stellate cells [108] that promote fibrosis. The use of the LXR inverse agonist does not lead to inflammation and does not elevate hepatic cholesterol while inhibiting triglyceride accumulation [28]. It inhibits the early step of NAFLD without promoting NASH. Nevertheless, how LXR inverse agonist may reduce lipogenesis without promoting cholesterol accumulation remains to be clarified. Importantly, FXR shows a very important role in the hepatic clearance of cholesterol too. FXR-deficient mice are highly sensitive to diet-induced steatosis, inflammation and fibrosis [109,110]. It is therefore possible that, under certain dietary

conditions, FXR rather than LXR promote the clearance of toxic sterols in NASH. FXR agonists are currently evaluated as potential drugs in the treatment of metabolic diseases [111]. Ongoing clinical trials are addressing the potential of FXR agonist in patients with NASH [111].

Conclusion

LXR is a receptor involved in the control of various physiological functions and therefore drugs targeting LXR show great potential for the treatment of various diseases [72]. However, the broad array of functions LXR regulates makes it a challenging receptor to be selectively targeted. LXR plays a major role in fatty acid homeostasis, cholesterol metabolism and regulate important inflammatory signals, all of which are likely to influence NAFLD that has become a major public health issue worldwide.

Small molecules influencing the hepatic or intestinal activity of the receptor may represent some clinical relevance. While such strategy should be considered with caution, one recent report shows that hepato-specific inhibition of LXR can be an efficient strategy to reduce lipogenesis and prevent NAFLD that occurs in response to a high fat diet [28]. Further work could investigate the efficiency of such approach and the consequences of chronic inhibition of LXR on the different aspects of hepatic functions including the beneficial signaling that results from lipogenesis [96,112,113].

The cross-talks of LXR with other NRs in the circadian control of lipid homeostasis also remains to be further investigated. In addition, extra-hepatic activity of LXR could be relevant to NAFLD. For instance, there is accumulating evidences for the importance of LXR in the intestine and for the role of gut derived signals on NAFLD [114–116].

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Review

Liver X Receptor: an oxysterol sensor and a major player in the control of lipogenesis

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ABSTRACT

De novo fatty acid biosynthesis is also called lipogenesis. It is a metabolic pathway that provides the cells with fatty acids required for major cellular processes such as energy storage, membrane structures and lipid signaling. In this article we will review the role of the Liver X Receptors (LXRs), nuclear receptors that sense oxysterols, in the transcriptional regulation of genes involved in lipogenesis.

Contents

Abbreviations: ABC, ATP-binding cassette; ACC, acetyl-CoA Carboxylase; ACL, ATP-citrate lyase; AF, transactivation function; AGPAT, 1-acylglycerol-3-phosphate acyltransferases; ASC-2, activating signal cointegrator-2; bHLH/LZ, basic-helix-loop-helix leucine zipper; CEPT1, choline/ethanolaminephosphotransferase 1; CERS, ceramide synthase; CDIPT, CDP-diacylglycerol-inositol 3-phosphatidyltransferase; CH25H, cholesterol 25 hydroxylase; CHPT1, choline phosphotransferase 1; ChoRE, carbohydrate response element; ChREBP, carbohydrate responsive element binding protein; CPT1, carnitine palmitoyl-transferase 1; DAG, diacylglycerol; DBD, DNA binding domain; DGAT, diacylglycerol acyltransferase; DNA, deoxyribonucleic acid; DOS, dioxidosqualene; ELOVL, elongation of very long chain fatty acid; FA, fatty acid; FACE, fatty acyl-CoA elongase; FASN, fatty acid synthase; FoxO, forkhead box-"Other"; FXR, farnesoid X receptor; GK, gluco kinase; GPAT, glycerol-3-phosphate acyltransferase; GSM, glucose sensing domain; GTF, general transcription factor; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDL, high density lipoprotein; HMGCR, 3-hydroxy-3-methylglutaryl CoA reductase; IR, insulin receptor; LBD, ligand binding domain; LCE, long chain fatty acyl elongase; LPA, lysophosphatidic acid; L-PK, liver pyruvate kinase; LXR, Liver X Receptor; LXRE, LXR responsive element; MD, malate dehydrogenase; ME, malic enzyme; MOS, monooxidosqualene; Mlx, Max like protein; NADPH, nicotinamide adenine dinucleotide phosphate; NAFLD, non alcoholic fatty liver disease; NCoR, nuclear receptor corepressor; NR, nuclear receptor; OAA, oxaloacetate; OSC, oxydosqualene cyclase; PC, phosphatityl-choline; PDK, phosphoinositide-dependent kinase; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PKA, protein kinase A; PP2A, protein phosphatase 2A; PPARα, peroxysome proliferator activated receptor alpha; PUFA, polyunsaturated fatty acid; RIP140, receptor-interacting protein 140; RNA, ribonucleic acid; RXR, Retinoid X Receptor; SCD, stearoyl-CoA desaturase; SE, squalene epoxydase; SHP, short heterodimer partner; SIK2, serine/threonine kinase salt-inducible kinase 2; Sin3, stress-activated MAP kinase interacting protein 3; SIRT1, NAD-dependent deacetylase sirtuin-1; SMRT, silencing mediator of retinoid and thyroid receptors; SPTLC, serine palmitoyltransferase; SRE, sterol response element; SREBP, sterol regulatory element binding protein; SULT2B1, sulfotransferase 2B1; TFIID, transcription factor II D; TG, triacylglycerol; VLDL, very low density lipoproteins; X5P, xylulose-5-phosphate.

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1. Introduction

De novo lipogenesis is a key-process that leads to the synthesis of fatty acids (FAs). A very little amount of FAs remains unesterified as free FAs. Most FAs are used for the synthesis of complex lipids such as phospholipids, ceramides, cholesterol esters or triglycerides and thereby play a major role in membrane structure, cell signaling and energy storage.

The regulation of *de novo* lipogenesis is highly sensitive to hormonal, nutritional, environmental and/or genetic factors. It has been widely investigated in the context of the raising concerns about obesity and associated metabolic diseases such as hepatic steatosis or non alcoholic fatty liver disease (Ferre and Foufelle, 2010; Postic and Girard, 2008).

Since in mammals the liver is the major site for lipogenesis, most of the work reviewed here refers to the described regulation of hepatic fatty acid synthesis. We also examine the evidences for the role of the Liver X Receptors (LXR) as oxysterol sensors and their biological function. Finally, the specific roles of LXRs in the regulation of hepatic lipogenesis is presented.

2. Lipogenesis: *de novo* synthesis of fatty acids

The main enzymes involved in FA biosynthesis are indicated in Fig. 1A. Because lipogenesis is primarily associated with the synthesis of triacylglycerols (TG; Fig. 1B) for fatty acid storage these pathways will extensively described, even though alternative metabolic fate of *de novo* synthesized FAs and some of their role in signaling will also be presented.

2.1. Critical steps in fatty acid biosynthesis

De novo lipogenesis occurs when glucose supply is high. For instance, under fed conditions, higher animals preferentially burn carbohydrates to generate ATP while the excess of carbohydrate is converted into FAs. Therefore, FA synthesis is tightly linked to glucose catabolism (Postic and Girard, 2008). Indeed, the complete oxidation of glucose, also called glycolysis, leads to the synthesis of acetyl-CoA, which subsequently, can enter the citrate cycle in the mitochondria. Thus citrate, an intermediate compound of citrate cycle, is exported from mitochondria. This results in an increase of cytosolic citrate, which can next be converted into acetyl-CoA. This reaction is catalyzed by the ATP-citrate lyase (ACL).

Acetyl-CoA is then carboxylated into malonyl-CoA by the acetyl-CoA carboxylase (ACC) which is the first critical and rate-limiting enzyme in *de novo* FA synthesis. There are two isoforms of ACC: ACC1 and ACC2. ACC1 is cytosolic and mainly expressed in lipogenic tissues such as the liver and the adipose tissue whereas ACC2 is mitochondrial and predominantly expressed in oxidative tissues (Abu-Elheiga et al., 2000). These two isoforms are encoded by two distinct genes (Abu-Elheiga et al., 1995, 1997) and are known to play different roles: ACC1 is involved in *de novo* lipogenesis while ACC2 is rather implicated in the repression of mitochondrial β-oxydation providing the pool of malonyl-CoA which acts as allosteric inhibitor of carnitine palmitoyl-Transferase I (CPT1). During FA synthesis, such production of malonyl-CoA represents an important feedback loop on FA catabolism since CPT1 is involved in the entry of FAs in the mitochondria for oxidation (McGarry and Brown, 1997).

Transgenic mice lacking ACC1 are not viable (Abu-Elheiga et al., 2005). However, the liver-specific deletion of ACC1 leads to a reduction of lipogenesis and triglyceride accumulation without affecting FA oxidation (Mao et al., 2006). ACC2 knockout mice are leaner than wild-type mice, resistant to high fat/high carbohydrate diet induced obesity and more sensitive to insulin. This occurs as a result of an increased FA oxidation in the heart, the skeletal muscle and the liver (Abu-Elheiga et al., 2001, 2003).

Cytosolic malonyl-CoA can be used for FA biosynthesis. This reaction is catalyzed by the fatty acid synthase (FASN) that represents the second enzyme in *de novo* FA synthesis. Its major product is the sixteen carbon saturated FA: palmitic acid (C16:0). FASN uses the malonyl-CoA as a primer, acetyl-CoA as a carbon donor and the NADPH as a reducing equivalent (Chirala and Wakil, 2004). FASN consists of two multifunctional polypeptides forming a homodimeric complex of 260 kDa in the cytoplasm. Each homodimer is composed by three catalytic domains in the N-terminal section (β-ketoacyl synthase (KS), malonyl/acetyltransferase (MAT), and dehydrase (DH)) and are separated by a core region of around 600 residues from four C-terminal domains (enoyl reductase (ER), β-ketoacyl reductase (KR), acyl carrier protein (ACP) and thioesterase (TE)) (Chirala et al., 2001; Smith, 1994). Transgenic mice lacking *Fasn* die *in utero* (Chirala et al., 2003). Liver-specific deletion of *Fasn* does not protect against hepatic FA accumulation. Indeed, when fed a low fat/high cholesterol diet, the *Fasn* liver-specific knockout mice develop a fatty liver, which seems to occur as result of a defect in FA oxidation (Chakravarthy et al., 2005).

2.2. Fatty acid elongation and desaturation

The main product of FASN is palmitic acid (C16:0). It can either be elongated or desaturated. Elongation of FAs involves the addition of two carbons to a fatty acyl-CoA using malonyl-CoA as the carbon donor and NADPH as the reducting agent. Elongation of very long chain fatty acid (ELOVL) proteins are membrane-bound enzymes located in the endoplasmic reticulum. To date, in mammals seven ELOVL proteins (ELOVL1–7) have been identified. ELOVL6 (also known as long chain fatty acyl elongase (LCE) and fatty acyl-CoA elongase (FACE)) catalyzes the conversion of palmitate (C16:0) to stearate (C18:0). It was first discovered as an up-regulated gene in transgenic mice over-expressing the sterol regulatory element binding protein (SREBP)-1c and SREBP-2 (Moon et al., 2001). This discovery has been further confirmed in another study, which identified the gene coding for ELOVL6 as a gene under the control of SREBPs (Matsuzaka et al., 2002). ELOVL6 is expressed in the liver and is thought to catalyze the elongation of palmitic acid (C16:0) and palmitoleic acid (C16:1 n-7, see below) to form stearic acid (C18:0) and vaccenic acid (C18:1 n-7), respectively (Matsuzaka et al., 2002; Moon et al., 2001). It has been reported that ELOVL6 can also catalyze the elongation of FAs consisting of twelve or fourteen carbons (Matsuzaka et al., 2002; Moon et al., 2001). Mice lacking *Elovl6* have been created by Matsuzaka et al. (2007). They show a reduction of the hepatic content in stearic (C18:0) and oleic acid (C18:1 n-9). These mice are resistant to diet-induced insulin resistance. However, they do not show amelioration of obesity or

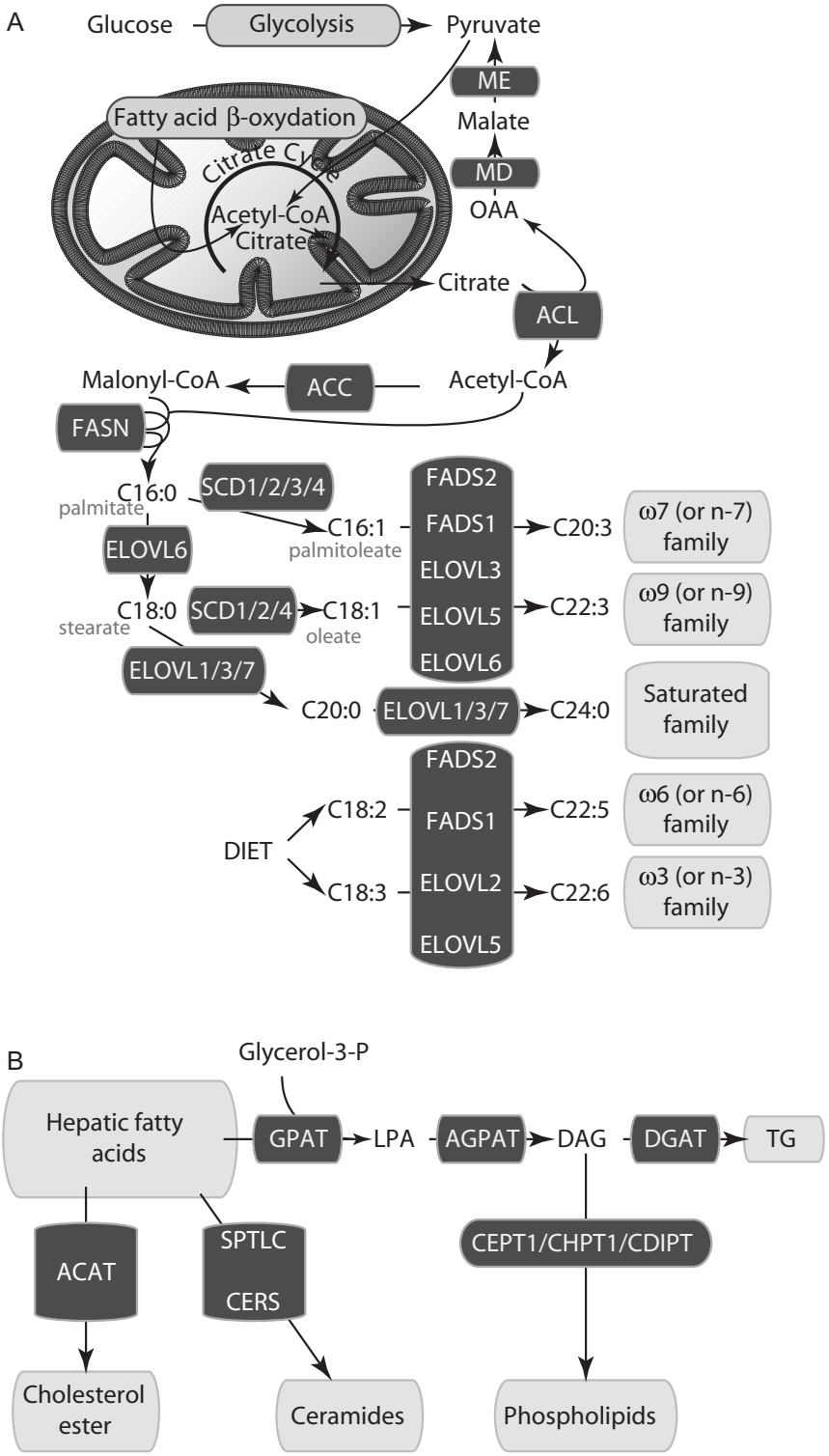

Fig. 1. *De novo* lipogenesis and triglyceride biosynthesis. (A) Acetyl-CoA, the precursor of palmitic acid biosynthesis is provided by the citrate cycle that uses glycolysis and pyruvate oxidative decarboxylation products or fatty acid β-oxidation. Mithochondrial acetyl-CoA is then condensed to form citrate and is exported to the cytosol. ATP citrate lyase (ACL) converts citrate to form oxaloacetate (OAA) and acetyl-CoA which is in turn carboxylated into malonyl-CoA by acetyl-CoA carboxylase (ACC). OAA is transformed into pyruvate through the action of two enzymes: malate dehydrogenase (MD) and malic enzyme (ME). The fatty acid synthase (FASN) leads to the synthesis of palmitate from the condensation of seven malonyl-CoAs. Palmitic acid (C16:0) can then be further elongated by elongation of very long chain fatty acids protein (ELOVL) 6 to form stearate (C18:0) or desaturated by stearoyl-CoA desaturase 1 (SCD1) to form palmitoleate (C16:1 n-7). Stearate can also be desaturated by SCD1 to form oleate (C18:1 n-9). Different of ELOVL and fatty acids (FAs) desaturase (FADS) can elongate and desaturate FAs including essential FAs supplied by the diet, to form the wide variety of FAs in the cell. (B) Triglyceride (TG) biosynthesis is supported by three enzymes Glycerol-3-phosphate acyltransferase (GPAT), 1-acylglycerol-3-phosphate O-acyltransferase (AGPAT) and diacylglycerol acyltransferase (DGAT) that catalyze the esterification of acyl-CoA on the first, second and third carbon of glycerol-3-phosphate respectively. GPAT and AGPAT products are lysophosphatidic acid (LPA) and diacylglycerol (DAG) respectively. FAs can also be incorporated into other complex lipids. Specific incorporation into various lipid classes is driven by rate-limiting enzymes in other pathways. Phospholipids are synthesized from DAG by several enzymes. Choline/ethanolaminephosphotransferase 1 (CEPT1) catalyzes the synthesis of phosphatidylcholine and phosphatidylethanolamine whereas choline phosphotransferase 1 (CHPT1) catalyzes the synthesis of phosphatidylcholine exclusively. CDP-diacylglycerol-inositol 3-phosphatidyltransferase (CDIPT) catalyzes the synthesis of phosphatidylinositol. FAs can also be incorporated into cholesterol by acetyl-CoA acetyltransferase to form a cholesteryl esters. Ceramides are synthesized by the incorporation of two FAs into serine and then sphinganine by serine palmitoyltransferase (SPTLC) and ceramide synthase (CERS) respectively.

hepatosteatosis. They also crossed *Elovl6*−/− mice with ob/ob mice and the pups showed a decreased hyperglycemia and improved insulin resistance compared to ob/ob pups.

Palmitic (C16:0) and stearic (C18:0) acid can then be desaturated by the stearoyl-CoA desaturase 1 (SCD1 also known as Δ9 desaturase) into palmitoleic (C16:1 n-7) and oleic acid (C18:1 n-9), respectively (Ntambi, 1999). The entire coding sequences of the SCD encoding genes as well as their promoter regions have been characterized in different species. Four SCD isoforms have been identified in mice (Kaestner et al., 1989; Miyazaki et al., 2003; Ntambi et al., 1988; Zheng et al., 2001). *Scd1* is expressed in various tissues including the liver and the adipose tissue (Ntambi et al., 1988), *Scd2* is mainly expressed in the brain and neuronal tissues (Kaestner et al., 1989), *Scd3* is specifically expressed in harderian and preputial gland, and in the sebocytes (Zheng et al., 2001). The expression of the mouse *Scd4* appears to be restricted to the heart. In the liver, the ELOVL6 product stearic acid (C18:0) and palmitic acid (C16:0) can be desaturated to form oleic acid (C18:1 n-9) and palmitoleic acid (C16:1 n-7) respectively. These FAs are the major components of membrane phospholipids, triglycerides and cholesteryl esters. This reaction is catalyzed by SCD1. The rat liver SCD1 was the first desaturase purified (Strittmatter et al., 1974). SCD1 is a 40 kDa intrinsic membrane protein anchored in the endoplasmic reticulum. This iron-containing enzyme catalyzes the biosynthesis of monounsaturated FAs that requires acyl-CoA, NADH, NADH reductase, cytochrome b5, phospholipid, and oxygen. Global (Ntambi et al., 2002) and liver-specific (Miyazaki et al., 2007) *Scd1* knockout mice have been generated by Ntambi's group. Global Scd1 knockout mice are lean and show a defect in the synthesis of lipids including triglycerides. They are protected from diet-induced obesity and insulin resistance (Ntambi et al., 2002) and from obesity induced by leptin deficiency (Cohen et al., 2002). A decrease in lipogenesis combined with an increase in FA oxidation are reported to mediate the protective effects of SCD1 deficiency (Cohen et al., 2002; Dobrzyn et al., 2004; Ntambi et al., 2002). Study of the mice lacking *Scd1* in the liver shows that this enzymes protects from obesity and steatosis induced by a high carbohydrate/very low fat diet (Miyazaki et al., 2007).

2.3. Triglyceride biosynthesis

Long chain acyl-CoA produced by *de novo* lipogenesis can be esterified on glycerol in order to form glycerolipids (Fig. 1B). Three steps lead to the incorporation of three acyl-CoAs on glycerol-3-phosphate. The first esterification is catalyzed by glycerol-3-phosphate acyltransferase (GPAT) and consists in the esterification of an acyl-CoA on the first carbon of glycerol-3-phosphate to form lysophosphatidic acid (LPA), which in turn can be transformed either in phospholipid or in triglyceride. Four GPATs belonging to the same family of acyltransferase have been described and encoded by four different genes (Cao et al., 2006; Chen et al., 2008; Ganesh Bhat et al., 1999; Harada et al., 2007; Nagle et al., 2008; Wang et al., 2007). GPAT1 and GPAT2 are located in the outer membrane of the mitochondria (Lewin et al., 2004) whereas GPAT3 and GPAT4 are located in the endoplasmic reticulum (Gimeno and Cao, 2008). Unlike GPAT2 and GPAT3, GPAT1 and GPAT4 are highly expressed in the liver. GPAT1 plays an important role in triglyceride biosynthesis as illustrated by the decreased liver TGs and VLDL secretion as well as plasma TGs in mice lacking (Hammond et al., 2002; Hammond et al., 2005). These mice are also resistant to obesity and insulin resistance induced by high-fat diet (Neschen et al., 2005). They also show a 40% and 30% decrease in hepatic palmitic acid in triglycerides and phospholipids respectively (Hammond et al., 2002). This highlights the preference of GPAT1 for palmitate esterification at the sn-1 position of glycerol-3-phosphate. It has also been reported that *Gpat1* knock-down in Ob/Ob mice results in the diminution of hepatic triacylglycerol and diacylglycerol as well as plasma glycemia (Xu et al., 2006). *Gpat4* knockout mice are also protected from high fat/high carbohydrate diet-induced obesity and from obesity induced by leptin deficiency (Vergnes et al., 2006). It seems that GPAT4 is less specific than GPAT1 and that it is able to esterify both saturated and unsaturated FAs into glycerol 3-phosphate to produce lysophosphatidic acid (Chen et al., 2008).

The next step consists in the esterification of another acyl-CoA on the sn-2 position of the LPA glycerol backbone in order to form phosphatidic acid (PA). This reaction is catalyzed by 1-acylglycerol-3-phosphate acyltransferases (AGPATs) proteins. To date, there are ten proteins with suspected AGPAT activity (AGPAT1–10) (Agarwal et al., 2006, 2007; Leung, 2001; Li et al., 2003; Sukumaran et al., 2009; Tang et al., 2006; Ye et al., 2005). However, only AGPAT1 and AGPAT2 have a clearly demonstrated enzyme activity (Leung, 2001). Furthermore, it seems that AGPAT2 is the isoform involved in acylation of LPA in the formation of TG. First, AGPAT2 has the strongest activity in *in vitro* assays when compared to AGPAT3-5,9 (Agarwal et al., 2007; Lu et al., 2005). Second, 80% of transgenic pups lacking *Agpat2* die after three weeks of age (Cortes et al., 2009), confirming that AGPAT2 plays a crucial role and cannot be substituted by other AGPATs. The other *Agpat* mRNA levels were measured and only slight increases were observed in mice lacking *Agpat2*. Total AGPAT enzymatic activity was reduced by 90% in the liver of these mice, confirming that AGPAT2 is responsible for the majority of the activity in the liver (Cortes et al., 2009). Finally, the preferential acyl-CoA incorporated in LPA by AGPAT2 is oleyl-CoA (C18:1 n-9). Several other acyl donors are incorporated including C14:0, C16:0, and C18:2 acyl-CoAs, with lower incorporation of C18:0 and C20:4 acyl-CoAs (Eberhardt et al., 1997; Hollenback et al., 2006). This is in accordance with TG composition in which sn-2 position is mainly composed of monoenoic and dienoic acyl groups rather than polyenoic acyl group generally enriched in sn-2 position of phospholipids (Glosset, 1996).

In order to form a triglyceride by esterifying another acyl-CoA on the sn-3 of PA, this one has to be dephosphorylated. This dephosphorylation is catalyzed by a family of proteins called phosphatidate phosphatase-1 enzymes (also known as LIPIN). Three enzymes belong to this family: LIPIN1, LIPIN2 and LIPIN3 (Carman and Han, 2009).

The last step in the triglyceride synthesis is catalyzed by the diacylglycerol acyltransferase (DGAT). To date, two enzymes sharing a DGAT activity have been discovered: DGAT1 and DGAT2 (Coleman and Lee, 2004). These two proteins are encoded by separate genes (Cases et al., 1998, 2001; Lardizabal et al., 2001; Oelkers et al., 1998). Under basal condition DGAT2 localizes in the endoplasmic reticulum. When oleic acid is provided DGAT2 localizes near the surface of lipid droplets where it co-localizes with mitochondria (Stone et al., 2009). DGAT1 is located in the endoplasmic reticulum (Cao et al., 2007), but it has also been reported that it also co-localizes with lipid droplets in *S. cerevisiae* (Sorger and Daum, 2002). Overexpression of either *Dgat1* or *Dgat2* leads to increased amounts of TGs in transfected cells. In cells over-expressing *Dgat1* the TG accumulation occurs as small lipid droplets around the cell periphery whereas in *Dgat2* transfected cells TGs are located in large cytosolic lipids droplets (Stone et al., 2004). DGAT1 and DGAT2 are both expressed in a wide variety of tissues including the liver (Cases et al., 1998, 2001). DGAT2 seems to have a greater activity than DGAT1 as over-expression of *Dgat2* leads to more important TG accumulation than in *Dgat1* over-expressing cells (Stone et al., 2004). Furthermore, transgenic mice lacking *Dgat2* present a lethal neonatal lipopenia not compensated by the presence of DGAT1. Transgenic mice lacking *Dgat1* are viable but present a reduction in adiposity and a resistance to high fat diet-induced obesity (Smith et al., 2000).

2.4. Lipogenesis and fatty acid signaling

As opposed to essential FAs of the n-6 and n-3 series, FAs synthesized through *de novo* lipogenesis are saturated or unsaturated FAs of the n-7 and n-9 series. Through a combination of elongation and desaturation mammalian cells can synthesize a wide number of FAs (Guillou et al., 2010). Depending on chain length and unsaturation, these fatty acids may become substrates for enzymes catalyzing the incorporation of acyl chains into more complex lipids (Fig. 1B). The selective acyl-chain incorporation into various lipid classes has been known for a long time. The development of new methods in the field of lipid biochemistry (Brown and Murphy, 2009; Clark et al., 2011; Ivanova et al., 2009; Shevchenko and Simons, 2010) brings further insights into the analysis of specific lipids and allows to raise novel and major questions as to how this occurs and how the acyl chains may influence signaling (Chakravarthy et al., 2009; Clark et al., 2011).

In the recent years, original approaches based on the development of various transgenic mouse models, on cutting-edge lipidomic analysis and systems biology have led to the proposal that key-lipogenic enzymes not only provide fatty acid for storage as TGs but intermediate metabolic signals. This is consistent with the different phenotypes observed in mouse models lacking the various enzymes required for TG synthesis. Exploiting this signaling pathway represents the concept of "Lipoexpediency" that introduces possible lipogenic signals as involved in protecting the organism against deleterious effects of acute lipogenesis itself (Lodhi et al., 2011). Lipoexpediency in whole body homeostasis has been extensively discussed (Lodhi et al., 2011). Here, we focus on lipoexpediency occurring in the liver.

Free palmitoleic acid (C16:1 n-7) has been identified as a "lipokine" reported to have beneficial effects on insulin resistance. It has been reported that increased amounts of this lipokine were correlated with insulin sensitivity (Cao et al., 2008). Transgenic mice lacking *Elovl6* are protected against diet-induced insulin resistance without any amelioration of obesity or hepatosteatosis (Matsuzaka et al., 2007). This resistance was attributed to improved insulin signaling, a benefic effect suggested to be correlated with palmitoleic acid availability (Matsuzaka et al., 2007).

As detailed previously, transgenic mice lacking *Fasn* in the liver are not protected from lipid accumulation when fed a low fat/high carbohydrate diet. These mice also showed a decrease in peroxysome proliferator activated receptor alpha (PPARα) target genes expression and a phenotype similar to those observed with the transgenic mice lacking *Pparα* (Chakravarthy et al., 2005), a nuclear receptor that is a master regulator of genes involved in FA oxidation. Chakravarthy et al. (2009) demonstrated that phosphatityl-choline (PC) containing palmitic acid (C16:0) and oleic acid (C18:1 n-9), two fatty acids derived from FASN activity, is a relevant ligand for PPARα. Therefore, *de novo* lipogenesis produces an endogenous ligand that binds to and activates PPARα thereby preventing fat accumulation through the induction of FA oxidation.

While we have focused on hepatic FA synthesis and liver-related phenotype of transgenic mice, it should be stated here that lipogenesis occurs to a lower extent in various tissues. Moreover, it is not only a critical event in energy storage and signaling in the context of metabolic related diseases. It is also clear that FA metabolism is critical for many other cellular functions.

3. Oxysterols as ligands for LXRs

3.1. LXRs, class II nuclear receptors

The Liver X Receptors are transcription factors which belong to the nuclear receptor (NR) superfamily, which comprises 49 members in mouse and 48 in human. There are two isoforms of LXR: LXRα (NR1H3) and LXRβ (NR1H2). Both have been discovered in 1995 (Teboul et al., 1995; Willy et al., 1995). They are class II NRs and form obligate heterodimer with Retinoid X Receptors (RXRs) the receptors for 9-*cis* retinoic acid (Repa and Mangelsdorf, 2000). The heterodimer LXR/RXR binds to the DNA (Fig. 2), on LXR responsive element (LXRE) composed by two direct repeat of the consensus sequence (AGGTCA) separated by four nucleotides (DR4). While LXRα is highly expressed in liver, intestine, kidney, and adipose tissue, LXRβ expression is expressed in many tissues (Auboeuf et al., 1997; Repa and Mangelsdorf, 2000).

As other nuclear receptors, LXRs are organized in different functional domains. The poorly conserved amino-terminal domain (A/B) contains a ligand independent transactivation function (AF-1), which stimulates a basal transcription even in the absence of a ligand. The central domain or DNA binding domain (DBD) is highly conserved and contains two zinc finger motifs, which interact with DR4 binding sites in the promoter of target genes. And finally, LXR contains a well-conserved carboxy terminal ligand binding domain (LBD) that exhibits a ligand-dependent transactivation function (AF-2). Upon ligand binding, the LBD interacts with different coregulators (for a review see Viennois et al., 2011).

In the absence of the ligand LXR/RXR binds to the DNA in the promoter of target genes and interacts with corepressors such as nuclear receptor co-repressor (NCoR) or silencing mediator of retinoid and thyroid receptors (SMRT) (Hu et al., 2003). Corepressors recruit proteins with histone deacetylases activity (HDACs), recruited through the interaction with the stress-activated MAP kinase interacting protein 3 (Sin3) (Jones et al., 2001). The DNA environment is then in a non-transcription permissive state and the transcription machinery cannot interact with the initiation site of transcription. Upon ligand binding to the LBD there is a modification in the conformation of LXR (Glass and Rosenfeld, 2000) which leads to the release of co-repressors (Hu et al., 2003) and the recruitment of co-activators such as activating signal cointegrator-2 (ASC-2) (Lee et al., 2008) or receptor-interacting protein 140 (RIP140) (Herzog et al., 2007) on the helix 12 of the LBD (Svensson et al., 2003). The histones are then acylated, the chromatin gets in a transcription-permissive state and the transcription machinery can be recruited and initiate transcription. Upon binding to a response element, LXR becomes acylated. It has been shown (Li et al., 2007b) that after the transcription of the target gene has occurred, LXR is deacetylated by NAD-dependent deacetylase sirtuin-1 (SIRT1), which leads to the ubiquitination of LXR and its degradation by the proteasome. This action of SIRT1 improves the turnover of LXR through activation/degradation cycle and enhances LXR activity (Li et al., 2007b).

LXRs have first been considered as orphan receptors because their natural ligands were unknown. However, Mangelsdorf's group first showed that oxidative derivatives of cholesterol, the oxysterols, induce LXRα activity in a gene reporter system (Janowski et al., 1996).

3.2. Origins and synthesis of oxysterols

Oxysterols have been first discovered in 1913 by Lifschutz (1913) as autoxidation products of cholesterol. These compounds have early been described as modulators of cholesterol metabolism through their influence on the sequestration of SREBPs in the endoplasmic reticulum thereby limiting the expression of genes involved in cholesterol synthesis (see for review Brown and Jessup, 2009). They also directly regulate the degradation of 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR), the rate-limiting enzyme in cholesterol synthesis (Brown and Jessup, 2009). Oxysterols are also activators of LXR (Janowski et al., 1996; Lehmann et al., 1997). Many different oxysterols are known, they all share a choles-

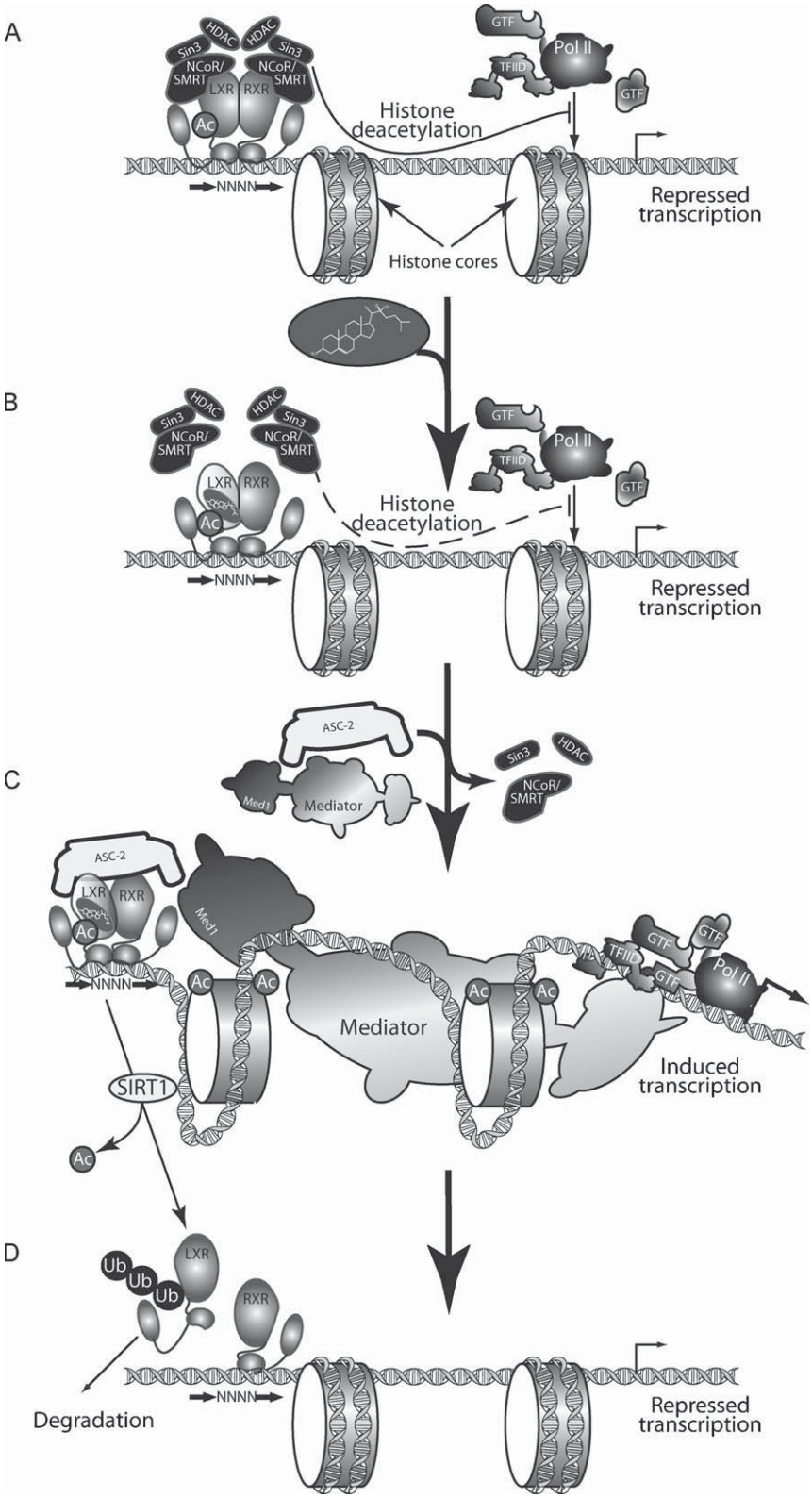

Fig. 2. Schematic representation of LXR activation. (A) In the absence of ligand, the Liver X Receptor (LXR)/Retinoid X Receptor (RXR) heterodimer is bound to a DR4 response element on the promoter of target genes. Co-repressors: nuclear receptor co-repressor (NCoR) or Silencing Mediator of Retinoid and Thyroid Receptors (SMRT), stress-activated MAP kinase interacting protein 3 (Sin3) and histone deacetylases (HDAC) are bound to the LXR/RXR heterodimer and keep the DNA in a non transcription-permissive state. (B) Upon binding of an agonist (for instance 22(R)-hydroxycholesterol) on the ligand binding domain (LBD) of LXR, there is a conformational change which leads to the departure of the co-repressors and recruitment of co-activators such as activating signal cointegrator-2 (ASC-2). (C) At the same time, several mechanisms including histone modifications and chromatin remodeling, allow RNA polymerase II and the transcriptional machinery including general transcription factor (GTF), transcription factor II D (TFIID), and the mediator complex, to increase the transcription of the target gene. (D) Next, NAD-dependent deacetylase sirtuin-1 (SIRT1) deacetylates LXR which leads to its ubiquitination and degradation by the proteasome. This mechanism seems to be important to start another transcription cycle of the target gene.

terol structure with an oxygen-containing functional group such as hydroxyl, keto or epoxyde group (for a review see Schroepfer, 2000). The oxygen-containing functional group can be added at the sterol ring or at the side chain of cholesterol. Oxysterols can be derived from non-enzymatic or from enzymatic oxidation of cholesterol or both.

There is a huge variety of oxysterols. Their origin has been reviewed by various authors (Brown and Jessup, 2009; Gill et al., 2008; Russell, 2000; Schroepfer, 2000). In addition, the specific impact of individual oxysterols on LXR may highly depend on genes and tissues. For instance, 5α,6α-epoxycholesterol was recently shown to exert both agonist or antagonist activities depending on LXR target genes and on cellular context (Berrodin et al., 2010). In the present manuscript we chose to focus on the formation of some oxysterols that have been described to occur through enzymatic pathways (Fig. 3). These oxysterols are 20(S)-hydroxycholesterol (Janowski et al., 1996), 22(R)-hydroxycholesterol (Janowski et al., 1996), 24(S)-hydroxycholesterol (Janowski et al., 1996; Lehmann et al., 1997), 25-hydroxycholesterol (Janowski et al., 1996), 27-hydroxycholesterol (Fu et al., 2001; Janowski et al., 1996) and 24(S),25 epoxycholesterol (Lehmann et al., 1997; Svensson et al., 2003) which are generally thought to be physiologically relevant LXR agonists in lipogenic tissues.

3.3. Biosynthesis of hydroxycholesterols

The formation of hydroxycholesterol is catalyzed by several enzymes (Fig. 3). CYP46A1 is a microsomal enzyme which catalyzes the reaction leading to the synthesis of 24(S)-hydroxycholesterol. This oxysterol is also called cerebrosol as it is found in large amounts in the brain (Bjorkhem, 2007). 27-Hydroxycholesterol is an intermediate in bile acid synthesis, it is produced by the mitochondrial enzyme CYP27A1 and it is the main oxysterol found in the circulation. Cholesterol 25 hydroxylase (CH25H) synthesizes the 25-hydroxycholesterol. Unlike other enzymes involved in oxysterol synthesis, which are cytochrome P450 members, CH25H is a di-iron enzyme located in the ER and the Golgi and found at low levels in most tissue (Russell, 2000). However, this oxysterol has also been

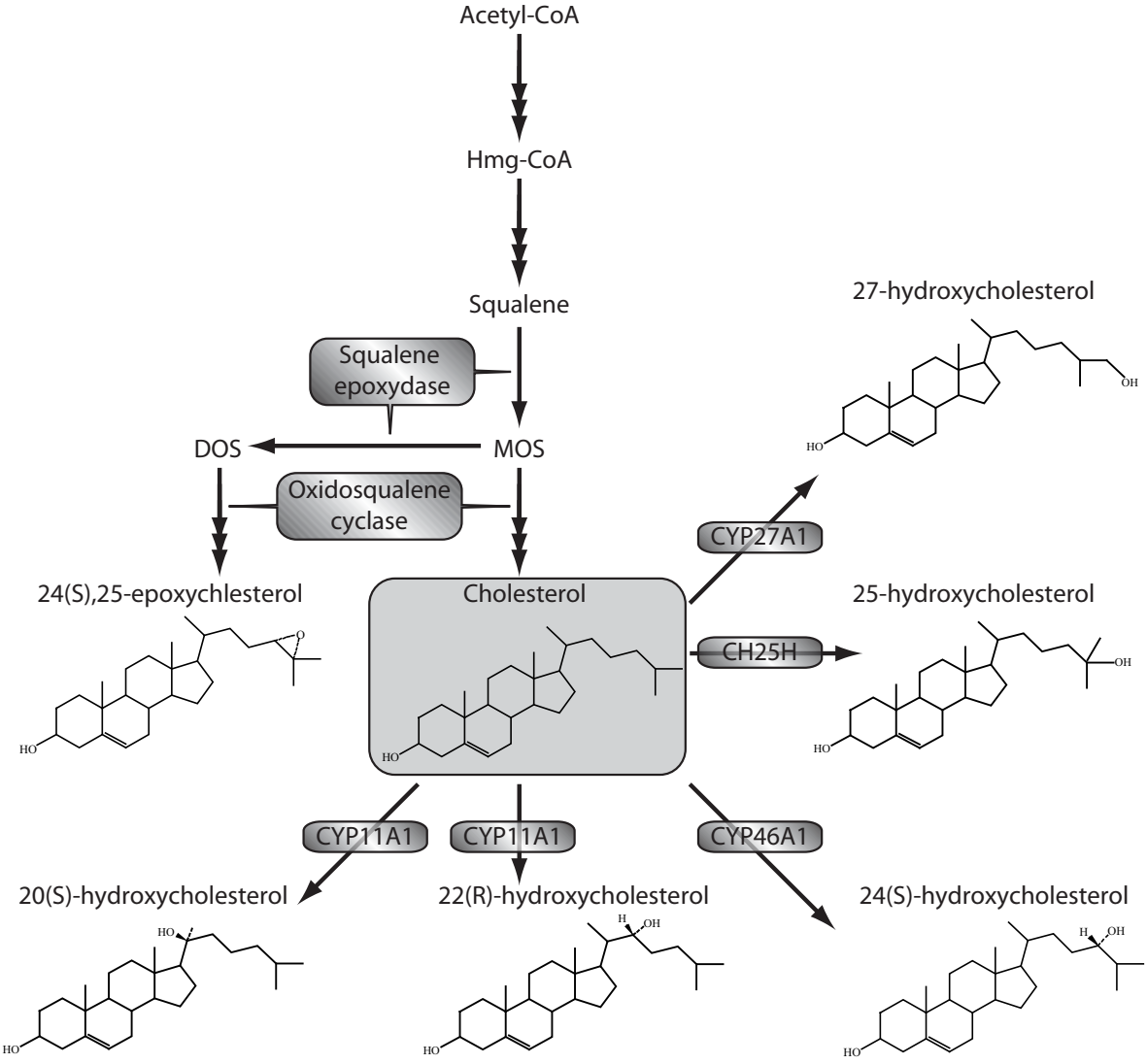

Fig. 3. Synthesis and structures of oxysterols with LXR agonist activity. 20(S)-hydroxycholesterol, 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, 25-hydroxycholesterol and 27-hydroxycholesterol are synthesized from cholesterol. Hydroxy groups are branched in different parts of the side chain of the cholesterol. The reactions that lead to their synthesis are catalyzed by CYP11A1 for both 20(S)-hydroxycholesterol and 22(R)-hydroxycholesterol and CYP46A1, CH25H and CYP27A1 for 24(S)-hydroxycholesterol, 25-hydroxycholesterol and 27-hydroxycholesterol respectively. 24(S),25-epoxycholesterol is synthesized in a "shunt pathway" which parallels and shares the same enzymes as the mevalonate pathway. Two enzymes are involved and control this pathway. Squalene epoxydase (SE) synthesizes monooxidosqualene (MOS) and dioxidosqualene (DOS). Oxydosqualene cyclase (OSC) catalyzes the first reaction of the pathway leading to the formation of cholesterol and 24(S),25-epoxycholesterol from MOS and DOS respectively. 24(S),25-epoxycholesterol have the same structure as cholesterol with an epoxy group branched on the carbons 24 and 25 of the side chain.

reported to appear through a non-enzymatic reaction (Smith, 1987) and through the activity of CYP3A (Honda et al., 2011). Finally, CYP11A1 catalyzes the formation of both 20(S)-hydroxycholesterol and 22(R)-hydroxycholesterol (Gill et al., 2008). However, these two compounds are intermediates in steroidogenesis and their synthesis mainly occurs in the adrenals (Gill et al., 2008).

Hydroxycholesterols have been reported in the mid 90s to activate LXR activity using gene reporter assays *in vitro*, for LXRα (Janowski et al., 1996) and for both isoforms (Lehmann et al., 1997). In 2007, Chen et al. (2007) brought another strong evidence *in vivo* for the activation of LXR by oxysterols. They showed that overexpression of the sulfotransferase SULT2B1 leads to an impaired LXR signaling both *in vitro* and *in vivo*. They also observed an impaired LXR activity in *Cyp46a1*, *Ch25h* and *Cyp27a1* triple knockout mice (Chen et al., 2007) demonstrating *in vivo* the role of oxysterols in the activation of LXRs. However, the expression of *Srebp-1c*, a LXR target gene, was still elevated in response to cholesterol feeding suggesting the presence of other endogenous ligands not synthesized by the deleted genes. Several studies showed that sulfated 25-hydroxycholesterol, the 25-hydroxycholesterol-3-sulfate is not only an inactivated oxysterol but has potent LXR antagonistic properties. Providing 25-hydroxycholesterol-3-sulfate to cell culture or over-expressing SULT2B1, the enzyme involved in generating 25-hydroxycholesterol-3-sulfate (Li et al., 2007a), leads to a decrease of LXR activity (Bai et al., 2010; Xu et al., 2010). Other sulfated oxysterols such as 5α,6α-epoxycholesterol-3-sulfates and 7-ketocholesterol-3sulfates are antagonistic ligands of Liver X Receptors (Song et al., 2001).

3.4. The mevalonate shunt pathway: another source of LXR ligands

24(S),25-epoxycholesterol is another activating ligand for LXRs. It has been discovered in 1981 by Nelson et al. (1981). As other oxysterols, 24(S),25-epoxycholesterol has the capacity to decrease cholesterol synthesis by reducing the activity of HMGCR (Dollis and Schuber, 1994; Saucier et al., 1985; Taylor et al., 1986), inducing its degradation (Song and DeBose-Boyd, 2004) as well as limiting the processing of SREBP-2 (Janowski et al., 2001; Wong et al., 2006) a transcription factor involved in cholesterol synthesis. Unlike previously mentioned hydroxycholesterols, 24(S),25-epoxycholesterol is not a cholesterol-derived oxysterol. Its established enzymatic biosynthesis occurs as a shunt in the mevalonate pathway which parallels the cholesterol biosynthesis pathway (Fig. 3). Therefore, 24(S),25-epoxycholesterol biosynthesis is under the same feedback control as cholesterol synthesis (Wong et al., 2007). This shunt pathway starts with the monooxidosqualene (MOS) which can be transformed into dioxidosqualene (DOS) by the squalene epoxydase (SE), also known as the squalene monooxygenase (SM). Remarkably, the degradation of this enzyme has very recently been reported to be under a proteasomal control regulated by cholesterol (Gill et al., 2011). The oxydosqualene cyclase (OSC) can then convert the cholesterol precursor, the MOS, and the 24(S),25-epoxycholesterol precursor, the DOS, into lanosterol and 24(S),25-epoxylanosterol respectively (Nelson et al., 1981). These two compounds are then transformed through several reactions to form either cholesterol or 24(S),25-epoxycholesterol (Panini et al., 1986). The enzymes involved in these reactions are shared by the two pathways. Several studies showed that this oxysterol activates LXR (Janowski et al., 1999; Lehmann et al., 1997). However, these studies relied on the addition of 24(S),25-epoxycholesterol to *in vitro* systems and cell culture. Later several approaches have also been used to modulate the amounts of endogenously produced 24(S),25-epoxycholesterol. Some of these approaches are based on the fact that OSC has a better affinity for DOS than for MOS (Boutaud et al., 1992). Statins, molecules used in therapy in order to decrease hypercholesterolemia and related cardiovascular diseases, are inhibitors of HMGCR an enzyme catalyzing the formation of mevalonate. Treatment of THP-1 macrophages with statins leads to the decreased synthesis of both 24(S),25-epoxycholesterol and cholesterol as well as the decrease of two typical LXR target genes: ABCA1 and ABCG1 (Wong et al., 2004). This impaired LXR response is rescued by adding exogenous 24(S),25-epoxycholesterol. These effects of statins seem to depend on the presence of cholesterol in the cells, since supplementing cells with cholesterol reverses the statin-mediated effects on LXR activity whereas depleting cellular cholesterol tends to strengthen the effect of statins (Wong et al., 2008b). Statins have also been used as a pretreatment in order to induce a burst on mevalonate pathway. This pretreatment on Chinese hamster ovary (CHO-7) cells leads to an increase of cholesterol synthesis paralleled with an increase of 24(S),25-epoxycholesterol synthesis and LXR signaling (Wong et al., 2008a). In both cases, increased or decreased mevalonate pathway flow, the 24(S),25-epoxycholesterol synthesis and cholesterol synthesis seem to parallel each other. The 24(S),25-epoxycholesterol appears as a compound that protects the cell against endogenous cholesterol (Wong et al., 2007). Other studies investigated the possibility of uncoupling 24(S),25-epoxycholesterol and cholesterol pathway to better elucidate the role of 24(S),25-epoxycholesterol. Because OSC shows a better affinity for DOS than for MOS, incomplete inhibition of this enzyme explains why the squalene epoxyde can be channeled into 24(S),25-epoxycholesterol pathways (Morand et al., 1997). Using partial inhibition of OSC allows to uncouple 24(S),25-epoxycholesterol synthesis from cholesterol synthesis as the MOS synthesized by SE accumulates and can be catalyzed once again by SE to form DOS. DOS will then be transformed into 24(S),25-epoxycholesterol. Indeed, OSC inhibitors was shown to induce a decrease of cholesterol synthesis as well as an increase of 24(S),25-epoxycholesterol synthesis and up-regulation of LXR activity in THP-1 human macrophages (Beyea et al., 2007; Wong et al., 2004), murine macrophage cell line (Wong et al., 2004) HepG2 (Morand et al., 1997), CHO-7 (Wong et al., 2008a). In 2007, Wong et al. (2008a) used another approach that evidenced the role of endogenous 24(S),25-epoxycholesterol. As a partial inhibition of OSC leads to increased synthesis of 24(S),25-epoxycholesterol, they overexpressed the gene coding for human OSC in CHO-7 cells. These cells are depleted from 24(S),25-epoxycholesterol and showed a decreased in LXR activity when compared to control cells. Interestingly, on the basis of experiments performed with statins in rat hepatoma cells it was also shown that a tonic activation of LXR by an oxysterol intermediate in the biosynthesis of cholesterol was required for the transcription of SREBP1c (DeBose-Boyd et al., 2001). All of these studies support the notion that cholesterol biosynthesis through its effect on 24(S),25-epoxycholesterol may influence LXR activity.

3.5. Roles of LXR in vivo

With the identification of oxysterols as physiological ligands of LXRs the possible role for these receptors in cholesterol metabolism was suspected. Transgenic mice lacking LXRα (Peet et al., 1998), LXRβ (Repa et al., 2000a) or both (Repa et al., 2000a) have been created thereby providing great tools to better understand the importance and significance of individual LXR isoforms *in vivo*. Evidences from the first studies with mice lacking LXRs supported the hypothesis that LXRs are involved in the regulation of cholesterol disposal making it an attractive drug target for the treatment of cholesterol related diseases. Synthetic high affinity compounds capable of activating LXRs have been developed. T0901317 (Schultz et al., 2000) and the GW3965 (Collins et al., 2002) are the most frequently used compounds to target LXR in research. Unlike GW3965, TO901317 is not strictly selective for LXR (Houck et al., 2004; Mitro

et al., 2007; Shenoy et al., 2004) (for a review on the LXR-ligand see Viennois et al., 2011). When administered *in vivo*, these synthetic ligands regulate a set of genes involved in reverse cholesterol transport from peripheral tissues to the liver (Costet et al., 2000; Kennedy et al., 2001; Repa et al., 2000b). LXRs are also involved in the regulation of genes involved in cholesterol (Peet et al., 1998; Schultz et al., 2000), bile acid (Chiang et al., 2001; Peet et al., 1998) and steroid synthesis (Cummins et al., 2006; Mouzat et al., 2009; Robertson et al., 2005; Volle et al., 2007). However, LXRs are not only involved in the control of whole-body sterol homeostasis. They have been shown to be central receptors in the integration of both metabolic and inflammatory signaling (reviewed in Zelcer and Tontonoz, 2006). The studies performed with transgenic mice have also allowed to evidence that LXRs are particularly important in atherosclerosis (Calkin and Tontonoz, 2010; Lo Sasso et al., 2010b), thrombosis (Spyridon et al., 2011) macrophage signaling (A-Gonzalez et al., 2009; Hong et al., 2011), but also in immunity (Bensinger et al., 2008; Cui et al., 2011; Villablanca et al., 2010; Zelcer and Tontonoz, 2006), reproduction (El-Hajjaji et al., 2011; Viennois et al., 2011), cell proliferation (Bensinger et al., 2008; Lo Sasso et al., 2010a), cancer (Pommier et al., 2010; Villablanca et al., 2010), Alzheimer's disease (Adighibe et al., 2006; Infante et al., 2010; Koldamova et al., 2005; Zelcer et al., 2007), and skin biology (Hanley et al., 2000; Jiang et al., 2006; Komuves et al., 2002). All the findings listed above have made LXRs major drug targets (Viennois et al., 2011). But the role of LXR in the regulation of fatty acid synthesis prevents the use of current LXR synthetic agonists as therapeutic agents. However, one group has reported the beneficial effect of a phytosterol-derived LXR agonist on plasma cholesterol without hypertryglyceridemic effect (Kaneko et al., 2003). In addition, a recent report has evidenced the tissue specific activation of LXR that promotes macrophage reverse cholesterol transport *in vivo* (Yasuda et al., 2010).

4. The major role of LXR in liver lipogenesis

Early studies showed that the use of T0901317 *in vivo* leads to a massive hepatic steatosis and increased triglycerides enriched very low density lipoproteins (VLDLs) secretion (Grefhorst et al., 2002). Moreover, transgenic mice lacking LXRα showed decreased expression of genes involved in lipogenesis (*Srebp-1c*, *Fasn*, *Scd1*) (Peet et al., 1998). Therefore, LXR has early been suspected to be a major regulator of FA synthesis. A better understanding of the role of LXR in the control of hepatic lipogenesis is a major issue as increased FA synthesis has been shown to contribute to the progression of non alcoholic fatty liver disease (NAFLD) (Donnelly et al., 2005).

4.1. LXR a master regulator of lipogenesis

LXRs appear to be direct regulators of the expression of critical genes involved in lipogenesis in the liver. However, it must be said that a recent report highlight tissue-specific effect of LXR on lipogenesis (Korach-Andre et al., 2011). The work referred to in the following paragraphs mainly relate on the pro-lipogenic effect of LXR action in the liver.

LXRE have been described on the promoter of *Fasn* (Joseph et al., 2002), *Acc* (Talukdar and Hillgartner, 2006) and *Scd1* (Chu et al., 2006). LXRβ seems to play a weaker role in lipogenesis than LXRα since transgenic mice lacking LXRα but not LXRβ show reduced lipogenic genes expression pattern when compared to the wild-type mice fed a high cholesterol diet (Repa et al., 2000a). It has also been shown that LXRs control the expression of two transcription factors involved in lipogenesis: SREBP-1c (Repa et al., 2000a) and the carbohydrate responsive element binding protein (ChREBP) (Cha and Repa, 2007). Therefore LXRs play both a direct and an indirect role in the regulation of lipogenesis (Fig. 4).

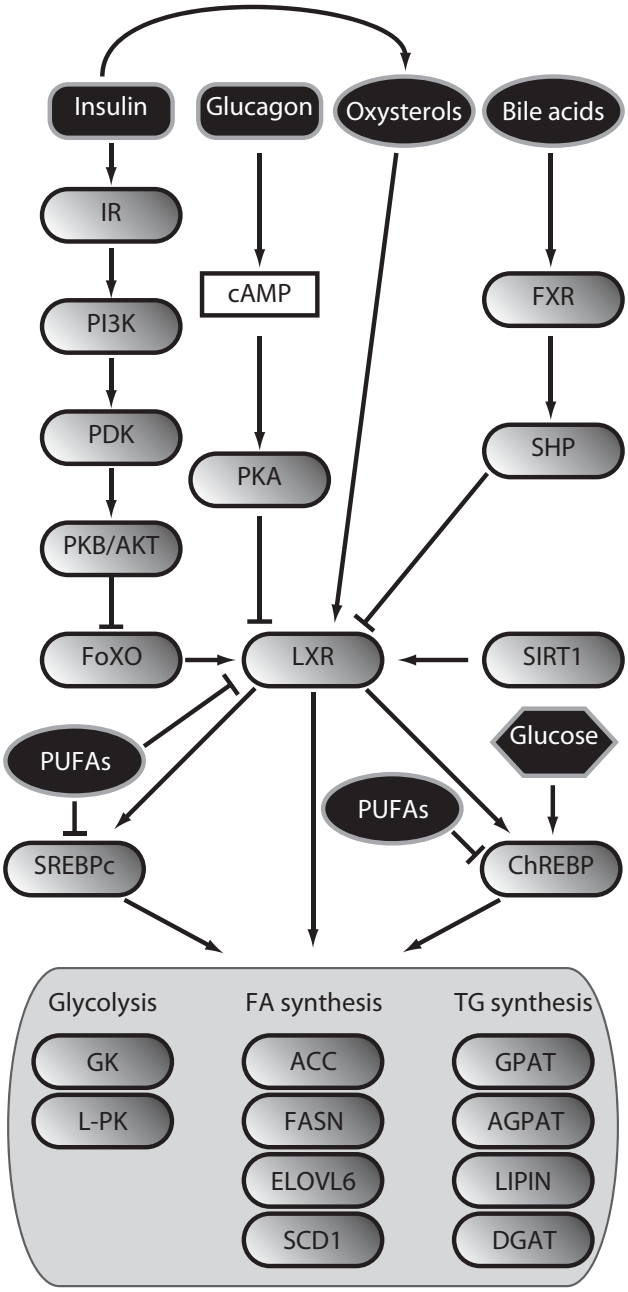

Fig. 4. Direct and indirect roles of LXR in the transcriptional control of hepatic lipogenesis by nutritional status. The genes coding for two lipogenic transcription factors: sterol regulatory element-binding protein 1c (SREBP-1c) and carbohydrate responsive element binding protein (ChREBP) are under the control of LXR. These three transcription factors regulate genes that are involved in glycolysis (GK, L-PK), fatty acid (FA) synthesis (ACC, FASN ELOVL6 and SCD1) and/or triglyceride (TG) synthesis (GPAT AGPAT, LIPIN and DGAT). The mechanisms are not fully described. ChREBP is activated by elevated glucose. Oxysterols are LXR ligands that can induce its activity. Other mechanisms can modify LXR activity. In the fed state, insulin regulates lipogenic genes including SREBP-1c however it seems that this regulation requires the presence of LXR. Insulin *via* the insulin receptor (IR), phosphatidylinositol 3-kinase (PI3 K) *phosphoinositide-dependent kinase* (PDK) protein kinase B (PKB/AKT) phosphorylates the Forkhead box O (FoxO) transcription factor and inhibits its inhibitory effect on LXR-dependant transcription of Srebp-1c. During fasting, glucagon level increases and, as a consequence, proteine kinase A (PKA), a mediator of glucagon/cAMP, represses the LXR induced expression of Srebp-1c. Bile acids also regulate LXR. Bile acids activate farnesoid X receptor (FXR) which in turn activates the small heterodimer partner (SHP) a nuclear receptor which lacks the DNA binding domain (DBD) common to most nuclear receptor. SHP is able to interact with LXR and represses the expression of LXR target gene. NAD-dependent deacetylase sirtuin-1 (SIRT1) is able to deacetylate LXR which leads to ubiquitination and degradation of LXR. This mechanism is important to "recycle" LXR and to enhance its transcriptional activity of its target genes. Polyunsaturated fatty acids (PUFAs) modulate the activity of LXR, ChREBP and SREBP-1c by distinct mechanism.

Two LXREs were identified in the promoter of *Srebp-1* (Chen et al., 2004) although usually most promoters of LXR target genes only have a single LXRE (Costet et al., 2000). SREBPs are transcription factors from the basic-helix-loop-helix leucine zipper (bHLH/LZ) transcription factor family and are located in the endoplasmic reticulum membrane as a precursor form. To influence transcription of their target genes, SREBPs must be proteolytically cleaved to release the NH2-terminal segment that can enter the nucleus (Wang et al., 1994). There are three SREBP isoforms in mammals which are designated SREBP-1a, SREBP-1c, and SREBP-2. SREBP-1a, and SREBP-1c are encoded by the same gene and differ only in their first exon (Shimomura et al., 1997; Yokoyama et al., 1993) whereas SREBP-2 is encoded by another gene (Hua et al., 1993; Miserez et al., 1997). SREBP-2 is involved in cholesterol synthesis, indeed expression of a dominant-positive truncated form of this protein leads to an increase of mRNA transcripts coding for enzymes involved in cholesterol synthesis and an increase of cholesterol synthesis (Horton et al., 1998). SREBP-1c plays a role in lipogenesis, as expression of a dominant-positive truncated form of SREBP-1c leads to triglycerides accumulation in the liver and an increase of lipogenic gene expression (Shimano et al., 1997, 1999). Transgenic mice expressing a dominant-positive truncated form of SREBP-1a show an increase in both cholesterol and triglycerides synthesis (Shimano et al., 1997), suggesting that SREBP-1a shares its effects with the two others SREBP isoforms. Truncated SREBP-1a has stronger effects on lipogenesis than truncated SREBP-1c (Shimano et al., 1997). However, SREBP-1a is expressed only at low levels in the livers of adult mice, rats, hamsters, and humans (Shimomura et al., 1997), which suggests that *in vivo* SREBP-1c is the main isoform involved in the control of lipogenesis in adults. The detailed mechanisms of SREBP-2 and SREBP-1a post-transcriptional activation in response to cellular sterol depletion is well-described (Brown and Goldstein, 2009; Goldstein et al., 2006). SREBP-1c cleavage does not occur in response to low cholesterol level. It is stimulated by insulin (Hegarty et al., 2005; Howell et al., 2009; Yabe et al., 2003) and by endoplasmic reticulum stress (Kammoun et al., 2009). However, it is clear that the LXR-SREBP-1c axis is very important for the effect of LXR on lipogenesis. Indeed, in mice with a liver-specific deletion of SREBP-1c a highly disminished response to LXR synthetic agonist and to fasting/refeeding was observed (Liang et al., 2002). The LXR-SREBP-1c axis is highly regulated. Interestingly, it has recently been reported that, in human, SREBP-1c regulates the expression of a MicroRNA that mediates a feedback loop on the auto-regulation (Laffitte et al., 2001) of LXRα expression (Ou et al., 2011).

Like SREBP-1c, ChREBP is also a bHLH/LZ transcription factor family that contributes to the regulation of lipogenesis (Denechaud et al., 2008b). It has been discovered in 2001 by Uyeda's group (Yamashita et al., 2001). The gene encoding for ChREBP is mainly expressed in liver, small intestine, kidney and white and brown adipose tissue (Iizuka et al., 2004). It acts as an heterodimer with Max like protein (Mlx) (Ma et al., 2006; Stoeckman et al., 2004). As SREBP-1c, ChREBP needs to be post-translationally modified and thus activated in order to exert its transcriptional activity in the nucleus. Under low concentration of glucose, ChREBP is phosphorylated and remains in the cytosol. Upon high carbohydrate feeding, ChREBP translocates into the nucleus. Even if ChREBP gene is under the transcriptional control of LXR it has been reported that only glucose induces its transcriptional activity (Denechaud et al., 2008a). To date, several activation mechanisms have been described. One requires dephosphorylation of ChREBP on its Ser-196 residue by the protein phosphatase 2A (PP2A) (Kabashima et al., 2003). This phosphatase is activated by xylulose-5-phosphate (X5P) (Kabashima et al., 2003), a metabolite of the pentose-phosphate pathway alimented by glucose. Another mechanism involves the glucose sensing domain (GSM), an evolutionary conserved domain located on the N-terminus part of ChREBP (Li et al., 2006). This GSM domain is reported to require glucose-6-phosphate (Li et al., 2010) to be activated. ChREBP activity in response to glucose also seems to involve its glycosylation (Sakiyama et al., 2010). Finally, a recent study has provided evidence for another molecular mechanism that regulates ChREBP activity. This mechanism involves the activity of p300, histone acetyltransferase (HAT) co-activator that coactivates glucose-mediated ChREBP induction of glycolytic and lipogenic gene expression by acetylating both histone and ChREBP itself (Bricambert et al., 2010). These authors have also identified the serine/threonine kinase salt-inducible kinase 2 (SIK2) as an upstream regulator of ChREBP through its effect on p300.

When activated, SREBP-1c and ChREBP translocate to the nucleus where they bind respectively to sterol response element (SRE) and carbohydrate response element (ChoRE) of their target genes and govern their expressions. SREs have been identified in the promoter of major genes involved in fatty acid synthesis: *Acc* (Lopez et al., 1996), *Fasn* (Latasa et al., 2000), *Elovl6* (Kumadaki et al., 2008), *Scd1* (Tabor et al., 1999). Similarly, ChoRE have been located on the promoters of *Acc* (O'Callaghan et al., 2001) and *Fasn* (Rufo et al., 2001). Moreover, SREBP-1c−/− mice and ChREBP−/− mice like LXRαβ−/− mice fed a standard diet show reduced expression of lipogenic genes (Cha and Repa, 2007; Iizuka et al., 2004; Liang et al., 2002; Repa et al., 2000a). LXR together with ChREBP and SREBP-1c belong to a network of nutrient sensing factors involved in the control of hepatic fatty acid synthesis.

4.2. Hormonal and nutritional regulation of LXR

Insulin signaling is essential to maintain glucose and lipid homeostasis in the fed state. It has been suggested that insulin activates LXR (Chen et al., 2004; Tobin et al., 2002). Furthermore, the lack of LXRs blunts the insulin induced expression of enzymes involved in fatty acid and cholesterol metabolism (Tobin et al., 2002). The use of a gene reporter assay showed that the induction of the expression of Srebp-1c by insulin requires the two LXREs in its promoter (Chen et al., 2004). It has also been suggested from experiments done in cell culture that a tonic activation of LXR by an endogenously produced sterol is required in order to maintain SREBP-1c expression (DeBose-Boyd et al., 2001). Brown and Goldstein's group also showed that insulin might lead to the synthesis of an endogenous ligand required for the activation of SREBP-1c by LXR (Chen et al., 2004). Another mechanism of LXR activation induced by insulin has been proposed. The hepatic over-expression of a constitutive active form of forkhead box-"Other" 1 transcription factor (FoxO1) leads to a decrease expression of *Srebp-1c* (Zhang et al., 2006). In the absence of any cellular stimulus FoxO transcription factors localize in the nucleus where they regulate transcription of their target genes. FoxOs are phosphorylated by the protein kinase B (PKB), a downstream target of insulin receptor, and are inactivated by relocalizing from the nucleus to the cytosol (Birkenkamp and Coffer, 2003). Zhang et al. (2006) postulated that constitutively active FoxO1 might impair LXR activity. Recently, it has been shown that active form of FoxO1 counteracts the binding of LXRα with the LXRE in the *Srebp-1c* promoter (Liu et al., 2010). This suppression of *Srebp-1c* expression by FoxO1 can be counteracted by the inactivation of FoxO1 by insulin (Liu et al., 2010).

During fasting, glucagon is secreted by the β-cells of the pancreatic islets in response to low blood glucose. In the liver, glucagon induces an increase of intracellular cAMP and induces downstream activation of the protein kinase A (PKA). This kinase is involved in several cellular mechanisms by phosphorylating its target proteins. PKA can phosphorylate LXR on its ligand binding domain and heterodimerization domain (Yamamoto et al., 2007). This leads to a decrease of LXR activity (Yamamoto et al., 2007). This finding is in accordance with an inhibition of lipogenesis induced by glucagon.

Essential fatty acids, also known as polyunsaturated fatty acids (PUFAs), of the n-3 and n-6 series can modulate lipids metabolism. Linoleic (C18:2 n-6) and α-linolenic acids (C18:3 n-3), the precursors of n-6 and n-3 fatty acid families respectively, cannot be synthesized in animals and must be provided by the diet. These precursors can be further desaturated and elongated to produce very long chain PUFAs such as arachidonic acid (C20:4 n-6) and docosahexaenoic acid (C22:6 n-3) (Guillou et al., 2010). It has been showed that the presence of PUFAs in the diet decrease the expression of genes coding for enzymes involved in lipogenesis such as *Fasn*, *Acc* and *Scd1* (Jump and Clarke, 1999). Whereas a deficiency in dietary PUFAs (Alwayn et al., 2004; Sekiya et al., 2003) or disruption of the very long chain PUFA synthesis pathway (Moon et al., 2009) leads to an increased lipogenesis and triglycerides accumulation in the liver. PUFAs repress the expression of SREBP-1c (Ou et al., 2001) and its post-transcriptional maturation (Hannah et al., 2001). Moreover, PUFAs also inhibit hepatic maturation of ChREBP (Dentin et al., 2005). PUFAs are also known to be able to bind to and activate certain nuclear receptors such as PPAR α (Gottlicher et al., 1992; Martin et al., 2007). It has also been showed that PUFAs bind to LXR and act as antagonists in *in vitro* settings and in cell culture (Ou et al., 2001; Svensson et al., 2003). Therefore PUFAs may not only repress lipogenic gene expression through SREBP-1c and ChREBP but also *via* LXR (Ou et al., 2001). However, this possibility is still very much debated since further studies performed in cell culture (Pawar et al., 2002, 2003) and *in vivo* (Pawar et al., 2003; Takeuchi et al., 2010) have provided results which are not consistent with such possibility. Beside the debated antagonistic effect of PUFAs on LXR activity, it has also been shown that certain PUFAs may repress transcription of *Srebp-1c* by reducing trans-activating capacity of LXR (Howell et al., 2009).

Other nutrient-sensing mechanisms that influence LXR-mediated promotion of fatty acid synthesis may involve the farnesoid X receptor (FXR, NR2H4) and the histone deacetylase SIRT1. FXR is activated by bile acids (Makishima et al., 1999). Upon activation, FXR induces the expression of the short heterodimer partner (SHP, NR0B2) (Goodwin et al., 2000), an atypical orphan nuclear receptor lacking a DNA-binding domain. It is known to act as a corepressor of many nuclear receptors. Brendel et al. (2002) showed that SHP interacts with the helix 12 of LXR and represses the expression of typical LXR target as well as reducing its activity in a gene reporter assay. SIRT1 acts in response to nutrient availability as a master switch in lipid and glucose homeostasis (Feige and Auwerx, 2007; Hou et al., 2008; Ponugoti et al., 2010). As described earlier, SIRT1 deacetylates LXR which leads to its ubiquitination and its degradation through the proteasome (Li et al., 2007b). This degradation is important for recycling LXR and maintaining its activity. Transgenic mice lacking *Sirt1* show similar characteristics as transgenic mice lacking LXR: decreased HDL-cholesterol and plasma triglycerides (Kalaany et al., 2005; Li et al., 2007b).

5. Conclusion

LXRs play an essential role in lipid homeostasis highlighted by its central position at the crossroad between cholesterol and fatty acid metabolism. It is a critical receptor in the control of various physiological functions that relates not only to metabolic and cardiovascular diseases such as obesity, atherosclerosis and diabetes, but also to other diseases such as dermatological and reproductive disorders, Alzheimer's disease and cancer. Therefore, LXRs show great potential as pharmacological targets and the development of selective LXR agonist without deleterious side effects such as those that occur as a consequence of elevated lipogenesis is a major challenge. Another challenge in the field is the development of highly sensitive biochemical approaches that will allow us to better understand the tissue-specific oxysterol metabolism. It is required to better delineate the role of the oxysterol-LXR axis in health and disease.

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Dual extraction of both mRNAs and lipids from a single sample

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Abstract Extractions of RNAs and lipids from a large number of biological samples represent time-consuming and costly steps required for both transcriptomic and lipidomic approaches. Most protocols rely on independent extractions of nucleic acids and lipids from a single sample, thereby increasing the need for biological material and the variability in data analysis. In this work, we investigated whether it would be possible to use standard RNA extraction procedures in order to analyze not only mRNA levels but also lipids from a single liver sample. We bring evidence that the organic phase, obtained when using standard reagents for RNA extractions can be kept to analyze lipids such as neutral lipids and fatty acids by gas chromatography.

Supplementary key words: transcriptomic, lipidomic, Liver X Receptor.

INTRODUCTION

Transcriptomic (Momin et al., 2011) and lipidomic (Fahy et al., 2005) analysis have both become accessible and essential tools for investigators in the field of lipid research. Most lipid analysis and gene expression assays involve independent, costly and time-consuming extractions.

Given that most mRNA extractions are based on a phase split between chloroform and phenol, we thought it would be worth evaluating whether such organic phase can be used for complementary lipid analysis. We developed a protocol adapted from Bligh and Dyer's extraction (Bligh and Dyer, 1959) and applied it to the remaining organic phase once nucleic acids have been extracted from the aqueous phase.

We used tissue and cell samples to assess the method we developed. Finally, the protocol was validated through analysis performed on samples from transgenic mice lacking the Liver X Receptor (LXR) (Repa *et al.*, 2000), a transcription factor that has been shown to play a central role in cholesterol and fatty acid metabolism (Calkin *et al.*, 2012).

MATERIAL AND METHODS

Animals

LXRα and LXRβ knockout mice (LXR$^{-/-}$) and their wild-type controls were maintained on a mixed-strain background (C57BL/6:129Sv), housed in a temperature-controlled room with a 12-h light, 12-h dark cycle and fed *ad libitum* with water and Global-diet 2016S (Harlan, Gannat, France). These mice have been extensively detailed in previous articles (Repa *et al.*, 2000) and were kindly provided by Dr D.J. Mangelsdorf (University of Texas, USA). All experiments were performed on age-matched male mice.

Cell culture

JWZ murine hepatic cells, also referred to as MuSH immortalized hepatocytes were kindly provided by Dr. J.P. Gray. Cells were cultured with L-glutamine (2 mM), penicillin (100 IU/ml), streptomycin (100 mg/ml), and BFS (10%). The culture medium was supplemented with dexamethasone (1 mM).

RNA extraction

Total RNA was extracted from liver samples or from mouse hepatic cell line. RNA was extracted with TRIzol® reagent (Invitrogen, Cergy Pontoise, France) according to the manufacturer's protocol or with the standard protocol described by Chomczynski and Sacchi (2006).

Extraction of lipid from the remaining organic phase after mRNA extraction

A sample of the organic phase obtained after RNA extraction was used for lipid extraction. We either carefully removed all the organic phase or 25 µL out organic phase and added them into a tube containing the appropriate internal standards and 2.5 mL of methanol. After vortexing, the sample

is filtered once on glass wool prior to addition of chloroform (2.5mL) and water (2mL). Next the samples are intensely mixed by vortexing for 10 sec and centrifuged for 2 min at 1500 g. The organic phase is next extracted, evaporated to dryness and resuspended in the appropriate solvent for lipid analysis.

Estimation of the organic phase volume in TRIzol extraction

The organic phase volume was estimated through the use of radiolabeled [1-^{14}C] palmitic acid (Perkin Elmer Life Sciences; Paris, France) added prior to extraction of mRNA from 20 liver samples weighing between 20 and 120 mg. Then, the organic phase volume was calculated from the amount of radioactivity found in 25µL of the organic phase as compared to the input measured by liquid scintillation counting (Tri-Carb 1600 TR; Packard, Meriden, CT, U.S.A.).

Standard lipid extraction

Triglyceride (TG), cholesterol, cholesterol esters and fatty acid assays were performed as described previously (Zadravec *et al.*, 2010). Briefly, following homogenization of tissue samples in methanol/5 mM EGTA (2:1, v/v), lipids corresponding to an equivalent of 1 mg of tissue were extracted according to Bligh and Dyer in chloroform/methanol/water (2.5:2.5:2.1, v/v/v), in the presence of the internal standards.

Neutral lipid analysis

Total lipids were resuspended in 50 µL of ethyl acetate and neutral lipids were analyzed by gas-liquid chromatography on a Focus Thermo Electron system using a Zebron-1 Phenomenex fused-silica capillary column (5 m, 0.32 mm i.d., 0.50 mm film thickness). Oven temperature was programmed from 200 to 350°C at a rate of 5°C/min, and the carrier gas was hydrogen (0.5 bar). The injector and the detector were at 315 and 345°C, respectively.

Fatty acid analysis

Fatty acids (FA) were analyzed as FA methyl ether as previously described (Zadravec *et al.*, 2010). To measure total FA methyl ester (FAME) molecular species, lipids corresponding to an equivalent of 1 mg of liver were extracted in the presence of glyceryl triheptadecanoate as an internal standard. The dried lipid extract was transmethylated with 1 ml of BF3 in methanol (1:20, v/v) for 150 min at 100°C, evaporated to dryness, and the FAMEs were extracted with hexane/water (3:1). The organic phase was evaporated to dryness and dissolved in 50µl ethyl acetate. One microliter of FAME was analyzed by gas-liquid chromatography on a 5890 Hewlett-Packard system (Hewkett-Packard, Palo Alto, CA, USA) using a Famewax fused-silica capillary column (30 m, 0.32 mm i.d., 0.25 mm film thickness; Restek, Belfast, UK). Oven temperature was programmed from 110 to 220°C at a rate of 2°C/min, and the carrier gas was hydrogen (0.5 bar). The injector and the detector were at 225 and 245°C, respectively.

Real-time qPCR

For RT, amplified RNA samples (2µg) were reverse-transcribed using SuperScript™ II reverse transcriptase (Invitrogen). Primers for SYBR Green assays are given in Table 1. Real-time amplifications were performed on an ABI Prism 7000 SDS (Applied Biosystems). All q-PCR data were normalized by TATA Box Binding Protein mRNA levels. Differential gene expression was calculated by the $\Delta\Delta C_T$ calculation method.

Statistical analysis

Differential effects were analyzed by a Student *t*-test. $P < 0.05$ was considered significant.

RESULTS

Comparison of RNA extraction protocols

We first compared the two RNA extraction protocols by measuring the mRNA level of various genes when measured from the same liver samples following two different mRNA extraction protocols. As shown in Figure 1A, we found that whatever the method used the mRNA level of three tested genes were similar. We also found that under such circumstances the amount of 28S RNA was similar.

Comparison of lipid extraction after RNA extraction

We next tested whether RNA extraction protocols commonly used also allowed subsequent lipid extraction and analysis. To address this point we measured abundant neutral lipids such as triglycerides, cholesterol and cholesterol esters from total liver lipid extract (Figure 1B). We compared the amount of these lipids to those obtained after a standard lipid extraction and found no significant differences between protocols. This observation suggests that lipid can be extracted and quantified from the remaining organic phase after a RNA extraction. Consistent with this first finding we next showed that such extraction also allows for total fatty acid estimations (Figure 1C).

Estimation of the organic phase volume

Next, we estimated the volume of the organic phase by using radiolabeled [1-^{14}C] palmitic acid. We checked that under conditions of mRNA extraction with TRIzol the upper phase contained no significant radiolabeling and determine the organic phase volume from the amount of radioactivity contained in 25µL carefully extracted from the organic phase. Through this, we estimated that the organic phase is about 560µL (data not shown). This tip allowed us not to try to extract all of the remaining organic phase for quantification of lipids.

Linearity of both RNA and lipid assays after dual extraction from a single sample

We then focused on one RNA extraction protocol with the widely used Trizol reagent. We determined whether within the range of tissue weight and cell number it is usually applied to subsequent RNA assays and lipid quantification would be linear. Again, we measured total RNA and major neutral lipids from both liver samples (Figure 2) and from cultured cells (Figure 3). We found that in both cases RNA and lipids can be reliably quantified.

Analysis of both hepatic mRNA and lipids reflecting the activity of the Liver X Receptor

To test the newly established method we focused on the activity of the nuclear receptor LXR. It is well-known that pharmacological activation of LXR strongly up-regulates the expression of the hepatic genes involved in fatty acid biosynthesis (Repa *et al.*, 2000, Ducheix *et al.*, 2013). The livers of mice which had been treated for five days with T0 were analyzed for mRNA and lipids. As expected we observed a marked increase in the expression rate-limiting genes in fatty acid biosynthesis such as Fatty Acid Synthase (*Fas*) and Stearoyl-CoA Desaturase 1 (*Scd*1) in mice treated with T0 (Figure 4A). This effect is largely dependent on the presence of LXR since similar increase was not observed in mice lacking both LXR isoforms (LXR-/-). Correlated with the increase in lipogenic gene expression, we observed that the liver triglyceride level was increased in wild-type but not in transgenic mice (Figure 4B). When we measured fatty acid proportion we also observed, consistent with the increase in SCD1 expression, an increase in the proportion of mono-unsaturated fatty acids (Figure 4B).

CONCLUSION

In this report we provide evidence that standard nucleic acid extraction protocol can be adapted to analyze lipids which are usually discarded (Figure 5). Such adaptation is reliable to analyze lipids extracted from tissue samples and from cultured cells. In addition, this approach may be particularly relevant to allow both transcriptome and lipid analysis from biopsies or whenever the amount of tissue is limiting.

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206 FIGURE LEGENDS

207

208 **Figure 1: Commercially available Trizol can be used to extract mRNA (A) total lipids**
209 **(B) and total fatty acids (C) from mouse liver samples.** Commercially available Trizol was
210 compared to Chomczynski & Sacchi method for mRNA extraction (A) and to Bligh & Dyer
211 for lipid extraction (B, C) from mouse liver samples. RNAs were analyzed by qPCR and
212 lipids were analyzed by Gas Chromatography. Data are the mean ± SEM. * Significant
213 difference from Bligh and Dyer by t-test (n= 6 samples per group). $P < 0.05$ was considered
214 significant.

215

216 **Figure 2: Trizol can be used to extract total lipids from a wide range of mouse liver**
217 **samples weigh.** Commercially available Trizol was used to extract mRNA (A) and
218 cholesterol (B), cholesterol esters (C), triglycerides (D) from mouse liver samples ranging
219 from 1 to 100 mgs. Bligh & Dyer was also used for cholesterol (E), cholesterol esters (F),
220 triglycerides (G) extraction from mouse liver samples from 1 to 100 mgs. RNAs were
221 analyzed by qPCR and lipids were analyzed by Gas Chromatography. Data are the mean ±
222 SEM (n= 6 samples per group).

223

224 **Figure 3: Trizol can be used to extract total lipids from a wide range of hepatocyte**
225 **numbers.** Commercially available Trizol was used to extract mRNA (A) and cholesterol (B),
226 cholesterol esters (C), triglycerides (D) from JWZ cells ranging from 1 to 5.10^6 cells. Bligh &
227 Dyer was also used for cholesterol (E), cholesterol esters (F), triglycerides (G) extraction from
228 mouse liver samples from JWZ cells ranging from 1 to 5.10^6 cells. RNAs were analyzed by

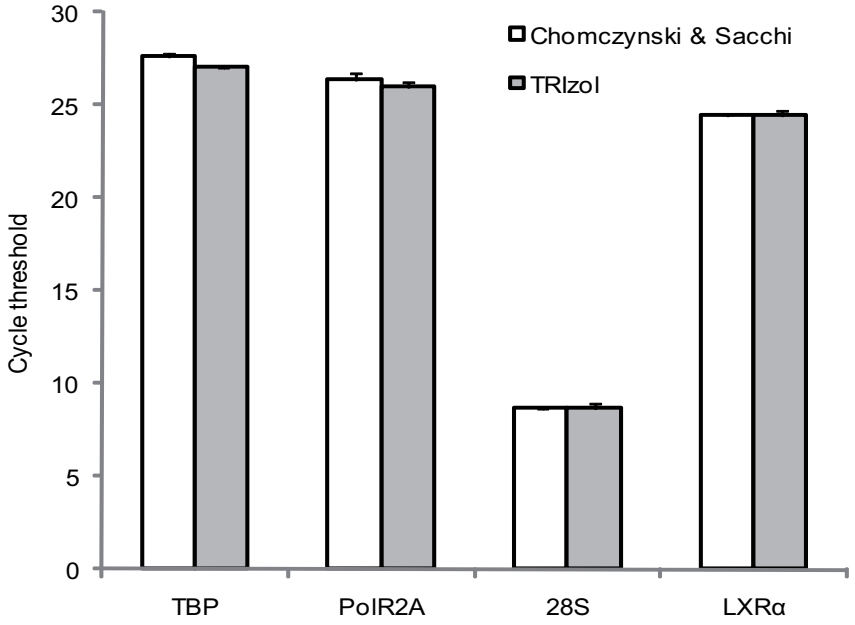
qPCR and lipids were analyzed by Gas Chromatography. Data are the mean ± SEM (n= 6 samples per group).

Figure 4: Trizol extraction of both hepatic mRNA and lipids allows detection of changes in gene expression (A) and liver lipids (B) in mice treated with T0901317. Commercially available Trizol was used to extract mRNA (A) and lipids (B) from mouse liver samples. RNAs for *Fas* and *Scd1* were analyzed by qPCR (A). Triglycerides and fatty acids were analyzed by Gas Chromatography. MUFA: Mono-Unsaturated Fatty Acids; SFA: Saturated Fatty Acids. Data are the mean ± SEM (n = 6 samples per group). * Significant effect of T0301317 by t-test. $P < 0.05$ was considered significant.

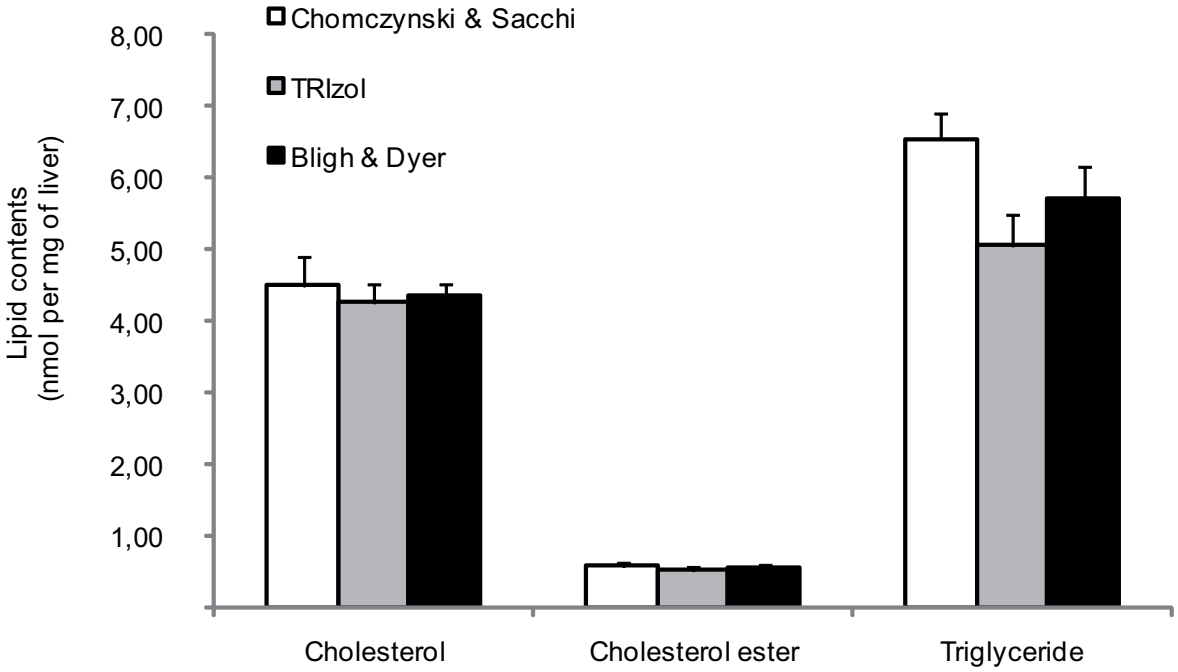
Figure 5: Dual extraction of both lipid and mRNA from a single sample.

Figure 1

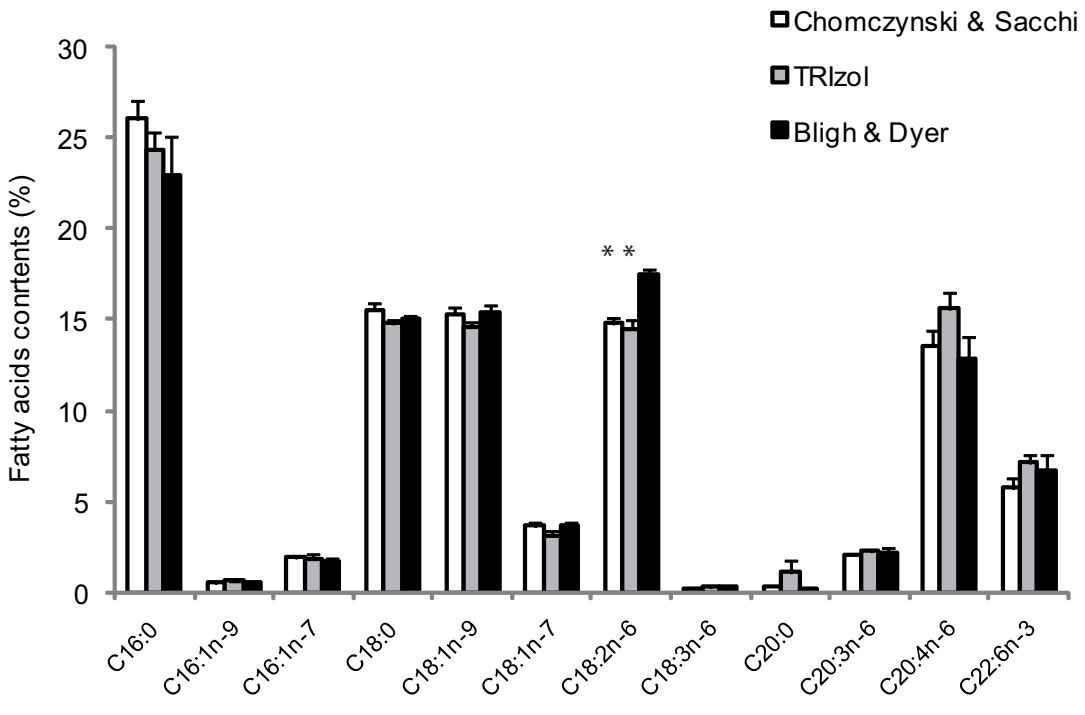
A


Chomczynski & Sacchi
TRIzol
Cycle threshold
30
25
20
15
10
5
0
TBP
PolR2A
28S
LXRα

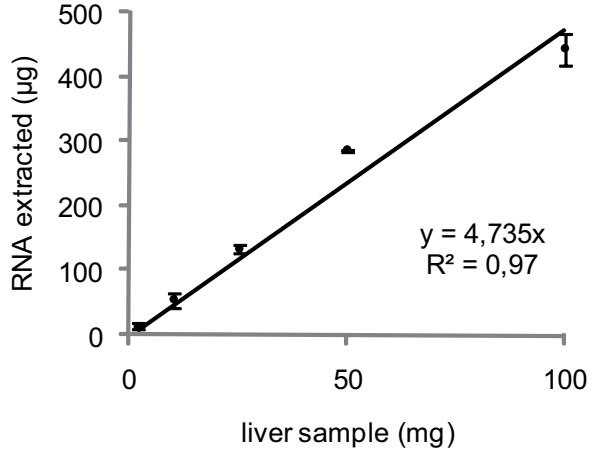
B


Chomczynski & Sacchi
TRIzol
Bligh & Dyer
Lipid contents
(nmol per mg of liver)
8,00
7,00
6,00
5,00
4,00
3,00
2,00
1,00
Cholesterol
Cholesterol ester
Triglyceride

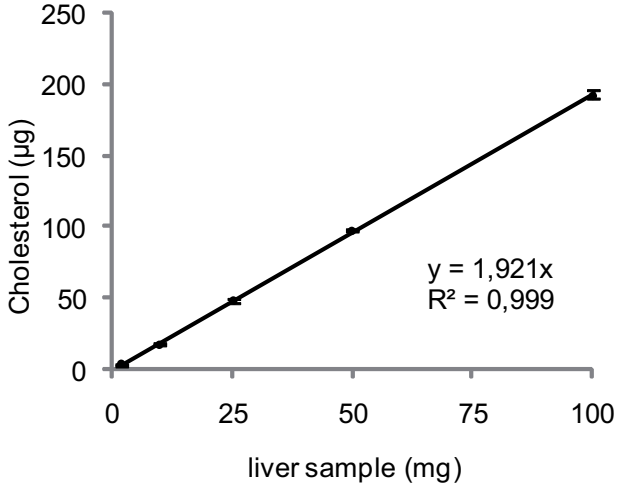
C


Chomczynski & Sacchi
TRIzol
Bligh & Dyer
Fatty acids contents (%)
30
25
20
15
10
5
0
* *
C16:0
C16:1n-9
C16:1n-7
C18:0
C18:1n-9
C18:1n-7
C18:2n-6
C18:3n-6
C20:0
C20:3n-6
C20:4n-6
C22:6n-3

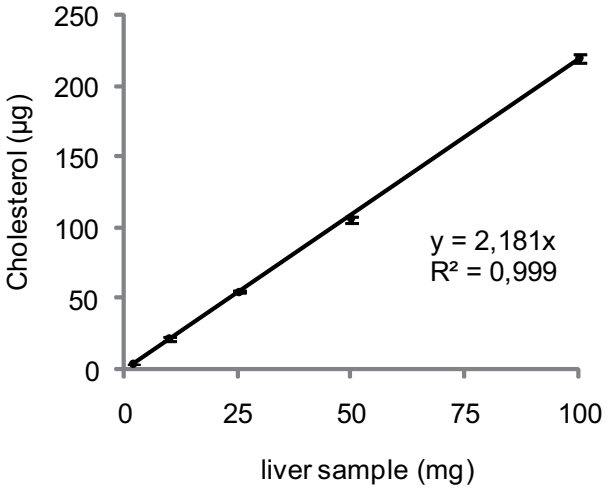
A


RNA extracted (µg)
500
400
300
200
100
0
0
50
100
liver sample (mg)
y = 4,735x
R² = 0,97

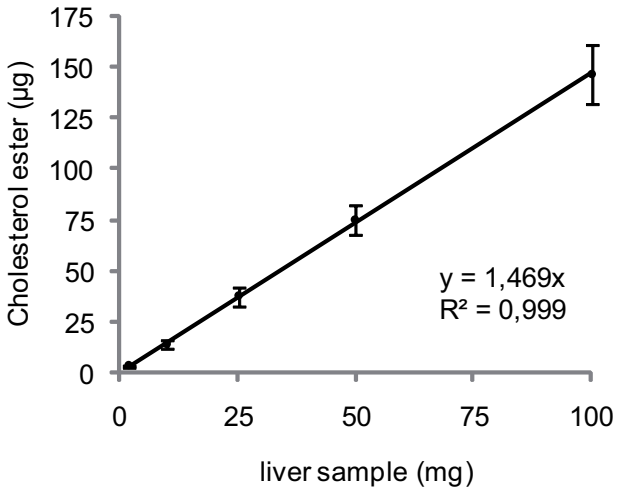
B


Cholesterol (µg)
250
200
150
100
50
0
0
25
50
75
100
liver sample (mg)
y = 1,921x
R² = 0,999

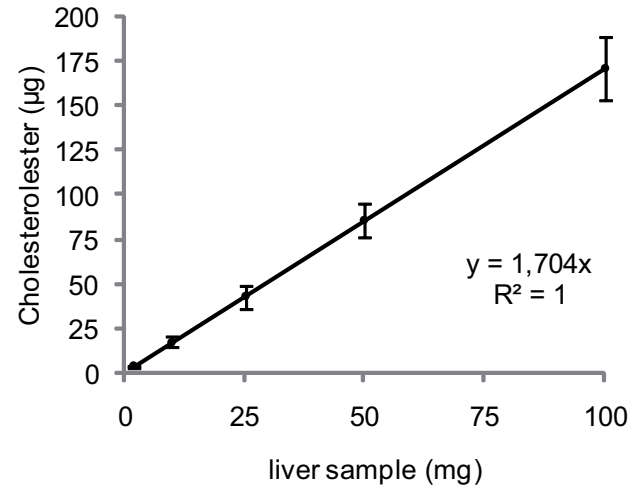
E


Cholesterol (µg)
250
200
150
100
50
0
0
25
50
75
100
liver sample (mg)
y = 2,181x
R² = 0,999

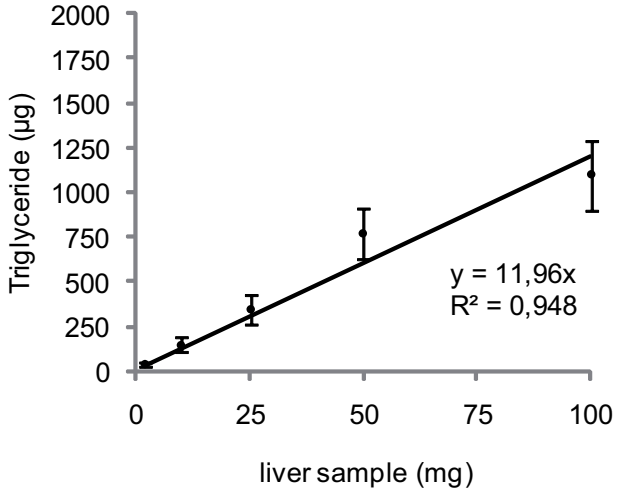
C


Cholesterol ester (µg)
175
150
125
100
75
50
25
0
0
25
50
75
100
liver sample (mg)
y = 1,469x
R² = 0,999

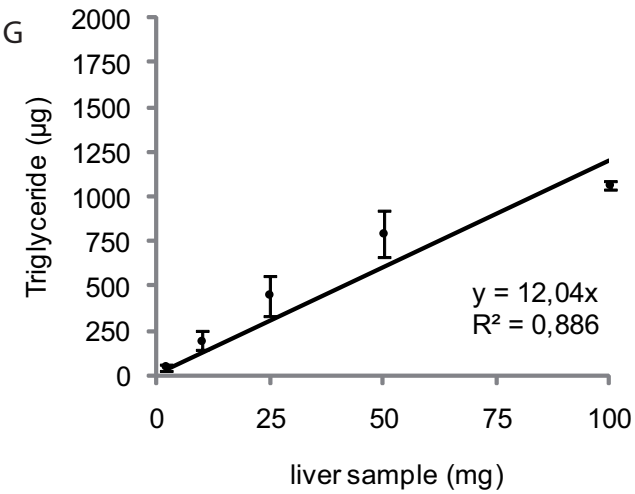
F


Cholesterolester (µg)
200
175
150
125
100
75
50
25
0
0
25
50
75
100
liver sample (mg)
y = 1,704x
R² = 1

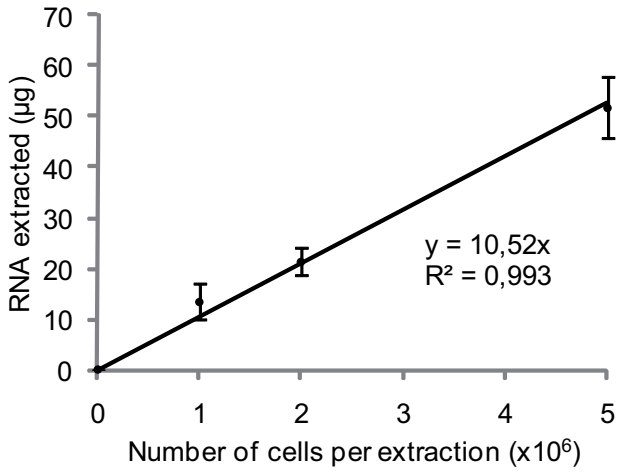
D


Triglyceride (µg)
2000
1750
1500
1250
1000
750
500
250
0
0
25
50
75
100
liver sample (mg)
y = 11,96x
R² = 0,948

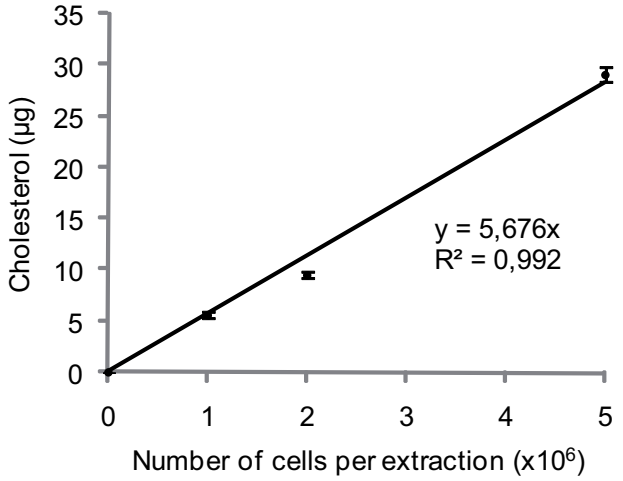
G


Triglyceride (µg)
2000
1750
1500
1250
1000
750
500
250
0
0
25
50
75
100
liver sample (mg)
y = 12,04x
R² = 0,886

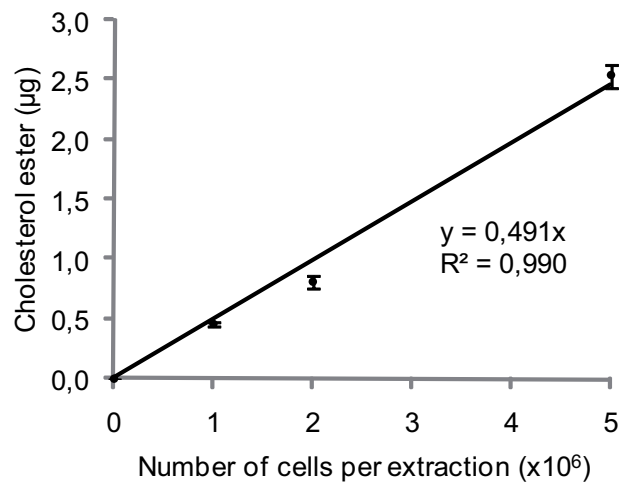
A


RNA extracted (µg)
70
60
50
40
30
20
10
0
0
1
2
3
4
5
Number of cells per extraction (x10⁶)
y = 10,52x
R² = 0,993

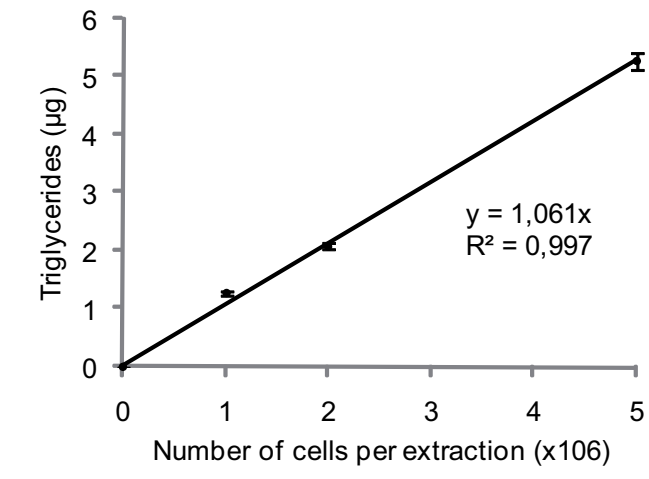
B


Cholesterol (µg)
35
30
25
20
15
10
5
0
0
1
2
3
4
5
Number of cells per extraction (x10⁶)
y = 5,676x
R² = 0,992

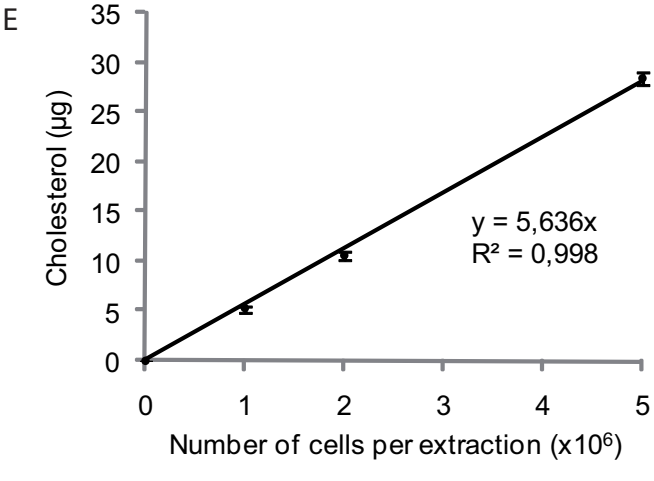
C


Cholesterol ester (µg)
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
1
2
3
4
5
Number of cells per extraction (x10⁶)
y = 0,491x
R² = 0,990

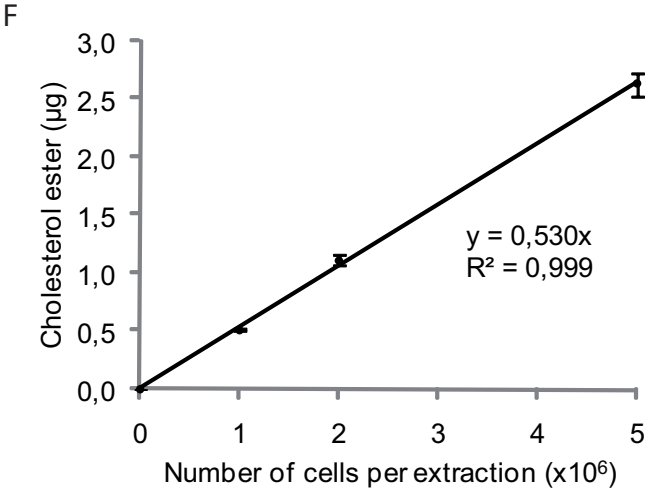
D


Triglycerides (µg)
6
5
4
3
2
1
0
0
1
2
3
4
5
Number of cells per extraction (x106)
y = 1,061x
R² = 0,997

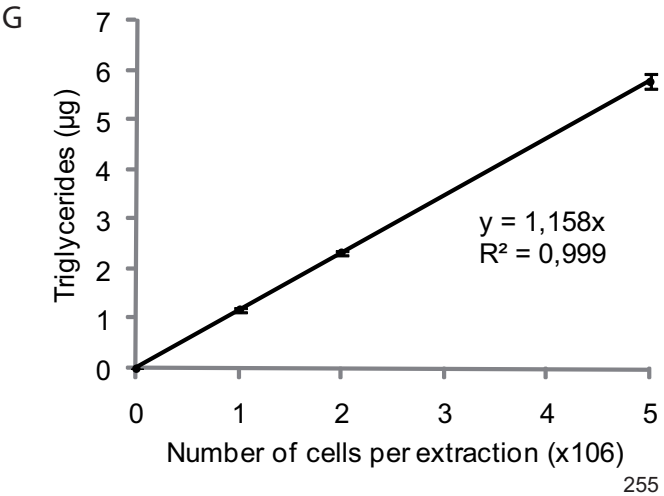
E


Cholesterol (µg)
35
30
25
20
15
10
5
0
0
1
2
3
4
5
Number of cells per extraction (x10⁶)
y = 5,636x
R² = 0,998

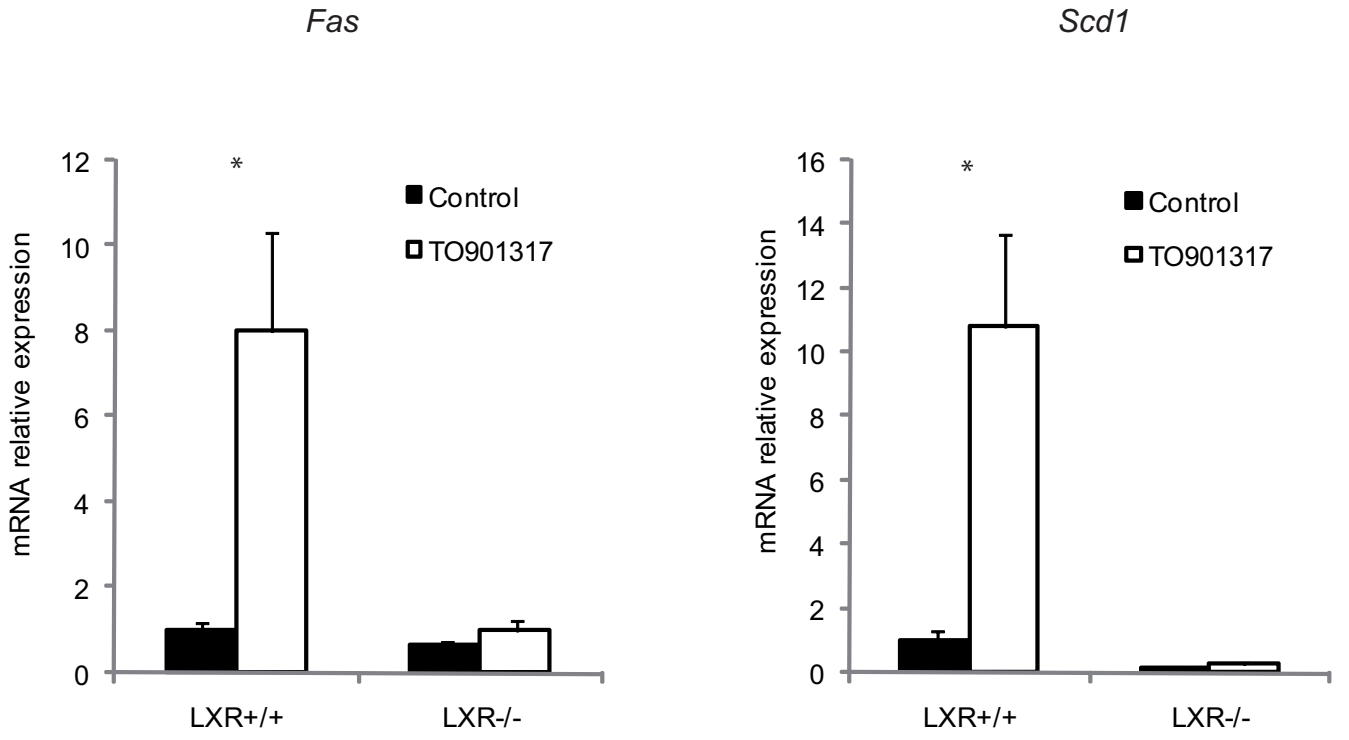
F


Cholesterol ester (µg)
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
1
2
3
4
5
Number of cells per extraction (x10⁶)
y = 0,530x
R² = 0,999

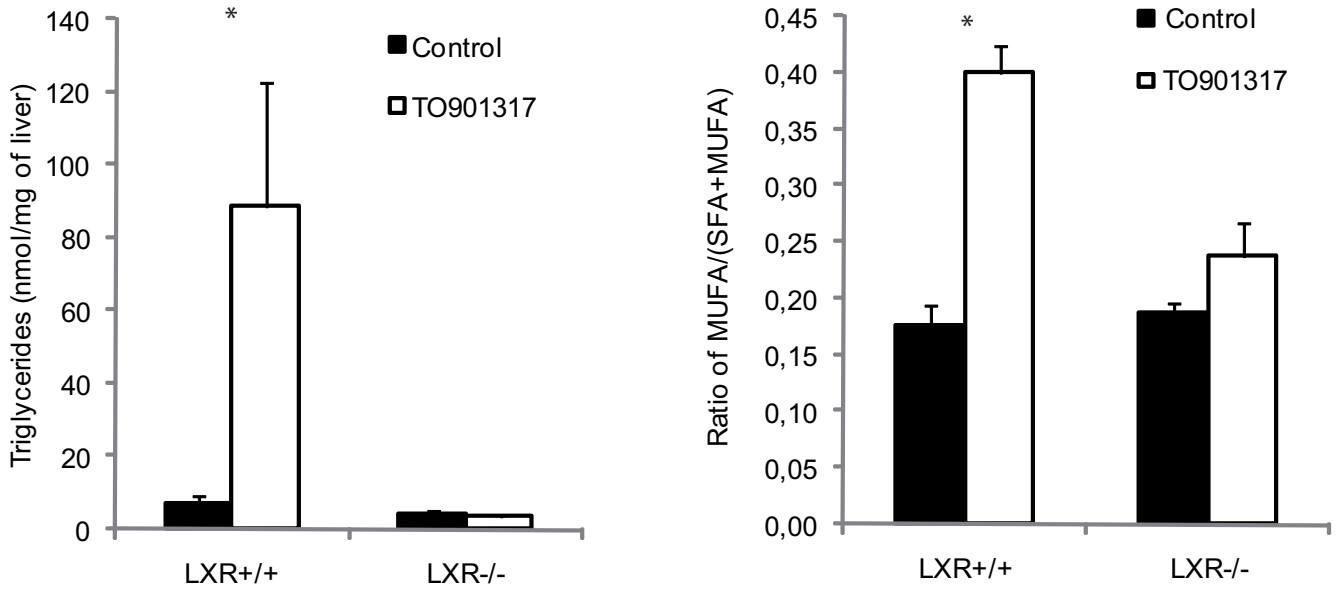
G

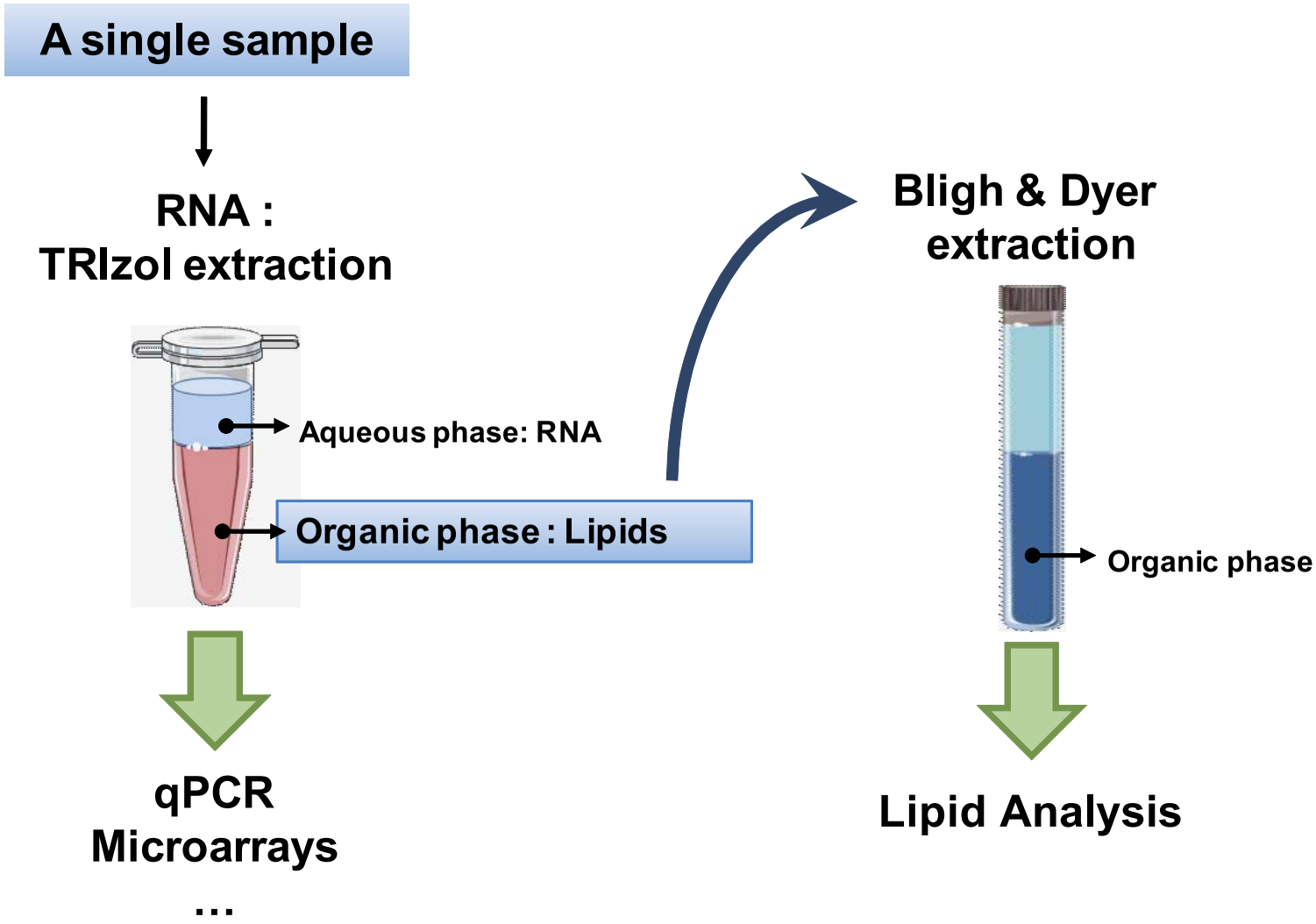

Triglycerides (µg)
7
6
5
4
3
2
1
0
0
1
2
3
4
5
Number of cells per extraction (x106)
y = 1,158x
R² = 0,999

A


Fas
Scd1
mRNA relative expression
Control
TO901317
*
LXR+/+
LXR-/-
0
2
4
6
8
10
12
14
16

B


Triglycerides (nmol/mg of liver)
Ratio of MUFA/(SFA+MUFA)
Control
TO901317
*
LXR+/+
LXR-/-
0
20
40
60
80
100
120
140
0,00
0,05
0,10
0,15
0,20
0,25
0,30
0,35
0,40
0,45


A single sample
RNA :
TRIzol extraction
Aqueous phase: RNA
Organic phase : Lipids
qPCR
Microarrays
…
Bligh & Dyer
extraction
Organic phase
Lipid Analysis

Low Doses of Bisphenol A Induce Gene Expression Related to Lipid Synthesis and Trigger Triglyceride Accumulation in Adult Mouse Liver

Alice Marmugi,[1,2] Simon Ducheix,[1,2] Frédéric Lasserre,[1,2] Arnaud Polizzi,[1,2] Alain Paris,[3] Nathalie Priymenko,[1,2] Justine Bertrand-Michel,[4] Thierry Pineau,[1,2] Hervé Guillou,[1,2] Pascal G.P. Martin,[1,2] and Laïla Mselli-Lakhal[1,2]

Changes in lifestyle are suspected to have strongly influenced the current obesity epidemic. Based on recent experimental, clinical, and epidemiological work, it has been proposed that some food contaminants may exert damaging effects on endocrine and metabolic functions, thereby promoting obesity and associated metabolic diseases such as nonalcoholic fatty liver disease (NAFLD). In this work, we investigated the effect of one suspicious food contaminant, bisphenol A (BPA), *in vivo*. We used a transcriptomic approach in male CD1 mice exposed for 28 days to different doses of BPA (0, 5, 50, 500, and 5,000 μg/kg/day) through food contamination. Data analysis revealed a specific impact of low doses of BPA on the hepatic transcriptome, more particularly on genes involved in lipid synthesis. Strikingly, the effect of BPA on the expression of *de novo* lipogenesis followed a nonmonotonic dose-response curve, with more important effects at lower doses than at the higher dose. In addition to lipogenic enzymes (Acc, Fasn, Scd1), the expression of transcription factors such as liver X Receptor, the sterol regulatory element binding protein-1c, and the carbohydrate responsive element binding protein that govern the expression of lipogenic genes also followed a nonmonotonic dose-response curve in response to BPA. Consistent with an increased fatty acid biosynthesis, determination of fat in the liver showed an accumulation of cholesteryl esters and of triglycerides. *Conclusion*: Our work suggests that exposure to low BPA doses may influence *de novo* fatty acid synthesis through increased expression of lipogenic genes, thereby contributing to hepatic steatosis. Exposure to such contaminants should be carefully examined in the etiology of metabolic diseases such as NAFLD and nonalcoholic steatohepatitis. (HEPATOLOGY 2012;55:395-407)

Changes in diet and lifestyle are leading causes for the emergence of the metabolic diseases associated with obesity. Recently, the hypothesis that a number of food contaminants acting as endocrine-disrupting chemicals may influence metabolic diseases has been proposed.[1]

Bisphenol A (BPA) is an endocrine disruptor highly prevalent in our environment. It is used as the monomer of polycarbonate plastics and epoxy resins.[2] The human population is widely exposed to low levels of BPA, primarily by way of the diet by migration from food and beverage containers.[2] 93% of urine samples

Abbreviations: Acc, acetyl-CoA carboxylase; BPA, bisphenol A; ER, estrogen receptor; FA, fatty acid; Fasn, fatty acid synthase; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptor; Scd1, stearoyl-CoA desaturase-1.

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Potential conflict of interest: Nothing to report.

Additional Supporting Information may be found in the online version of this article.

collected from the National Health and Nutrition Examination Survey (NHANES III) cohort revealed detectable levels of BPA.[3] As a protective measure the U.S. Environmental Protection Agency and the European Food Safety Agency have established a tolerable daily intake (TDI) of 50 μg/kg/day derived by applying an uncertainty factor of 100 to the no-observed-adverse-effect level (NOAEL) of 5,000 μg/kg/day mainly based on liver and reproductive toxicity. However, recent animal studies revealed that exposure to environmentally relevant BPA doses below the TDI alters biological functions such as reproductive functions, brain functions, and metabolic processes by interfering with endocrine signaling pathways.[4]

Recent epidemiological studies showed an association between urinary levels of BPA and the prevalence of diabetes, cardiovascular diseases, and elevated markers of liver toxicity.[5,6] These studies pointed to metabolic disorders as a potential impact of exposure to low doses of BPA. In agreement with this hypothesis, experimental evidence has accumulated that BPA can alter several aspects of metabolic functions in rodents. Animal studies showed an increased body weight in offspring of mothers exposed to BPA during gestation and/or lactation period.[7] The increase in body weight was more pronounced and persistent in females than males and the effects were stronger at low compared with high doses of exposure. Such nonmonotonic dose-response relationship have been reported for many actions of BPA.[8-11] How perinatal BPA exposure may exert these effects remains to be determined, but potential target tissues of BPA action including adipose tissue and pancreas have been studied. Gestational exposure to BPA was shown to increase adipose tissue mass at weaning associated with adipocyte hypertrophy and overexpression of lipogenic genes.[9,10,12] Low BPA doses were also shown to increase leptin and to decrease adiponectin secretion.[9,13] *In vitro* studies documented an increased lipid accumulation and adipocyte differentiation after exposure of 3T3L1 preadipocytes to BPA and other endocrine-disrupting chemicals.[14-16]

Nadal and colleagues showed that BPA increases insulin synthesis and secretion with concurrent impacts on glucose homeostasis.[17,18] *In vivo* injection of 1, 10, or 100 μg/kg/day of BPA to adult male mice resulted in a significant dose-dependent decrease in glycemia in parallel to an increase in insulin from 30 minutes after injection.[19] Isolated islets of pancreatic β-cells exposed to a range of BPA doses showed increased insulin content following an inverted U-shape dose-response curve.[20] The same group recently reported on similar effects in pregnant mice and their offspring exposed to 10 or 100 μg/kg/day of BPA.[21]

Thus, both the adipose tissue and the pancreas have emerged as important targets of low BPA doses. Despite the important roles of the liver in whole body energy homeostasis, little is known about the hepatic impacts of exposure to environmentally relevant doses of BPA. Here we evaluated the effects of oral exposure to 50 μg/kg/day (TDI) or 5,000 μg/kg/day (NOAEL) of BPA on mouse liver transcriptome. Initial genome-wide microarray screenings evidenced a predominant impact of low BPA doses on lipid biosynthesis pathways. Using a wide range of doses, we showed that these effects are specific to low, environmentally relevant doses of BPA and correlate with an increased hepatic accumulation of neutral lipids.

Materials and Methods

Animals and Treatments. Six-week-old male CD1 mice (Charles River, Les Oncins, France) divided into five groups (n = 6/group) were administered BPA by way of the diet for 28 days (housing at 22 ± 2°C, 12-hour light/dark). A standard diet (ingredients from SAFE Diet, Augy, France) was formulated from maize starch (49%), saccharose (24.4%), casein (14%), minerals mix (5%), peanut oil (2.5%), rapeseed oil (2.5%), cellulose (2%), vitamins mix (0.5%), and methionine (0.1%). BPA (4,4′-dihydroxy-2,2-diphenylpropane, CAS# 80-05-7, Sigma-Aldrich, France) was incorporated in the diet at 0 (controls), 0.05, 0.5, 5, or 50 ppm. Considering a diet consumption of 10% of the body weight per day, this corresponds to an oral exposure of 0 (controls), 5, 50 (TDI), 500, or 5,000 μg of BPA/kg BW/day (NOAEL), respectively. *In vivo* studies were conducted under E.U. guidelines for the use and care of laboratory animals and were approved by an independent ethics committee.

Blood and Organ Sampling. Blood was collected at the submandibular vein in heparin-coated capillaries. Plasma was prepared by centrifugation (2,000*g*, 10 minutes) and kept at −80°C until use. Following euthanasia, the liver and the perigonadic white adipose tissue (pWAT) were removed, weighed, dissected, snap-frozen in liquid nitrogen, and stored at −80°C until use. Sampling was performed on two consecutive days (n = 3 mice/group per day) but no block effect was statistically evidenced.

Gene Expression Studies. Total RNA was extracted with TRIzol reagent (Invitrogen, Cergy Pontoise, France). Transcriptomic profiles were obtained using

Agilent Whole Mouse Genome microarrays (4 × 44k) following the manufacturer's instructions. Microarray data and all experimental details are available in the Gene Expression Omnibus (GEO) database (accession GSE26728). For real-time quantitative polymerase chain reaction (qPCR), total RNA samples (2 μg) were reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Courtaboeuf, France). Primers for SYBR Green assays are presented in Supporting Table 1. Amplifications were performed on an ABI Prism 7300 Real Time PCR System (Applied Biosystems). qPCR data were normalized by TATA-box binding protein (TBP) messenger RNA (mRNA) levels and analyzed with LinRegPCR.[22]

Immunoblot Analysis. Protein extracts were prepared using the Proteo-Jet cytoplasmic and nuclear extraction kit (Fermentas, Saint-Rémy-lès-Chevreuses, France). Following separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), liver proteins were probed with primary antibodies from Cell Signaling (β-actin: 4970; lamin A/C: 2032; ACLY: 4332; ACLY-P: 4331; ACC: 3662; FAS: 3189), Abcam (estrogen receptor [ER]: ab16460; GK: ab37796), Santa Cruz Biotechnology (LXR: sc-13068; SCD1: sc-14719), Lab Vision (SREBP-1c: MS-1207-P1ABX), Novus Biological (CHREBP: nb400-135), and secondary antibodies from Biotium (CF680 or CF770-labeled). G6PASE antibody was a gift from Dr. Gilles Mithieux.[23] The images were analyzed on the Odyssey Infrared Imaging system (Li-Cor, Lincoln, NE). Band intensities were normalized to those of β-actin or lamin A/C.

Biochemical Assays. Hepatic lipid content and FA composition were determined as described.[24] Plasma levels of triglycerides, glucose, total cholesterol, low- or high-density lipoprotein (LDL, HDL) cholesterol were determined on a biochemical analyzer, COBAS-MIRA+. Plasma insulin was assayed with the ultrasensitive mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Crystal Chem, Downers Grove, IL).

Histology. Frozen liver samples were embedded in Neg 50 (Fisher Scientific, Courtaboeuf, France). Sections (5 μm, Leica RM2145 microtome, Nanterre, France) were stained with Oil-Red-O and hematoxylin/eosin and visualized with a Leica DFC300 camera (Leica).

Statistical Analysis. All data were analyzed using R (www.r-project.org). Microarray data were processed with Bioconductor packages (www.bioconductor.org) as described in GEO entry GSE26728. Genes with q-value $\leq$ 0.1 were considered differentially expressed between BPA-treated and control animals. The enrichment of Gene Ontology (GO) Biological Processes was evaluated using a conditional hypergeometric test (GOstats package). For data other than microarray data, differential effects were analyzed by analysis of variance (ANOVA) followed by Student's t tests with a pooled variance estimate. $P \leq 0.05$ was considered significant.

Results

Low BPA Doses Increase Plasma Insulin. Male CD1 mice were exposed for 4 weeks to 0, 5, 50, 500, or 5,000 μg/kg/day of BPA by way of the diet. BPA exposure had no effect on body weight gain and relative liver weight (Fig. 1A). However, a significant increase in pWAT weight was observed in the animals exposed to 50 μg/kg/day (Fig. 1A). Plasma insulin levels were significantly increased following exposure to 5, 50, and 500 μg BPA/kg/day (Fig. 1B) with a maximal effect at the lowest dose. BPA had no significant effect on plasma glucose and total, LDL- or HDL-cholesterol levels. The animals exposed to 500 μg BPA/kg/day displayed a significant increase in plasma triglyceride levels (Fig. 1B). To evaluate whether these observations were specific to a mouse strain and of a mode of BPA exposure, we performed an experiment in C57BL/6J mice exposed to the same BPA doses by way of the water. Although the modulations were generally of lower amplitude than in CD1 mice, the results obtained in this independent experiment were consistent with those presented here (Supporting Fig. 1).

Effects of BPA Reference Doses on Liver Transcriptome. Using microarrays, we compared the transcriptome of liver samples from mice exposed to BPA reference doses (TDI: 50 μg/kg/day and NOAEL: 5,000 μg/kg/day) to those from control animals. The global impact of BPA-TDI or BPA-NOAEL is illustrated by the distribution of raw P-values for gene expression changes between BPA-treated groups and the control group (Fig. 2A). The overabundance of low P-values reflects the amplitude of the impact on the transcriptome. Exposure to BPA-TDI (174 unique genes differentially expressed compared with controls: 108 upregulated and 66 down-regulated; Supporting Table 2) had a stronger impact on liver transcriptome compared with BPA-NOAEL (0 genes with q-value $\leq$10%). A heatmap of the average intensities for the corresponding 196 unique oligonucleotide probes illustrates the specific impact of BPA-TDI on the expression of these genes compared with BPA-NOAEL. Among the up-regulated genes the nine GO categories

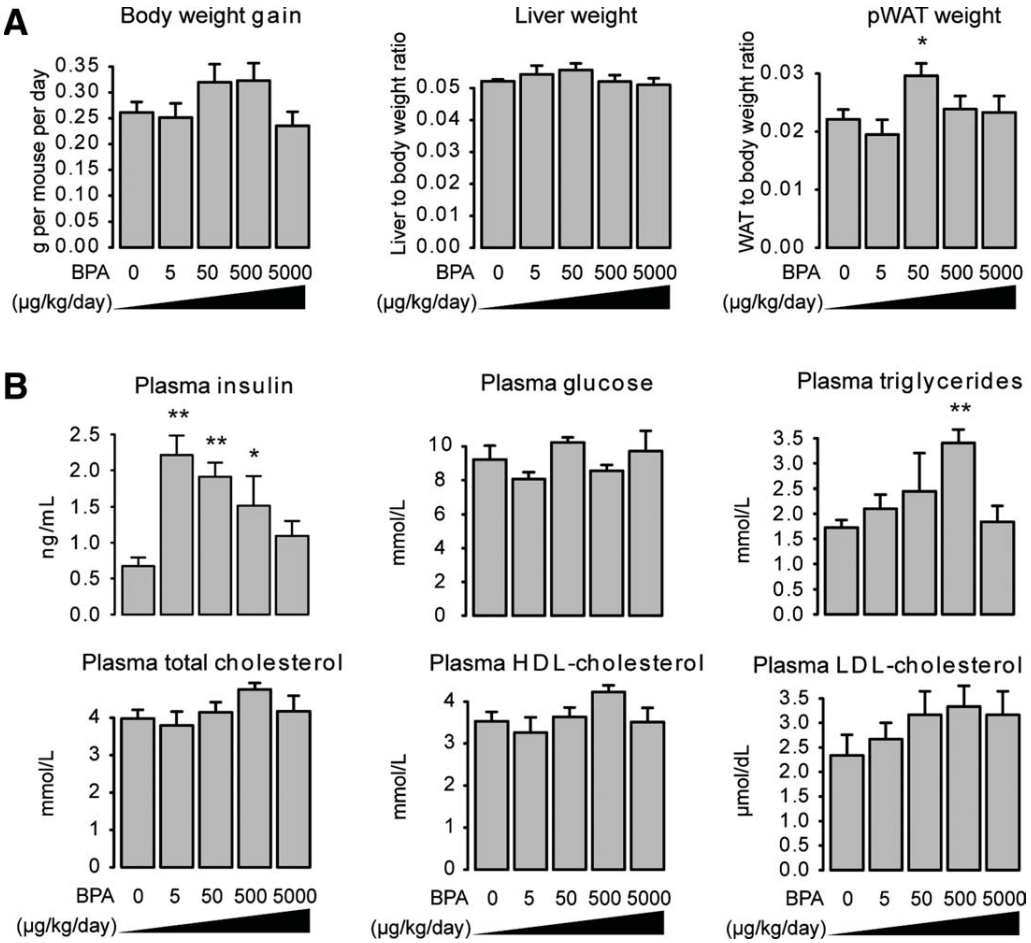


Fig. 1. Effects of different doses of BPA on body weight gain, on liver and adipose tissue weight, and on plasma parameters. (A) Body weight gain, liver weight (relative to body weight), and perigonadic white adipose tissue (pWAT) weight (relative to body weight) of male CD1 mice exposed orally for 28 days to different BPA doses (0, 5, 50, 500, and 5,000 μg/kg/day, n = 6 animals/group). (B) Quantification of plasma insulin, glucose, triglycerides, cholesterol (total, HDL, LDL) from the same animals.

significantly overrepresented (q-value $\leq$ 10%) were all related to lipid biosynthesis (Fig. 2B). Consistently, genes with increased expression at BPA-TDI included genes involved in *de novo* fatty acid (FA) synthesis (Acly: ATP citrate lyase, Acaca: Acetyl-CoA carboxylase alpha, Acacb: Acetyl-CoA carboxylase beta, Fasn) and elongation (Elovl6: long-chain FA elongase 6), in triglyceride synthesis (Gpat: glycerol-3-phosphate acyltransferase) and cholesterol synthesis (Mvd: mevalonate (diphospho) decarboxylase, Lss: lanosterol synthase). The most strongly induced gene at BPA-TDI was Pnpla3 (patatin-like phospholipase domain containing 3), a gene whose function is still poorly understood but whose genetic variability has been associated with the severity of nonalcoholic steatohepatitis (NASH).[25] Another member of this family, Pnpla5 (patatin-like phospholipase domain containing 5) was also induced at the TDI. The Thrsp-Spot14 (thyroid hormone responsive Spot14 homolog) is the second most strongly induced gene at BPA-TDI versus control. Its overexpression was previously shown to increase lipogenesis in human hepatocytes.[26] To identify enriched functional categories among the regulated genes independently of the q-value/FDR threshold, we used gene set enrichment analysis (GSEA, data not shown). Results of GSEA for the up-regulated genes also pointed to increased lipogenesis as the main and specific impact of BPA-TDI. Interestingly, GSEA identified an enrichment of peroxisome proliferator-activated receptor alpha (PPARα) target genes involved in FA oxidation among the down-regulated genes for both BPA reference doses.

Low BPA Doses Induce Hepatic Gene Expression Related to Lipid Biosynthesis. Based on microarray results, we evaluated by qPCR the effects of a wide range of BPA doses (0, 5, 50, 500, and 5,000 μg/kg/day) on the expression of genes related to hepatic lipid metabolism. Figure 3 illustrates that the effects of BPA

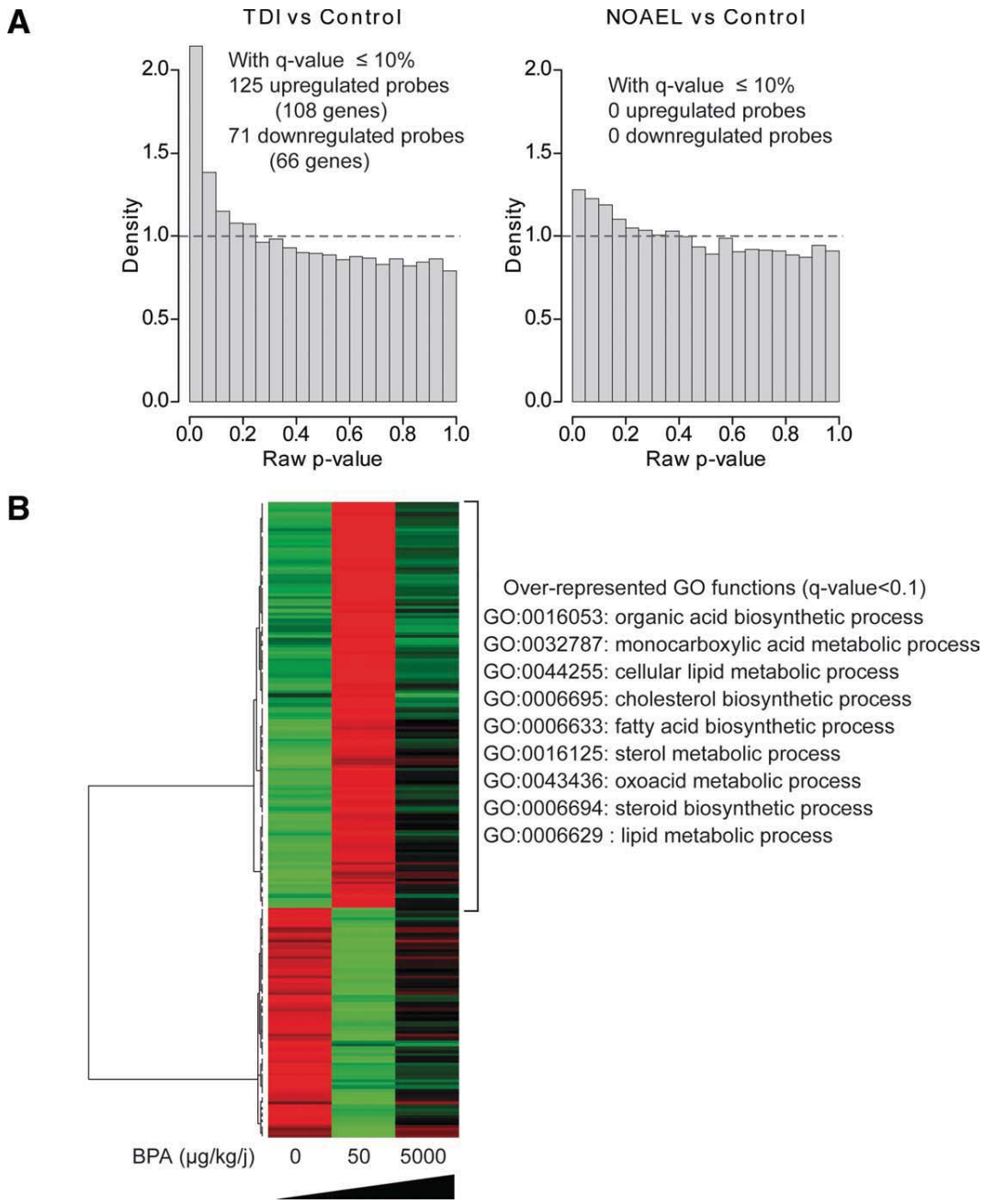
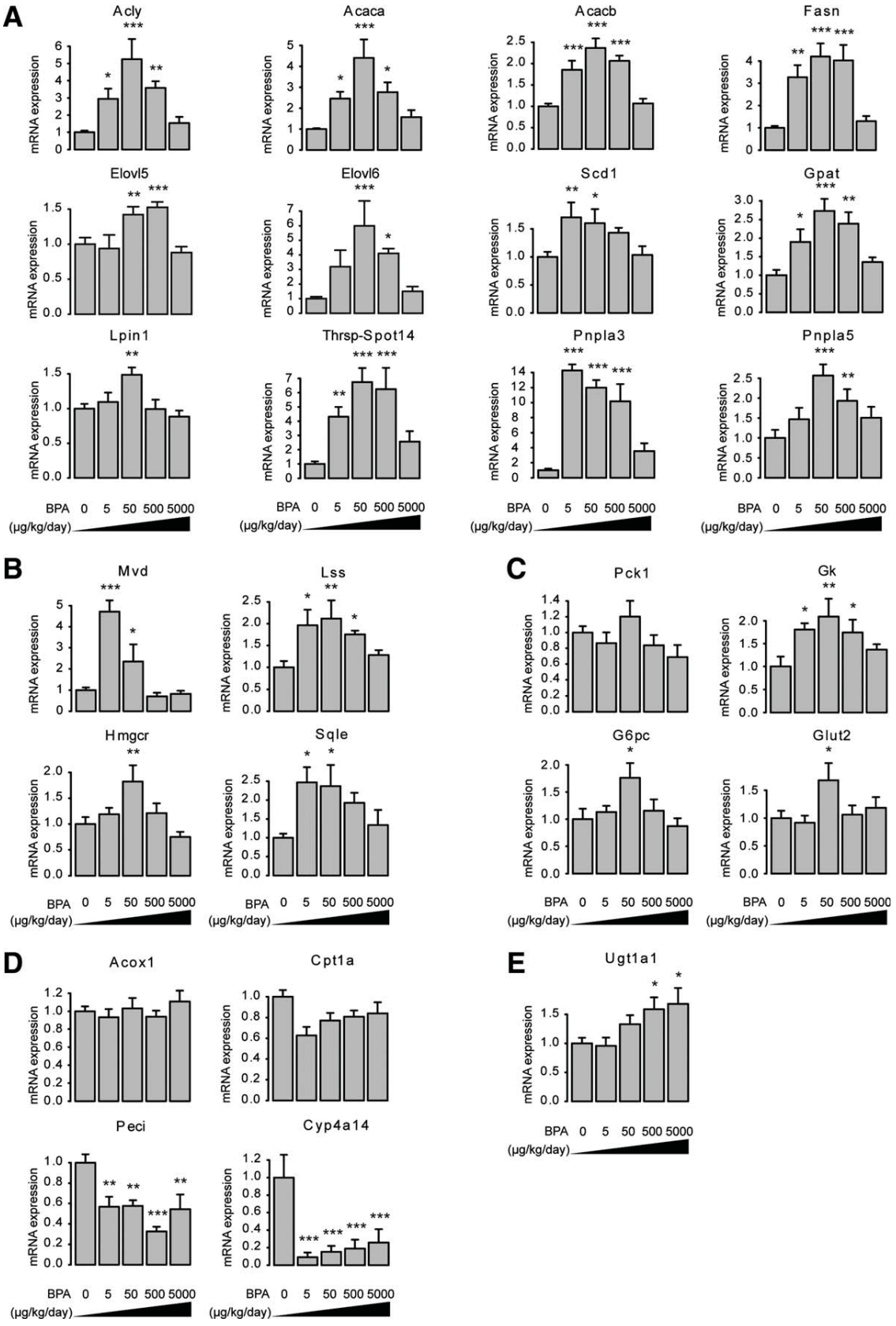


Fig. 2. Effects of BPA reference doses on liver transcriptome. mRNAs were extracted from the livers of male CD1 mice (n = 6/group) exposed or not to a low (TDI: 50 μg/kg/day) or high dose (NOAEL: 5,000 μg/kg/day) of BPA. Hepatic transcriptomes were analyzed using Agilent Whole Mouse Genome microarrays (4 × 44K). (A) Distribution of raw *P*-values corresponding to the comparison between BPA-treated and control group. The dashed lines illustrate the flat histogram that is expected in the absence of BPA effect. The overabundance of low *P*-values (peak on the left of the histogram) illustrates graphically the global impact of the treatments on the transcriptome. The number of significant (q-value $\leq$10%) up- and down-regulated probes and of the corresponding number of genes are indicated. (B) The heatmap for the 196 probes significantly regulated at BPA-TDI versus control illustrates the specific impact of the low dose (50 μg/kg/day) compared with the high dose (5,000 μg/kg/day). Red and green colors indicate values above and below the mean, respectively. Black color indicates values close to the mean. Only the mean values for each group are represented in the heatmap but the hierarchical clustering was obtained from individual values using 1-Pearson correlation coefficient as distance and the Ward's criterion for agglomeration. Analysis of GO biological functions significantly enriched (q-value $\leq$10%) among the up-regulated genes clearly pointed to processes linked to lipid metabolism as the most robustly enriched.

on key enzymes involved in lipogenesis (Fig. 3A), cholesterol biosynthesis (Fig. 3B), and to a lesser extent in glucose metabolism (Fig. 3C) follow a nonmonotonic dose-response relationship. Key microarray findings were confirmed for Acly, Acaca, Acacb, Elovl6, Fasn, Thrsp-Spot14 (Fig. 3A), Mvd, Lss (Fig. 3B), Gpat,


A
Acly
Acaca
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Fasn
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Scd1
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mRNA expression
BPA 0 5 50 500 5000
(μg/kg/day)
B
Mvd
Lss
Hmgcr
Sqle
C
Pck1
Gk
G6pc
Glut2
D
Acox1
Cpt1a
Peci
Cyp4a14
E
Ugt1a1

Fig. 3.

Pnpla3, and Pnpla5 genes (Fig. 3A). Similar patterns of expression were also observed for Elovl5 (FA elongation), Scd1 (synthesis of monounsaturated FA), Lpin1 (triglyceride synthesis, Fig. 3A), Hmgcr, and Sqle (cholesterol biosynthesis, Fig. 3B). Because hepatic glucose and lipid metabolism are tightly linked, we analyzed the expression of genes involved in glucose homeostasis. A similar effect of BPA was observed for both the phosphoenolpyruvate carboxykinase 1 (Pck1) and the glucose-6-phosphatase (G6pc), which are involved in gluconeogenesis (Fig. 3C). The mRNA expression of glucokinase (Gk) which regulates glycolysis was also increased (Fig. 3C). An induction of the main hepatic glucose transporter (Glut2) was also observed (Fig. 3C). These effects on glucose metabolism-related genes were almost exclusively significant at BPA-TDI and were of more modest amplitude compared with those affecting genes involved in lipid metabolism.

Based on GSEA results, we evaluated the effects of BPA exposure on the expression of genes involved in FA oxidation. BPA had no effect on the expression of Acox1 or Cpt1a involved in peroxisomal and mitochondrial β-oxidation, respectively (Fig. 3D). However, all BPA doses reduced the expression of Peci involved in the metabolism of unsaturated FA and of Cyp4a14, two target genes of PPARα (Fig. 3D).

We also studied the impact of BPA on the mRNA expression of genes involved in FA uptake and very low-density lipoprotein (VLDL) secretion. The results obtained did not suggest an upregulation of these pathways at low BPA doses (Supporting Fig. 2).

Finally, we searched for a more classical monotonic dose-response relationship between BPA exposure and gene expression. This led us to show that the expression of UDP glucuronyltransferase 1a1 (Ugt1a1), an enzyme involved in the phase II metabolism of xenobiotics and hormones, including estradiol is dose-dependently increased by BPA (Fig. 3E).

Western blot analysis for key lipogenic proteins (ACLY and its more active form phosphorylated on Ser454: ACLY-P, ACC, FAS, and SCD1), for GK, and for G6PASE showed protein levels consistent with the mRNA changes (Fig. 4).

Effects of BPA on Hepatic Transcription Factors. In order to gain insight into the transcriptional mechanisms which could contribute to the effects of BPA on liver gene expression, we measured the expression of different transcription factors involved in the regulation of hepatic energy metabolism. These included several nuclear receptors: PPARα; the adipogenic regulator PPARγ; PPARβ/δ; liver X receptor alpha (LXRα); ERα; constitutive androstane receptor (CAR); pregnane X receptor (PXR), and the hepatocyte nuclear factor 4α (HNF4α). BPA had no significant effect on the expression of Pxr and Hnf4α (Fig. 5A). The expression of Car was highest in control mice and was significantly reduced in mice exposed to 5 and 50 μg BPA/kg/day (Fig. 5A). On the opposite, ERα expression was lowest in control mice and was significantly increased in mice exposed to 5 and 50 μg/kg/day (Fig. 5A). We did not detect the expression of ERβ in liver samples. Pparα expression was decreased almost 3-fold in mice exposed to 5 or 500 μg BPA/kg/day only (Fig. 5A). Pparβ/δ expression was significantly increased by about 50% in mice exposed to 500 μg BPA/kg/day but a trend toward increased expression was also observed at 5 and 50 μg/kg/day (Fig. 5A). Pparγ and Lxrα expression were clearly increased by 4- and 2-fold, respectively, in the liver of mice exposed to BPA-TDI only (Fig. 5A). We also measured the expression of sterol regulatory element binding protein 1c (SREBP-1c), a major regulator of *de novo* lipogenesis,[27] of sterol regulatory element binding protein 2 (SREBP-2), which regulates cholesterol metabolism,[28] and of carbohydrate response element binding protein (ChREBP), a transcriptional regulator of glucose and lipid metabolism.[29] The expression of Srebp-1c,

Fig. 3. Effects of BPA exposure on hepatic gene expression related to lipogenesis, cholesterol biosynthesis, glucose metabolism, and fatty acid oxidation. Hepatic mRNAs from male CD1 mice exposed to different doses of BPA (0, 5, 50, 500, and 5,000 μg/kg/day) were used to assay by qPCR the relative expression of genes involved in (A) lipogenesis (Acly: ATP citrate lyase, Acaca: acetyl-CoAcarboxylase alpha, Acacb: acetyl-CoAcarboxylase beta, Fasn: fatty acid synthase, Elovl5 and Elovl6: elongation of long chain fatty acids family members 5 and 6, Scd1: stearoyl-CoA desaturase-1, Gpat: glycerol-3-phosphate acyltransferase, Lpin1: Lipin 1, Thrsp-Spot14: thyroid hormone responsive Spot14 homolog, Pnpla3 and Pnpla5: patatin-like phospholipase domain containing 3 and 5) (B) cholesterol biosynthesis (Mvd: mevalonate (diphospho) decarboxylase, Lss: lanosterolsynthase, Hmgcr: Hmg-coenzyme A reductase and Sqle: squalene epoxidase) (C) glucose metabolism (Pck1: phosphoenolpyruvate carboxykinase 1, Gk: glucokinase, G6pc: glucose-6-phosphatase, and Glut2: glucose transporter 2) (D) fatty acid oxidation (Acox1: acyl-coenzyme A oxidase 1, Cpt1a: carnitine palmitoyltransferase 1A, Peci: peroxisomal 3,2-trans-enoyl-CoA isomerase, Cyp4a14: cytochrome P450, family 4, subfamily a, polypeptide 14) (E) Ugt1a1: UDP-glucuronosyltransferase 1 polypeptide A1. All data were normalized to TBP (TATA-box binding protein) mRNA expression levels. Values shown are the mean $\pm$ standard error of the mean (SEM) ($n = 6$ per group). Data were analyzed by ANOVA followed by post-hoc Student's test with a pooled variance estimate. Asterisk denotes a significant difference compared with control condition (0 μg/kg/day of BPA): $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

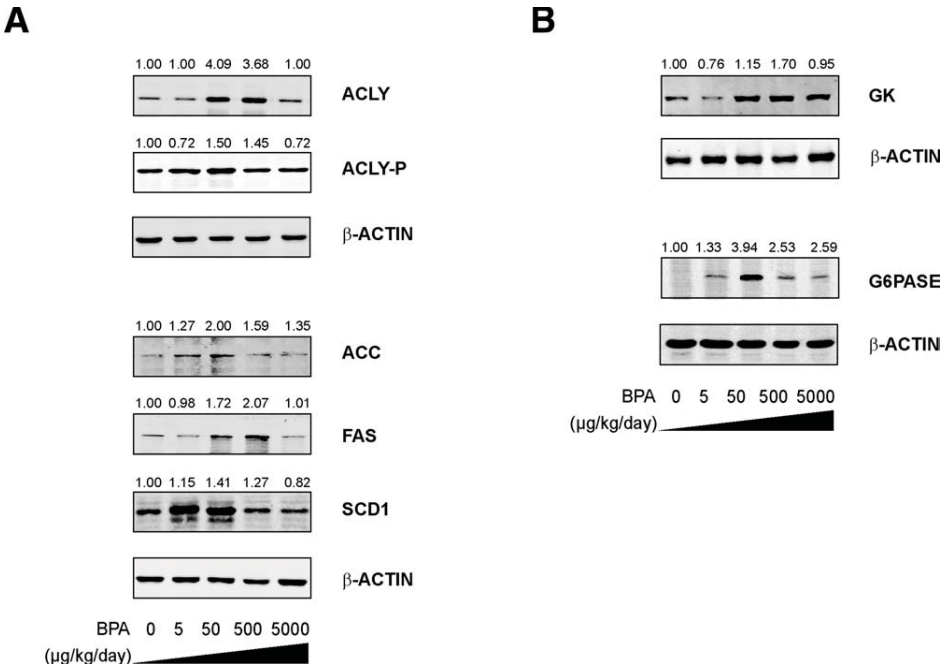

Fig. 4. Western blot analysis of the effect of BPA exposure on hepatic protein expression. Cytoplasmic protein extracts were prepared from the livers of control and BPA-exposed animals (0, 5, 50, 500, and 5,000 μg/kg/day; n = 3 to 6 animals/group). The protein extracts were subjected to SDS-PAGE and transferred on nitrocellulose membranes. Hepatic proteins were probed using antibodies specific to (A) key enzymes involved in fatty acid biosynthesis: ATP citrate lyase (ACLY), the more active form of ACLY phosphorylated on Ser454 (ACLY-P), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase-1 (SCD1) and (B) key enzymes involved in glucose metabolism: glucokinase (GK) and glucose 6-phosphatase (G6PASE). β-Actin was used as loading control. A representative image is shown for each protein. The values indicated above each image are the mean values obtained for 3 to 6 animals per group.

Srebp-2, and Chrebp exhibited an inverted U-shaped dose-response profile under the effect of BPA (Fig. 5B). This was also the case for insulin induced gene 1 (Insig1), but not for insulin induced gene 2 (Insig2), two negative regulators of SREBP-2 and SREBP-1c processing, respectively (Fig. 5B). The analysis by western blot of nuclear protein levels for ER and for the key regulators of lipogenesis SREBP-1C, CHREBP and LXR confirmed a specific effect of low BPA doses on the active protein levels of these transcription factors (Fig. 5C).

Effects of BPA on Hepatic Lipids and FA Composition. To evaluate the consequences of increased expression of lipogenic genes, we stained hepatic neutral lipids with Oil-Red-O. The representative pictures in Fig. 6A illustrate a greater accumulation of lipids in the liver of mice exposed to BPA compared with control livers. Lipid droplets were larger and more numerous in the livers of mice exposed to BPA-TDI compared with those exposed to BPA-NOAEL. The quantification of liver lipid content confirmed these observations. BPA had no effect on hepatic total free cholesterol content (not shown). Liver triglycerides were significantly increased by approximately 60% and 65% in mice exposed to 50 and 500 μg BPA/kg/day, respectively, compared with control mice (Fig. 6B). Additionally, mice exposed to BPA-TDI also showed a significant increase in hepatic cholesteryl esters (Fig. 6B). The analysis of hepatic FA composition (Fig. 6C; Supporting Table 3) showed that exposure to 50 or 500 μg BPA/kg/day resulted in accumulation of palmitic (C16:0) and oleic acids (C18:1n-9), the major constituents of triglycerides and cholesteryl esters. Conversely, the proportions of polyunsaturated FA and of C18:0, which are found at higher levels in phospholipids, were reduced at these doses. Despite increased Elovl6 mRNA expression, the C18:0/C16:0 ratio was decreased at these doses. This may result from a combined increased synthesis of C16:0 by FAS and the efficient desaturation/elongation of C18:0 (as illustrated by the increased C18:1n-9/C18:0 ratio, Fig. 6D), both producing substrates for triglyceride synthesis.

Discussion

Our results show that the oral exposure of adult male mice to low BPA doses increases plasma insulin and hepatic mRNA and protein expression related to lipid biosynthesis. This correlates with increased liver lipids after 4 weeks of exposure. Most significant

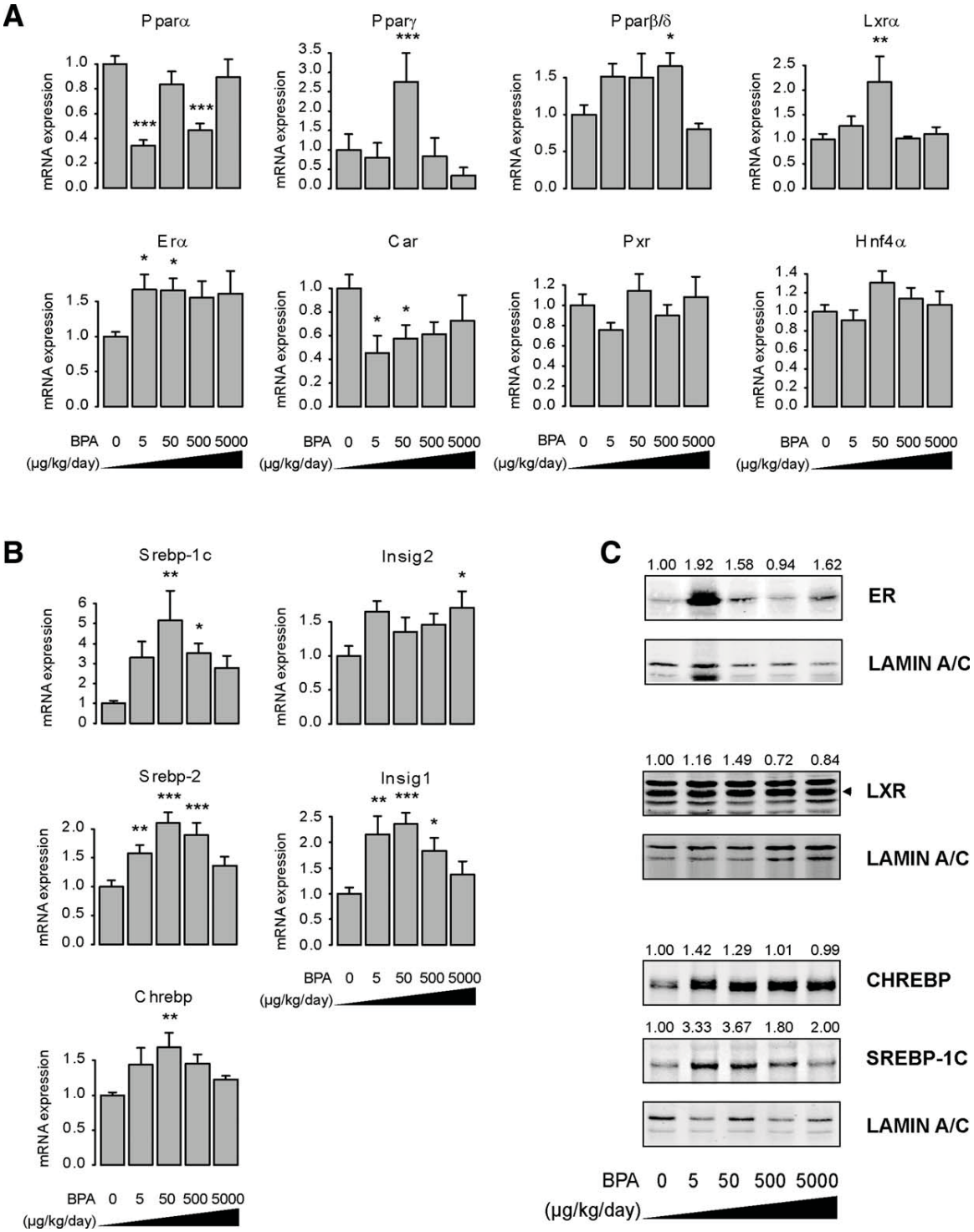

Fig. 5. Effects of low BPA doses on the expression of master transcriptional regulators of hepatic lipid and glucose homeostasis. Hepatic mRNAs from male CD1 mice exposed to different doses of BPA (0, 5, 50, 500, and 5,000 μg/kg/day) were used to assay by qPCR the relative mRNA expression of (A) nuclear receptors: the peroxisome proliferator-activated receptors alpha (Pparα), gamma (Pparγ), beta/delta (Pparβ/δ), the liver X receptor alpha (Lxrα), the estrogen receptor alpha (ERα), the constitutive androstane receptor (Car), the pregnane X receptor (Pxr), and the hepatocyte nuclear factor 4α (Hnf4α) (B) and of other transcriptional regulators of hepatic lipid and glucose metabolism: the sterol regulatory element binding transcription factor 1c (Srebp-1c) and its associated factor encoded by the Insulin induced gene (Insig2), the sterol regulatory element binding transcription factor 2 (Srebp-2) and its associated factor encoded by the insulin induced gene 1 (Insig1), and the carbohydrate response element binding protein (Chrebp). All data were normalized to TBP (TATA-box binding protein) mRNA expression levels. Values shown are the mean $\pm$ SEM (n $=$ 6 per group). Data were analyzed by ANOVA followed by post-hoc Student's test with a pooled variance estimate. Asterisk denotes a significant difference compared with control condition (0 μg/kg/day of BPA): $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (C) Immunoblots for ER, LXR, CHREBP, and SREBP-1c were performed as described in the legend of Fig. 4 except that nuclear proteins were extracted and analyzed and that LAMIN A/C was used as a loading control. The values indicated are the mean of the values obtained for 3 animals per group.

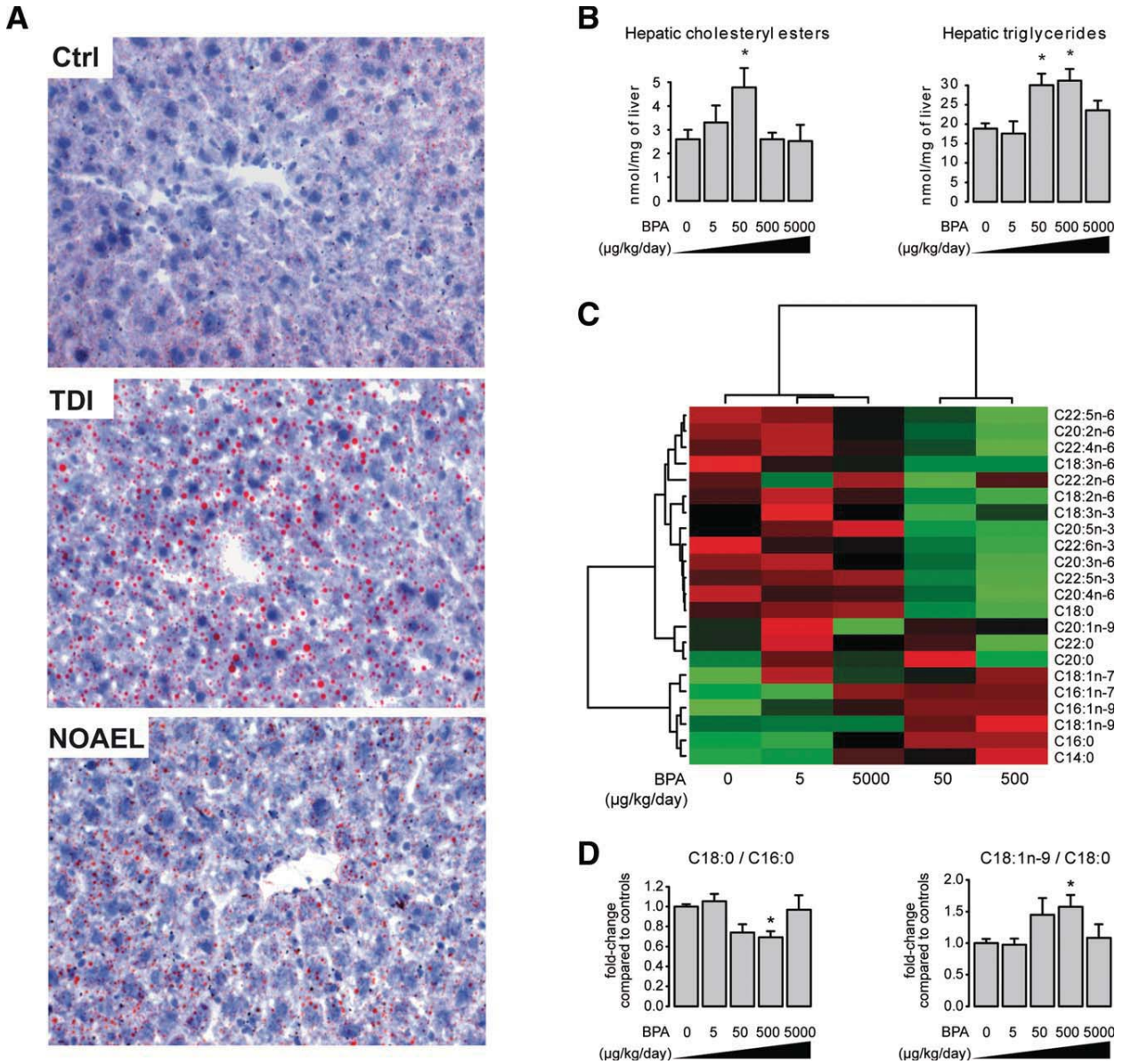

Fig. 6. Accumulation of triglycerides and cholesteryl esters and changes in the fatty acid profile in the livers of mice exposed to BPA low doses. (A) Oil-Red-O-staining of neutral lipids realized on histological sections of livers from mice exposed or not to a BPA low dose (TDI: 50 μg/kg/day) or high dose (NOAEL: 5,000 μg/kg/day). Neutral lipids appear in red (original magnification $\times$200). (B) Neutral lipids were extracted from the liver of mice exposed to different doses of BPA (0, 5, 50, 500, and 5,000 μg/kg/day) by way of their diet. After extraction, lipids were analyzed by gas chromatography. The presence of internal standards enabled to quantify neutral lipids. Values shown are the mean $\pm$ SEM (n = 6 per group). Data were analyzed by ANOVA followed by post-hoc Student's test with a pooled variance estimate. *$P < 0.05$: significant difference compared with control condition (0 μg/kg/day of BPA). (C) The hepatic fatty acid composition of mice exposed to the five BPA doses (n = 5 or 6 per group) were determined by gas chromatography of fatty acid methyl esters. The heatmap and the dendrograms were obtained as described in the legend of Fig. 2. The clustering of the groups clearly identifies a specific impact of exposure to 50 and 500 μg BPA/kg/day on the fatty acid profile. It is characterized by increased proportions of saturated and monounsaturated fatty acids and reduced proportions of polyunsaturated fatty acids and C18:0. (D) A significant decrease in the C18:0/C16:0 ratio and a concomitant increase in the C18:1n-9/C18:0 ratio are observed in the livers of animals exposed to 500 μg BPA/kg/day.

effects were observed for BPA doses within one order of magnitude around the current TDI of 50 μg/kg/day. Conversely, virtually no effects were observed at the NOAEL (5,000 μg/kg/day). Agencies for risk assessment have established a "safe" TDI for BPA at 50 μg/kg/day, but several studies have revealed that exposure to environmentally relevant BPA doses below the TDI alters various biological functions, including reproductive, behavioral, metabolic, and immune systems.[4] However, the molecular mechanisms underlying these

low-level responses are still unknown. It was proposed that down-regulation of receptors at higher hormone or xenoestrogen levels may contribute to shape these nonmonotonic curves. Some of BPA's actions, including insulin production by the pancreas, were attributed to its ability to bind to nonclassical membrane estrogen receptor as well as the G-protein coupled-receptor 30 (GPR30) and to act through nongenomic pathways.[20,30] Interestingly, we observed that, contrary to lipid metabolism genes, Ugt1a1 expression displayed a dose-dependent increase in response to BPA (Fig. 3E). Human UGT1a1 mRNA expression has been previously reported to be increased by low BPA doses in HepG2 cells.[31] This phase II enzyme is involved in the metabolism of endogenous estrogens[32] and has also been shown to catalyze BPA glucuronidation at high substrate concentration.[33] Whether the modest increase in Ugt1a1 expression can interfere with the action of BPA and/or endogenous estrogens may be doubtful, but it suggests that different pathways with different sensitivities to BPA are targeted depending on the dose of exposure.

The effects of BPA on insulin expression and secretion have been described.[17] Our results strongly suggest that the effects of BPA on insulin production by the pancreas translate to transcriptional and functional consequences in the liver. Indeed, insulin is known to increase glycolysis and lipogenesis by way of both post-translational protein modifications and transcriptional mechanisms.[34] SREBP-1c plays a major role in the regulation of these genes in response to insulin.[35] LXR is thought to contribute to the effect of insulin on Srebp-1c gene expression.[36] LXR also directly regulates the expression of lipogenic genes.[37] Additionally, insulin also stimulates the proteolytic processing of SREBP-1c,[38] leading to increased mature nuclear form and subsequent induction of lipogenic gene expression. In addition to insulin, glucose stimulates glycolytic and lipogenic gene expression by activating the ChREBP,[29] which is itself under the transcriptional control of LXR.[39] Insulin also induces the expression of Spot14, which is required for induction of hepatic lipogenesis by thyroid hormone and insulin[40,41] and of Pnpla3 by way of SREBP1-c.[42] SREBP-2 expression and activity are primarily regulated by low sterol levels but were also reported to respond to increased insulin levels.[43,44] SREBP-2 is a major transcriptional regulator of genes involved in cholesterol biosynthesis, including Hmgcr and Sqle.[45] Thus, insulin is likely to contribute to a large number of the regulations observed following BPA exposure. However, although the expression of some genes (e.g., Pnpla3) parallels plasma insulin levels, many other gene expression patterns do not strictly follow the insulin profile. Moreover, the up-regulation of genes involved in neoglucogenesis (G6pc and Pck1, Fig. 3C) is unexpected in the context of high insulin levels. We cannot rule out that other mechanisms, independent of insulin and possibly involving direct effects of BPA on the liver, may contribute to the transcriptional impacts of low BPA doses reported here.

We have shown an accumulation of liver triglycerides and cholesteryl esters together with associated changes in hepatic FA composition in the animals exposed to low BPA doses. Among the mechanisms potentially involved in these effects (increased FA uptake, impaired secretion, increased lipogenesis, or reduced oxidation), our results point to an activation of lipogenesis and cholesterol biosynthesis as the major mechanism involved, potentially associated with an inhibition of FA oxidation. Simple hepatic lipid accumulation is generally considered a benign and reversible process that does not invariably progress to a more serious condition. However, inappropriate regulation of hepatic *de novo* lipogenesis is now believed to facilitate the generation of lipotoxic lipid intermediates that could contribute to the pathogenesis of NASH.[46] NAFLD is strongly linked to overnutrition, underactivity, and insulin resistance,[47] but many other factors initiating hepatic steatosis or supporting the progression of NAFLD to NASH have been proposed.[48] These include biologic or synthetic hepatotoxins, bacterial endotoxins, and exposure to industrial petrochemicals. Because hepatic steatosis may lead to more severe pathologies such as NASH and fibrosis, the effects of environmental pollutants on liver functions should be carefully examined.

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Regulation of LPCAT3 by LXR

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ABSTRACT

In this work we analyzed the transcriptome profiles of chicken hepatoma cells (LMH) in response to T0901317, a pharmacological agonist of the liver X receptor (LXR). Through an *in silico* search for LXRE (LXR response element) consensus sequences in the promoter of genes whose expression was shown to be sensitive to TO901317, we identified a LXRE in the promoter of the LPCAT3 (lysophosphatidylcholine acyltransferase 3). This motif is highly conserved between species. We further investigated the regulation of this gene and showed that the expression of LPCAT3 was induced both in chicken and human hepatoma cells (LMH and HuH-7, respectively) in response to T0901317. Transactivation and electrophoretic mobility shift assays allowed us to locate a functional LXRE in the chicken LPCAT3 promoter. Altogether these data evidence for the first time that the chicken LPCAT3 gene is a direct target of LXR and therefore suggest a new role for LXR in phospholipid homeostasis.

1. Introduction

Liver X receptor alpha (LXRα), also referred to as nuclear receptor subfamily 1, group H, member 3 (NR1H3), and LXRβ (NR1H2) are members of the nuclear hormone receptor superfamily that are functional when heterodimerized with retinoid x receptors (RXRs) and can be activated by both RXRα and LXR ligands (Lu et al., 2001). RXRα/LXR heterodimers regulate the transcription of their target genes by binding to specific response elements (LXREs) that contain a hexameric nucleotide direct repeat spaced by four bases (DR4). They are known to be activated by both RXRα ligands (i.e. retinoids) and oxysterols (oxidized derivatives of cholesterol), including 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol and 24,25(S)-epoxycholesterol (Janowski et al., 1996; Lehmann et al., 1997).

LXRα has first been found to be implicated in the control of cholesterol homeostasis in mammals (Gill et al., 2008). The first LXRE was identified in the promoter of cholesterol 7α-hydroxylase (Lehmann et al., 1997). While early studies on LXRs focused on their role in cholesterol metabolism, LXRα was also found to be involved in the control of lipogenesis. In mice, targeted disruption of LXRα results in a reduced expression of a number of lipogenic genes including fatty acid synthase (FASN), acetyl CoA carboxylase (ACACA), steroyl CoA desaturase (SCD), sterol regulatory element binding protein 1 (SREBP-1, Peet et al., 1998) and carbohydrate-responsive element-binding protein (ChREBP; Cha and Repa, 2007). Both SREBP-1 and ChREBP are lipogenic transcription factors. Therefore, LXRα acts as a master lipogenic factor that regulates the expression of other transcription factors critical for the sensitivity of fatty acid synthesis to insulin and glucose (Denechaud et al., 2008). LXRα also directly governs the expression of genes encoding lipogenic enzymes in rodents as FASN (Joseph et al., 2002; Demeure et al., 2009) and SCD1 (Chu et al., 2006) contain functional LXRE.

The present work aimed at identifying new LXR target genes. To this end, we performed both a transcriptome and a bioinformatics analysis. This led us to identify LPCAT3 as a likely LXR target, which was subsequently confirmed through the localisation of a functional LXRE.

2. Materials and methods

2.1. Cell culture

The chicken primary hepatocellular carcinoma epithelial cell line (LMH) was obtained from ATCC (cat #CRL-2117). LMH cells were grown in Waymouth's medium (Invitrogen) supplemented with 10% foetal calf serum (FCS), 100 IU/ml penicillin, and 100 mg/ml streptomycin. The human hepatocellular carcinoma cells (HuH-7; provided by the unit 6061-INSERM-France) were grown in William's medium (Invitrogen), 10% FCS, 1.6 ml BSA 30%, 500 nM hydrocortisone 21-hemisuccinate sodium salt (Sigma-Aldrich), 1 μg/mL Insulin (Sigma-

Abbreviations: LMH, chicken hepatoma cell line; HuH-7, human hepatoma cell line; DNA, deoxyribonucleic acid; cDNA, complementary DNA; RNA, ribonucleic acid; PCR, polymerase chain reaction; qPCR, quantitative PCR; bp, base pairs.

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Aldrich). All cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

For gene expression analysis, LMH and HuH-7 cells were cultured in triplicate in 25 cm² culture flask for 48 h and then treated with 20 μM T0901317 (Sigma-Aldrich), 100 μM Wy14,643 (Interchim) or vehicle for 24 h. Total RNA was extracted with TRIzol® reagent (Invitrogen) according to the manufacturer's instructions. Quality and concentration of extracted RNA were assessed using a 2100 Bioanalyzer (Agilent Technologies).

2.2. Microarray analyses

The 20 K chicken array was produced by ARK-Genomics (Roslin Institute – UK: http://www.ark-genomics.org), and the array design was published in the ArrayExpress (Parkinson et al., 2007) repository with Accession No A-MEXP-820 ArrayExpress 2003 and in the Gene Expression Omnibus with the name GPL5480 (Barrett et al., 2007). The functional annotations were obtained by a bioinformatics procedure developed by SIGENAE (INRA-France; Casel et al., 2009).

All the procedures for microarray analyses (labelling, hybridization, detection of the fluorescence signals, normalization) were as previously described by Desert et al. (2008). We finally analyzed 7 microarrays: 4 and 3 for control and T0901317 conditions respectively. The significance of gene expression differences between control and treated cells were analyzed by analysis of variance with "aov" function. The *p*-values were corrected according to the false discovery rate (FDR) procedure of Benjamini–Hochberg (Benjamini and Hochberg, 1995). Gene expression difference was declared significant if its adjusted *p*-value was $p<0.01$.

2.3. Bioinformatics

Custom PERL scripts were developed to automate the LXRE detection procedure. *Gallus gallus* (GGA) genomic DNA sequences (including 5000 bp upstream and downstream sequence from the start of the first exon) of the identified genes were extracted from the Ensembl website using the GGA Ensembl ID. The orthologous genomic sequences of *Bos taurus* (BTA), *Canis familiaris* (CAF), *Homo sapiens* (HSA), *Mus musculus* (MMU) and *Rattus norvegicus* (RNO) were automatically extracted (1-to-1 and 1-to-many ortholog type) using the Ensembl Compara API. The DNA sequences were analyzed by the STAN software (Nicolas et al., 2005) using LXRE patterns designed from previous results found in the literature (Costet et al., 2000; Repa et al., 2000; Chiang et al., 2001; Laffitte et al., 2001; Zhang et al., 2001; Joseph et al., 2002). For STAN LXRE detection, the LXRE pattern syntax was: ("A"|"T"|"G") , "G" , ("A"|"T"|"G") , "T" , ("T"|"C") , "A" , 4...4 , ("A"|"C") , "G" , ("T"|"G") , ("T"|"G"|"C") , "C" ,("A"|"G"), see Nicolas et al. (2005) for details on the syntax used in STAN. Considering the huge volume of results obtained with such LXRE analysis, the strategy was first to select genes with a LXRE detection hit in each species. The second selection criteria was genes with an LXRE detection hit in the same upstream region between species (one third on RE conservation; Odom et al., 2007) and in the same orientation (i.e. 5' to 3' for every gene). Finally, all the detected LXRE sequences in each species were aligned to verify the pattern conservation with previously published LXRE.

2.4. Plasmid constructions

LPCAT3 promoter was cloned using the *Gallus gallus* genomic sequence to design primers, LPCAT3pF: 5′-GTGGGTTCTCCAGCAAAAGA-3′ and LPCAT3pR: 5′-CGCTCAGTAAGGCAAAGGTT-3′ for the PCR amplification from genomic DNA. The fragment cloned spanned from −1189 to −191 of the chicken LPCAT3 promoter (relative to the transcription start site from exon 1). First, this fragment was subcloned into PCR3.1 plasmid (Invitrogen) by TA cloning procedure. The promoter insert of PCR3.1 plasmid was then cloned into 98/376 sites of pGL2 basic plasmid (Promega), upstream the luciferase reporter gene, to create pGL2-LPCAT3-Luc. Site-directed mutagenesis of pGL2-LPCAT3-Luc plasmids were performed with the QuikChange II Site-Directed Mutagenesis Kit (Stratagene) using the following mutagenic primers (only forward primers are shown), 5′-ACGCGCCTCTT**TTTCT**CGAGTAACC**T**CTACCCGACC-3′ and 5′-GCCTCTT**TTTCT**CGAGT**TGCGT**CCTACCCGACCACAT-3′ (the LXRE sequences are underlined and mutated bases in bold) to create the pGL2-LPCAT3-mu1-Luc and pGL2-LPCAT3-mu2-Luc constructs, respectively. The pCMV-gallusLXRα and pCMV-rattusLXRα expression plasmids were generously given by Urs Meyer (Handschin et al., 2001) and Peter Tontonoz (Joseph et al., 2002), respectively.

2.5. Transactivation assay

For transactivation assay, transient transfections of LMH and HuH-7 cells were performed in triplicate in 6 well plates using the FuGENE lipofection protocol (Roche). Each well was transfected with 200 ng of reporter plasmid, 20 ng of pCMV-bgal reference plasmid containing a bacterial β-galactosidase gene and, depending on the conditions, 1800 ng of pCMV-rattusLXRα expression vector (HuH-7) or 200 ng of pCMV-gallusLXRα expression vector (LMH). A PGEMT plasmid was added to reach a total DNA content of 2 μg in each well. After 24 h following transfection, the cells were washed once with PBS, and incubated with fresh medium. After 48 h following transfection, the cells were lysed (Lysis buffer—Promega). Cell lysates were stored at −80 °C before analyses. After centrifugation 5 min at 12,000 *g*, supernatants (10 μl) were incubated 5 s in the presence of 40 μl of luciferase assay buffer (Promega) including luciferin (470 μM). Luciferase activity was determined using a luminometer (Veritas Turner Biosystems). The β-galactosidase activity was measured by hydrolysation of Ortho Nitro Phenyl Galactopyranoside (Sigma-Aldrich) and used to normalize the luciferase activity.

2.6. Real time quantitative RT-PCR assay

LPCAT3 mRNA levels were measured by SYBR Green real time quantitative PCR with the following primers: 18S: 5'-TTAAGTCCCTGCCCTTTGTACAC-3'; 5'-CGATCCGAGGACCTCACTAAAC-3'; LPCAT3: 5'-GGATGTTTATGGGTTATTCTCTGG-3'; 5'-TCTGTTGGCTGTTATTCTGCTT-3'. Reverse transcription (RT) was carried out using the high-capacity cDNA archive kit (Applied Biosystems) according to the manufacturer's protocol with 10 μg of total RNA. A 1/20 (LPCAT3) or 1/80 (18S) dilution of each RT reaction was further used for real time quantitative PCR (qPCR) in an iCycler iQ Multicolour Real-Time PCR Detector (Bio-Rad) programmed to conduct one cycle (95 °C for 15 min) and 40 cycles (95 °C for 15 s and 59 °C for 45 s). cDNA samples were mixed with 12.5 μl AB solute SYBR Green Mix (ABgene) and 300 nM, of specific reverse and forward primers. The presence of a unique product was checked by the melting curve program. PCR runs were performed in triplicates.

Two-fold serial dilutions from a pool of the cDNA samples were systematically added on each microplate for the calibration curve and determination of the amplification rate of the Taq polymerase. As the amplification rates for all genes were equal to 1 and similar to the 18S one, the gene expression level could be normalized relative to the 18S expression level as follows: $\Delta Ct = CTgene - CT18S$.

2.7. Electrophoretic mobility shift assays

Double-stranded oligonucleotides were labeled by filling in overhanging 5'-ends using the Klenow enzyme in the presence of [α-32P] dCTP. The sequences of the double-stranded DNA used as probes or competitors were: LPCAT3-LXRE: GCGCCTCT**TGCCCG**CGAG**TAACCC**CTAC; Mu1-LPCAT3-LXRE: GCGCCTCT**TTTTCT**CGAG**TAACCT**CTAC; Mu2-

LPCAT3-LXRE: GCGCCTCT**TTTTCT**CGAG**TTGCGT**CTAC and FASN-LXRE: CGTGCCGC**TGACCT**GTGG**TAACCT**CGGCGCCGCG (with direct repeat in bold and mutated nucleotides underlined). 20 μg of total proteins of LMH cell lysate were mixed with 5 ng of 32P-labelled double-stranded oligonucleotides (200000 cpm) in a volume of 20 μl of binding buffer (0.7 mM NA2HPO4 pH 7.2, 4 mM MgCl2, 0.07 mM EDTA, 4 mM spermidine, 1 μg herring sperm DNA, 1 μg of poly(dI·dC) and 5% glycerol (v/v)). The reactions were incubated for 30 min on ice. For competition experiments, 5- or 50-fold molar excess of unlabeled competitor DNA relative to labeled DNA was added to the reaction mixture 10 min before the addition of the labeled probe. For supershift assay, 15 μg of anti-flag monoclonal antibody (Sigma-Aldrich) was added to the proteins 15 min before incubation. DNA and DNA-protein complexes were resolved on 5% nondenaturing polyacrylamide gels in 0.25× Tris-borate-EDTA (TBE) buffer. Following electrophoresis, the gels were dried and subject to storage phosphor autoradiography.

2.8. Statistical analysis

Statistical significance of differences between two groups was determined by Student's *t* test. $p \leq 0.05$ are considered to be significant.

3. Results

In order to look for new LXR targets we investigated gene expression when treating LMH cells with T0901317, a synthetic LXRα ligand/agonist. The RNA from control and treated cells were hybridized on a chicken 20 k oligo microarray. The genes whose expression was significantly affected by the T0901317 treatment (*p*-value<1%) were then selected for LXRE detection, using STAN software (Nicolas et al., 2005). Amongst the genes tested, LPCAT3 (for which expression induction by the TO9013174 treatment was 1.5× through microarray measurement) was identified as carrying a putative LXRE highly conserved in most species (Fig. 1). In addition, for all species, the putative LXRE location is approximately the same (about 500 bases upstream the start of coding sequence mentioned by Ensembl). The oligonucleotide (5'-TTCTTTCATTCACTGCTATGTGTCC-TAATTCAGTTTCTCATCCTGAGGCTTATGGGTCGTACAGTCACGG-3') LPCAT3 annotation was checked through several blast alignments against nucleotides databases. This 70 bases oligonucleotide has a 100% homology with the chicken LPCAT3 gene sequence (GenBank ID: XM_416516) and 81% with the mice LPCAT3 homologous (GenBank ID: AB294194). In addition, the protein is highly conserved (73% identity) between chicken, mice and human.

The microarray results were next confirmed by real time PCR. T0901317 treatment significantly increases LPCAT3 expression by two fold in LMH and 4.5 fold in HuH-7 (Fig. 2). A treatment with Wy14,643 (a PPARα agonist) also induces the LPCAT3 expression by a factor 1.7 and 2.7 in LMH and HuH-7 cells, respectively. In addition, HuH-7 cells were co-transfected by a vector expressing LXRα and a construction of LPCAT3 promoter (with a LXRE mutated or not) upstream of the luciferase gene. As a result of the LXRα overexpression, the luciferase activity was induced. While when this putative LXRE is mutated, the luciferase activity is abolished (Fig. 3), clearly indicating that this response element is crucial for LPCAT3 expression. Therefore, to test whether LXRα binds this response element, a gel mobility shift analysis was performed (Fig. 4). The promoter of chicken fatty acid synthase, a direct target of LXRα in chicken (Demeure et al., 2009), was used as control. A single shift band was observed with LPCAT3 LXRE probe when tested with chicken LXRα. A 50-fold molar excess of unlabeled LPCAT3 LXRE competed for the binding of the complex whereas the same molar excess of the two competitor cold probes (mu1-LPCAT3-LXRE and mu2-LPCAT3-LXRE) induced a weak competition or failed to compete, demonstrating the specificity of the protein binding. The complex was supershifted upon the addition of

Species	position	LXRE
BTA	-419/-403	**AGGTTA**ctct**CGGTCG**
CAF	-487/-471	**AGGTTA**ctct**CGGTCG**
GGA	-394/-378	**GGGTTA**ctcg**CGGGCA**
HSA	-371/-355	**AGGTTA**ctcc**CGGTCG**
MUS	-426/-410	**AGGTTA**ctct**CGGTCG**
RNO	-436/-420	**AGGTTA**ctct**CGGTCG**
Consensus		**aGGTTA**nnnn**CGGtCg**

Fig. 1. LPCAT3 LXRE sequences identified by bioinformatics in different species. The sequence position is relative to the ATG. The LXRE is a direct repeat 4 (DR4) pattern composed of a direct repeat of six nucleotides (bold upper case) separated by four anonymous nucleotides (lower case). Nucleotides differing from the consensus sequence are underlined. BTA: bovine; CAF: dog; GGA: chicken; HSA: human; MUS: mice; RNO: rat.

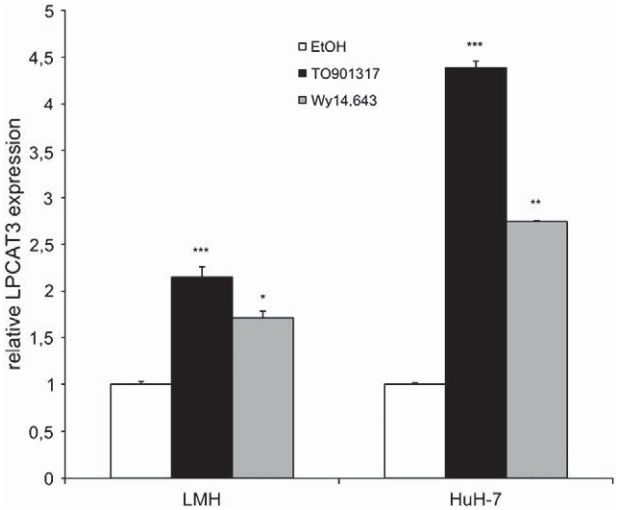

Fig. 2. Effect of TO901317 and Wy14,643 treatments on LMH and HuH-7 cells on LPCAT3 expression. LMH cells were treated with 20 μM of TO90317, 100 μM of Wy14,643 or ethanol for 24 H. mRNA levels were quantified by real time quantitative RT-PCR. ***$p<0.001$ versus ethanol control condition. **$p<0.01$ versus ethanol control condition. *$p<0.05$ versus ethanol control condition.

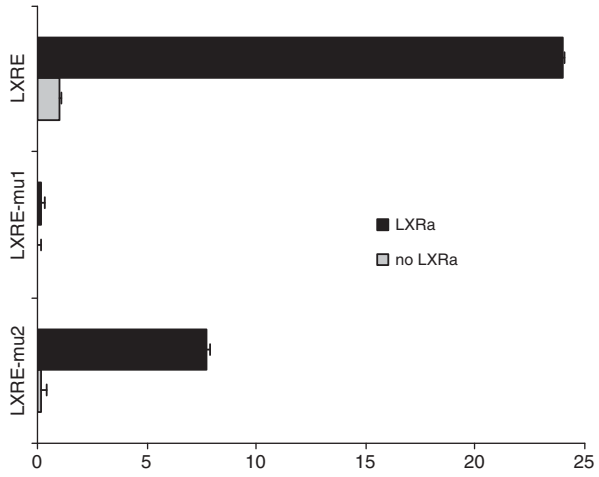

Fig. 3. Fold induction of pGL2-LPCAT3 luciferase activity by LXRα in HuH-7. Cells were transiently co-transfected with the wild type pGL2-LPCAT3-Luc reporter (LXRE), the pGL2-LPCAT3-mu1-Luc reporter or the pGL2-LPCAT3-mu2-Luc reporter construction containing specific mutations in the LXRE (LXRE-mu1: 5-ACGCGCCTCTT**TTTCT**CGAG-TAACC**T**CTACCCGACC-3; LXRE-mu2: 5-GCCTCTT**TTTCT**CGAGT**TGCGT**CCTACCCGACCA-CAT-3 with LXRE sequences underlined and mutated bases in bold) along with the expression vector p3X-Flag empty (no LXRα) or containing the LXRα cDNA (chLXRα). Luciferase activities normalized to β-galactosidase activity are relative to the LXRE + noLXRα condition.

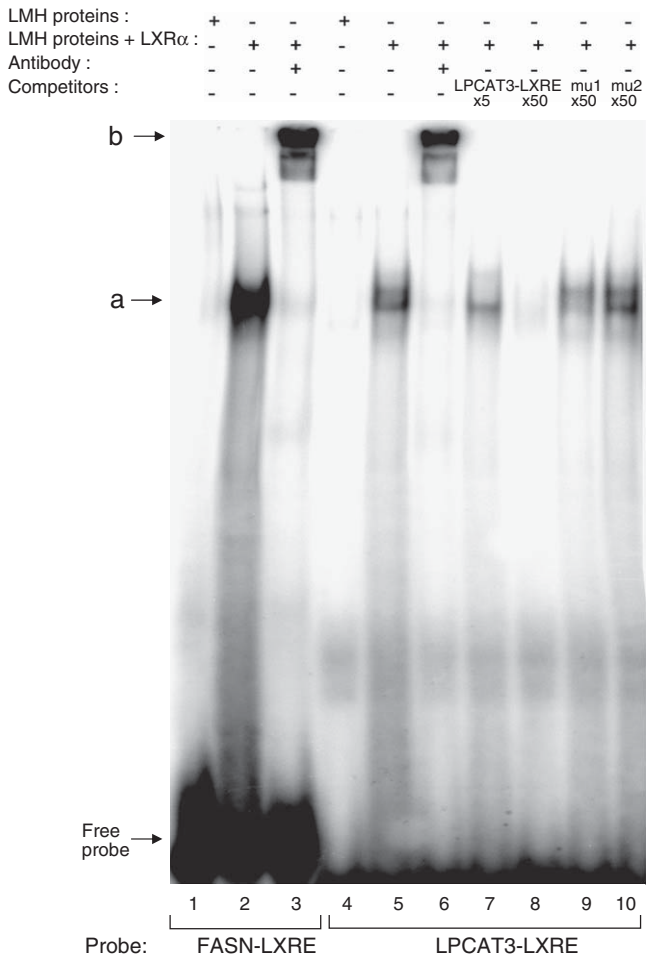

Fig. 4. Electrophoretic mobility shift assay (EMSA) demonstrating the binding of chicken LXRα to LPCAT3 LXRE. ^{32}P-Labeled DNA probes containing LPCAT3 LXRE of the chicken promoter (lanes 4–10), or the LXRE of the chicken FASN promoter (lanes 1–3) were incubated with 20 μg of LMH cells protein lysates. LMH cells used were either control (lanes 1 and 4) or LMH cells transiently transfected with chicken p3x Flag-LXRα (lanes 2–3 and 5–10). Competition for binding to receptor proteins were performed with non-radiolabeled LXRE added in excess (5–50 times unless indicated) and with mutated LPCAT3 LXRE (mu1LXRE (lane 9) and mu2LXRE (lane 10)). Transfected LMH cells proteins were incubated with antibody against p3x Flag (lanes 3 and 6). Arrows depict DNA/LXRα complex (a) and supershift (b).

an antibody directed against the 3xFlag epitope. This confirms the binding of chicken LXRα to LPCAT3 LXRE probe.

4. Discussion

Aiming at detecting new LXR target genes we used a microarray experiment in order to search for genes potentially sensitive to LXR agonist in chicken cell culture. This approach allowed the identification of a large group of LXR sensitive genes. To further look for genes specifically regulated by LXR we next proceeded to an *in silico* study. A LXRE pattern was designed from previous results found in the literature (Costet et al., 2000; Repa et al., 2000; Chiang et al., 2001; Laffitte et al., 2001; Zhang et al., 2001; Joseph et al., 2002) and compared to other species. Two criterions were used to test the different candidate genes: first the LXRE pattern had to be identified in each of the six species we analyzed and second the putative LXRE had to be located in the same upstream region for each orthologs. These criterions are very stringent as (i) a LXRE differing for only one nucleotide from the pattern will not be selected and (ii) it has been described that only a third of the binding sites are located in the same upstream region between orthologous genes (Odom et al., 2007). Using this strategy, LPCAT3 was identified further suggesting that it is a LXR target gene.

LPCAT3, also named MBOAT5 encodes a lysophosphatidylcholine acyltransferase. Various LPCATs exist with specific tissue distributions and substrates (MacDonald and Sprecher, 1991; Choy et al., 1997). LPCAT3 seems to be the major enzyme contributing to lysophosphatidylcholine acyltransferase activity in the liver (Zhao et al., 2008) and is highly conserved among species. LPCATs main function is the re-acylation of fatty-acyl chain at the sn-2 position of phosphatidylcholine (PC) after its hydrolyzation by a specific phospholipase A2 family member. Therefore, LPCAT3 is probably an important player in regulating membrane fluidity and phospholipid dependent signalling.

To further investigate the regulation of LPCAT3 by LXR we next used *ex vivo* and *in vitro* methods. The role of LXR in LPCAT3 expression regulation was evidenced through cell culture experiments, using LXR agonist, LXRα transfection and promoter-reporter transactivation assays. Next, the demonstration that LXRα binds LPCAT3 promoter was made through a gel shift experiment.

Altogether our results establish for the first time that LPCAT3 is specifically regulated by LXRα. This nuclear receptor has a central role in hepatic fatty acids metabolism (Repa et al., 2000; Joseph et al., 2002; Cha and Repa, 2007). LPCAT3 appears as a new LXR sensitive gene involved in fatty acid metabolism. The exact role of LPCAT3 remains unclear, however most studies of substrate specificity showed that arachidonyl-CoA (20:4-n-6) is the better substrate (Zhao et al., 2008; Gijon et al., 2008; Jain et al., 2009). Therefore, LPCAT3 could be considered as a major regulator of free arachidonic acid levels (Perez-Chacon et al., 2009).

In conclusion, the finding that LXRα regulates the expression of LPCAT3, an enzyme that contributes to the PC metabolism in the liver suggests that LXRα might be involved in new aspects of fatty acid homeostasis.

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Résumé

Rôle du Liver X Receptor dans la régulation transcriptionnelle de la lipogenèse.

Chez les mammifères, la lipogenèse ou synthèse *de novo* des acides gras joue un rôle essentiel à l'homéostasie énergétique. Elle est particulièrement active dans le foie. Le Liver X Receptor (LXR) est un récepteur nucléaire de classe II qui est impliqué dans la régulation de l'expression de gènes importants dans cette voie métabolique. Au niveau hépatique, LXR régule directement l'expression de certains gènes de la lipogenèse et aussi l'expression des facteurs de transcription SREBP-1c et ChREBP intervenant respectivement dans la réponse hépatique à l'insuline et au glucose.

Les ligands naturels de LXR sont les oxystérols, des dérivés oxygénés du cholestérol. Aussi, LXR est avant tout considéré et connu comme un senseur du cholestérol. Au cours de ces travaux nous nous sommes intéressés *in vivo* au rôle de LXR dans la régulation transcriptionnelle de la lipogenèse hépatique en fonction de différents stimuli: pharmacologiques, inflammatoires et nutritionnels.

Par une approche pharmacologique, nous avons étudié la régulation croisée avec entre LXR et le récepteur activé par les prolifférateurs de peroxisome (PPARα). Nous avons aussi montré que l'inflammation intestinale est un puissant inhibiteur de la lipogenèse hépatique. Enfin, nous avons mis en évidence le rôle de LXR dans la régulation de la lipogenèse en réponse à une carence en acides gras essentiels et à un régime riche en fructose.

Mots clés : Liver X Receptor, foie, lipogenèse, récepteur nucléaire, NAFLD

Abstract

Role of the Liver X Receptor in the transcriptional regulation of lipogenesis.

In mammals, lipogenesis or *de novo* fatty acid synthesis plays an essential part in energy homeostasis. It is particularly active in the liver. The Liver X Receptor (LXR) is a class II nuclear receptor that regulates the expression of important genes involved in this pathway. In the liver, LXR directly controls the expression of lipogenic genes and also the expression of transcription factors such as SREBP-1c and ChREBP required for the hepatic response to insulin and glucose respectively.

Natural ligands for LXR are oxysterols, which are oxygenated derivatives of cholesterol. Therefore, LXR is primarily considered and known as a cholesterol sensor. In this work, we were interested in the role of LXR in the transcriptional control of hepatic lipogenesis *in vivo* in response to distinct stimuli: pharmacological agonists, gut inflammation and changes in diet composition.

Through a pharmacological study, we highlighted the cross-talk between LXR signaling and the regulation of the Peroxisome Proliferator Activated Receptor (PPARα). We have also evidenced that experimentally induced colitis induces a potent inhibition of hepatic lipogenesis. Finally, we have shown that LXR is involved in the regulation of lipogenesis in response to essential fatty acid deficiency and to high fructose.

Key words: Liver X Receptor, liver, lipogenesis, nuclear receptors, NAFLD