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**STUDIES ON THE PHENOTYPE OF LXRβ-/- MICE:**
FROM MALABSORPTION
TO AMYOTROPHIC LATERAL SCLEROSIS

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Stockholm 2008
“What we are doing is just a drop in the ocean. But the ocean would be less because of that missing drop.”

(Mother Teresa)
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>LXR</td>
<td>Liver X Receptor</td>
</tr>
<tr>
<td>ABC</td>
<td>ATP Binding Cassette</td>
</tr>
<tr>
<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
</tr>
<tr>
<td>AF</td>
<td>Activation function domain</td>
</tr>
<tr>
<td>DBD</td>
<td>DNA binding domain</td>
</tr>
<tr>
<td>LBD</td>
<td>Ligand binding domain</td>
</tr>
<tr>
<td>RXR</td>
<td>Retinoid X Receptor</td>
</tr>
<tr>
<td>LXRE</td>
<td>Liver X Receptor response element</td>
</tr>
<tr>
<td>SMRT</td>
<td>Silencing mediator of retinoic acid receptor</td>
</tr>
<tr>
<td>CoR</td>
<td>Nuclear receptor corepressor</td>
</tr>
<tr>
<td>CYP7</td>
<td>Sterol 7-hydroxylase</td>
</tr>
<tr>
<td>SREBP</td>
<td>Sterol regulatory element binding protein</td>
</tr>
<tr>
<td>CETP</td>
<td>Cholesterol ester transfer protein</td>
</tr>
<tr>
<td>PLTP</td>
<td>Phospholipid transfer protein</td>
</tr>
<tr>
<td>PEPCK</td>
<td>Phosphoenolpyruvate carboxykinase</td>
</tr>
<tr>
<td>G6P</td>
<td>Glucose 6-phosphatase</td>
</tr>
<tr>
<td>CYP8B1</td>
<td>Sterol 12α-hydroxylase</td>
</tr>
<tr>
<td>CYP27</td>
<td>Sterol 27-hydroxylase</td>
</tr>
<tr>
<td>HFD</td>
<td>High fat diet</td>
</tr>
<tr>
<td>SOD1</td>
<td>Superoxide dismutase 1</td>
</tr>
<tr>
<td>OXTR</td>
<td>Oxytocin receptor</td>
</tr>
<tr>
<td>PGF2α</td>
<td>Prostaglandin F 2α</td>
</tr>
<tr>
<td>PCNA</td>
<td>Proliferating cell nuclear antigen</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>PDC</td>
<td>Parkinson’s dementia complex</td>
</tr>
<tr>
<td>TDP-43</td>
<td>TAR-DNA binding protein</td>
</tr>
<tr>
<td>AQP-1</td>
<td>Aquaporin-1</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
</tbody>
</table>
LIST OF PUBLICATIONS

This thesis is based on the following publications, referred to in the text by their roman numerals:


II. **Gabbi C**, Kim HJ, Hultenby K, Bouton D, Toresson G, Warner M, Gustafsson JÅ. Pancreatic exocrine insufficiency in LXRβ-/- mice is associated with a reduction in aquaporin-1 expression. PNAS 2008; Sep 19 (Epub ahead of print)
ABSTRACT

Liver X Receptors, LXRα and LXRβ (NR1H2) are nuclear receptors belonging to the superfamily of ligand-activated transcription factors with a key role in the control of lipid and glucose metabolism. Studies of the phenotype of LXRα-/− mice and LXRβ-/− mice have shown that these nuclear receptors have specific and distinct roles, although they share very high sequence homology (78%). In particular, unlike LXRα-/− mice, LXRβ-/− mice are resistant to obesity when fed with a high fat diet and have a decreased gonadal fat pad during standard diet feeding. In addition, they develop a motor neuron disease that resembles amyotrophic lateral sclerosis by the age of 7 months. The studies in this thesis are aimed at investigating the mechanisms through which loss of LXRβ leads to disease.

The first study investigated the pathogenesis of motor neuron disease in LXRβ-/− mice. It has been shown that in the spinal cord of patients with amyotrophic lateral sclerosis, there is an accumulation of sphingomyelin, ceramides and cholesterol esters: phytosterols are known ligands of LXRβ and are secreted into the intestinal lumen by ABCG transporters. These transporters are LXR-regulated genes. In the first study we treated LXRβ-/− mice with β-sitosterol, a known motoneuron toxin thought to be involved in ALS. After 3 weeks of treatment, in LXRβ-/− mice but not in wild type littermates, there was a reduction in the number of motor neurons in the lumbar region of the spinal cord and in the surviving motor neurons there were aggregates of ubiquitin and TDP-43. In addition, in the substantia nigra there was a loss of tyrosine hydroxylase-positive dopaminergic neurons and an increase in the number of activated microglia. Brain cholesterol concentrations were higher in LXRβ-/− than in their
wild type counterparts, and treatment with \( \beta \)-sitosterol reduced brain cholesterol in both WT and LXR\( \beta \)-/- mice. Levels of the intestinal transporters, ABCG5/8 and Niemann-Pick C1 Like 1, were not affected by the loss of LXR\( \beta \) and/or treatment with \( \beta \)-sitosterol. In conclusion, these data indicate that multiple mechanisms are involved in the sensitivity of LXR\( \beta \)-/- mice to \( \beta \)-sitosterol: activation of microglia, accumulation of protein aggregates in the cytoplasm of large motor neurons, and depletion of brain cholesterol.

The second paper examined the resistance to gain weight of LXR\( \beta \)-/- mice. We demonstrated that these mice at 11 months of age on a standard diet, exhibit a pancreatic exocrine insufficiency that could explain their resistance to gain weight. Moreover, we showed a marked reduction in the expression of the water channel aquaporin-1 in pancreatic ducts of LXR\( \beta \)-/- mice. This reduction is likely responsible for the thick and dense secretion visualized in electron microscopy and for the pancreatic insufficiency. With a specific antibody we demonstrated the presence of LXR\( \beta \) in the nuclei of epithelial cells in pancreatic ducts and by treatment of wild type mice with an LXR agonist we could demonstrate an increase in the level of mRNA of aquaporin-1. These findings suggest that LXR\( \beta \) plays an important role in controlling pancreatic juice secretion through the regulation of water transport.

Interestingly, patients affected by ALS also exhibit exocrine insufficiency. Thus the two major characteristics of LXR\( \beta \)-/- mice may be part of a single syndrome whose common etiology stems from defective LXR\( \beta \) signaling.
2. INTRODUCTION

2.1 Nuclear receptors and reverse endocrinology

Nuclear receptors are transcription factors that act as intracellular receptors for small biologically active molecules such as lipids and steroid hormones. In contrast to conventional extracellular receptors that bind to peptide ligands and activate a cascade of cytoplasmic kinases, nuclear receptors are intracellular, have lipophilic ligands and directly regulate the expression of target genes by binding to specific DNA sequences (response elements) in the promoters of target genes. Each response element consists of a consensus sequence (AGGTCA) of single or double elements in a direct, everted or inverted repeat involving binding of NRs as monomers, homodimers or heterodimers [1].

Forty-eight members of the nuclear receptor superfamily have been identified in humans. Nuclear receptors share a canonical structure, composed of functionally distinct domains (Figure 1A): the N-terminal activation function 1 (AF1) domain, highly variable in sequence and length [2, 3]; the very conserved DNA-binding domain (DBD) that contains two zinc fingers, involved not only in DNA binding but also in receptor dimerization [4]; and the C-terminal ligand binding domain (LBD) with a key role in ligand binding, nuclear localization, receptor dimerization and interaction with coactivators and corepressors [5, 6]. Between the DBD and LBD is the hinge domain that provides flexibility between these two domains. AF2 is a part of LBD whose different conformations are dictated by the structure of the ligand bound in the ligand binding pocket and accordingly recognized by coactivators or corepressors.
Over the past 15 years, thanks to the highly conserved sequence homology of the DBD of nuclear receptors, it has been possible to discover many previously unknown nuclear receptors. So, endocrinology has been fascinatingly “reversed” [7]: the characterization of cloned-receptor sequences took place before the identification of natural specific ligands and even before the discovery of any functional role and tissue distribution of many of these nuclear receptors. This reverse endocrinology has, as final step, the identification of pharmaceutical compounds that can selectively modulate the activity of nuclear receptors and be a promising treatment for diseases in which nuclear receptors or their target genes are involved.

2.2 Liver X Receptors

Liver X Receptor α, LXRα (NR1H3) and LXRβ (NR1H2) are nuclear receptors belonging to the superfamily of ligand-activated transcription factors with a key role in the control of lipid metabolism and inflammation.

Initially the LXRs were considered “orphans” because they were cloned on the basis of sequence homology with other receptors and their ligands were unknown. They have since been “adopted” by oxygenated cholesterol metabolites that at physiological concentrations are able to bind to and activate these receptors. The most potent ligands for LXRs are 22-hydroxycholesterol, 24(S)-hydroxycholesterol, 24(S), 25-epoxycholesterol and 27-hydroxycholesterol [8]. Recently, high concentration of D-glucose has also been reported to activate LXRs in vitro [9]. In addition, two nonsteroidal synthetic compounds, GW3965 and T0314407 have been identified as potent and orally active agonists for both LXRs [10, 11]. Although subtype-specific ligands have not been synthesized, a
subset of natural bile acids has been reported to activate LXRα [12] whereas N-Acylthiadiazolines have selectivity for LXRβ but with modest potency [13].

Levels of LXRα mRNA are high in the liver, adipose tissue, adrenal glands, intestine, kidney, macrophages and lung, while LXRβ mRNA is widely distributed throughout the body with high levels in the developing brain [14, 15].

LXRs form obligate heterodimers with Retinoid X Receptor (RXR) [16]. The LXR/RXR complex that can be activated by ligands of either partner, binds to LXR-responsive elements (LXREs) consisting of a direct repeat (DR4) of the core sequence 5’-AGGTCA-3’ separated by 4 nucleotides on the promoter of target genes [17]. In the absence of ligands (Figure 1B), LXRs bind to the cognate LXRE in complex with corepressors like SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) and N-CoR, (nuclear receptor corepressor) [18, 19]. In this condition the transcriptional activity of the receptor is suppressed. In the presence of ligands (Figure 1C), LXR undergoes a conformational change that induces the release of the corepressors, recruitment of specific coactivators and then the transcription of target genes [20].

LXR target genes are involved not only in cholesterol, triglyceride, and bile acid metabolism but also in inflammatory response and energy balance. LXRs act as “sterol-sensors”: when the cellular concentration of oxysterols increases, LXR induces the transcription of genes that protect the cell from cholesterol overload.
**Figure 1:** panel A shows the canonical structure of nuclear receptors; in panel B, LXR is interacting with corepressors in absence of ligands; in panel C, with ligands, LXR binds to coactivators and promotes the transcription of target genes.
In the liver, LXR activation promotes cholesterol elimination in two different ways: first, through its conversion to bile acids, by increasing the expression of CYP7A1, cholesterol 7α-hydroxylase, the rate-limiting enzyme in the classical pathway of bile acid biosynthesis [21]; second, by promoting the transcription of genes for ATP binding cassette transporters, ABCG5 and ABCG8 that transport cholesterol from the hepatocytes to the bile canaliculi [22, 23]. At the same time, hepatic LXR activation also increases the synthesis of fatty acids and triglycerides by up-regulating sterol regulatory-binding protein 1c (SREBP-1c), the key regulator of hepatic lipogenesis [11] and other genes in the pathway like fatty acid synthase, acyl-CoA carboxylase and stearoyl-CoA desaturase 1 [24, 25]. For this reason, treatment with LXR agonists usually increases hepatic and serum levels of triglyceride, an unwanted side effect for this class of pharmaceuticals.

Protection from cholesterol accumulation by LXR activation is also provided through the control of “reverse cholesterol transport”. In macrophages, LXR ligands induce the expression of ABCA1, ABCG1 and ABCG4 transporters that promote the efflux of cholesterol to high density lipoproteins [26-29]. In addition LXRs promote the transcription of apolipoproteins that may act as cholesterol acceptors like ApoE and ApoC in macrophages or ApoD in adipose tissue [30-32]. Moreover, LXRs positively regulate enzymes involved in lipoprotein remodeling like lipoprotein lipase, cholesterol ester transfer protein (CETP) and the phospholipid transfer protein (PLTP) [33, 34].

In addition to cholesterol and triglyceride metabolism, LXR activation also influences glucose homeostasis. In particular, in the liver, LXR agonists decrease glucose output and increase glucose utilization by inducing
glucokinase and repressing phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) [35], while in white adipose tissue, GLUT4, an insulin dependent glucose transporter, is induced [36].

Besides their important physiological roles as antiatherogenic and antidiabetogenic receptors, LXRs are also immunomodulatory receptors. The antinflammatory action of LXRs is evident from the down-regulation of i-NOS, COX2, IL6 and IL1β that occurs in LXRα-/-β-/- mice. So far no LXRE has been identified on the promoters of these repressed genes and the mechanism through which LXRs exert these immunomodulatory effects remains to be elucidated [37].

2.3 Phenotype studies on mice lacking LXRβ isoform

A few years after the cloning of LXRs, the identification of oxysterols as LXR activators and the isolation of an LXRE in the promoter of CYP7A1, immediately raised the hypothesis of a key regulatory role of these nuclear receptors in cholesterol homeostasis. Recently, in vivo studies on the phenotype of LXRα-/- and LXRβ-/- mice have not only confirmed this theory, but also demonstrated specific and distinct roles for each isoform in controlling lipid and glucose metabolism, inflammation and proliferation.

2.3.1 Production of LXR deficient mice

Both LXRα-/- and LXRβ-/- mice used in our studies were created with the Cre-Lox recombination strategy [38]. This technique is used to delete specific segment of DNA flanked by LoxP sites, with the action of the Cre-recombinase (Cre). This enzyme is a site specific DNA recombinase that
catalyzes the splicing of DNA between 2 LoxP sites, consisting of a Cre-specific-recognition sequence of 23 pb [39]. For LXRβ targeting vector, LoxP cassettes were inserted into intron 4 and intron 6 in order to obtain a deletion of exon 5 and 6 that encode the DBD. The targeting vector for LXRα was obtained by inserting LoxP cassettes into introns 3 and 5 to obtain deletion of the DBD in exon 4 and 5. After electroporation of embryonic stem cells with the vector, and injection of recombinant ES-cell clones into C57BL/6 blastocysts, the mating of mice carrying the hypomorphic LXR alleles with Cre-transgenic deleter mice was performed. This Cre mouse strain was produced by injection of DNA fragment containing Cre gene under the transcriptional control of a human cytomegalovirus promoter into pronuclei of fertilized eggs. It mediates deletion of Lox-P flanked genes in all tissues including germ cells [39]. In the liver, no LXRβ transcripts were detected in LXRβ−/− mice while in LXRα−/− mice a transcript lacking exon 4 and 5 were formed.

LXRα−/−β−/− mice were obtained by several mating steps starting with mice carrying hypomorphic LXRα alleles and Cre-transgenic LXRβ−/− mice [40].

2.3.2 Metabolic phenotype

The most studied phenotype involves all the metabolic alterations that the three different genotypes, LXRα−/−, LXRβ−/− and LXRα−/−β−/−, demonstrate during dietary challenge. On standard rodent diet (Table 1), LXRα−/− mice differ from WT mice, first in the amount of perigonadal fat, which tends to be reduced with decreased adipocyte size [41] and second in the serum and hepatic lipid profile. Serum total cholesterol and VLDL TG are in normal range while LDL-CH is increased in parallel with a reduction in HDL-CH [40].
Table 1. Metabolic modifications in LXRα-/- mice during dietary challenge

<table>
<thead>
<tr>
<th></th>
<th>STANDARD CHOW DIET</th>
<th>2% CHOLESTEROL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sex</td>
<td>age</td>
</tr>
<tr>
<td><strong>Body Weight</strong></td>
<td>normal</td>
<td>12</td>
</tr>
<tr>
<td><strong>Perigonadal Fat</strong></td>
<td>tendency to be reduced</td>
<td>MF</td>
</tr>
<tr>
<td><strong>Hepatic Triglyceride</strong></td>
<td>tendency to be reduced</td>
<td>-</td>
</tr>
<tr>
<td><strong>Hepatic Cholesterol</strong></td>
<td>normal</td>
<td>-</td>
</tr>
<tr>
<td><strong>Serum Cholesterol</strong></td>
<td>normal</td>
<td>-</td>
</tr>
<tr>
<td><strong>LDL</strong></td>
<td>tendency to be increased</td>
<td>-</td>
</tr>
<tr>
<td><strong>HDL</strong></td>
<td>tendency to be reduced</td>
<td>-</td>
</tr>
<tr>
<td><strong>VLDL</strong></td>
<td>normal</td>
<td>-</td>
</tr>
<tr>
<td><strong>LDL/HDL ratio</strong></td>
<td>increased</td>
<td>-</td>
</tr>
<tr>
<td><strong>Serum Triglycerides</strong></td>
<td>normal</td>
<td>-</td>
</tr>
<tr>
<td><strong>Serum Glucose</strong></td>
<td>normal</td>
<td>F</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>BA excretion</strong></td>
<td>normal</td>
<td>MF</td>
</tr>
<tr>
<td><strong>BA pool</strong></td>
<td>reduced</td>
<td>MF</td>
</tr>
<tr>
<td><strong>Ratio CA/MCA</strong></td>
<td>reduced</td>
<td>MF</td>
</tr>
<tr>
<td><strong>Leptin</strong></td>
<td>normal</td>
<td>-</td>
</tr>
<tr>
<td><strong>Adiponectin</strong></td>
<td>normal</td>
<td>-</td>
</tr>
<tr>
<td><strong>Resistin</strong></td>
<td>normal</td>
<td>-</td>
</tr>
</tbody>
</table>
Glucose tolerance is the same as in WT mice, as is hepatic cholesterol; triglycerides in the liver are slightly reduced [40]. Interestingly, when LXRα-/− mice are fed with a diet containing 2% of cholesterol (Table 1), they are unable to regulate cholesterol catabolism in the liver, where, as a consequence, cholesterol esters accumulate as soon as 8 days after the start of feeding [21, 38]. WT rodents have the capacity to metabolize high concentrations of dietary cholesterol, mainly by increasing the activity of cholesterol 7α-hydroxylase, the rate limiting enzyme in the classical pathway of bile acid biosynthesis. Excess cholesterol is then eliminated by its conversion to bile acids. This mechanism is missing in LXRα-/− mice that even on a standard diet exhibit a decreased bile acid pool and a low ratio between cholic acid and muricholic acid [21]. Furthermore, during cholesterol feeding in these mice, serum total cholesterol and LDL-CH are significantly increased while CH-HDL is reduced; liver transaminases are higher than in WT mice but after 90 days they decrease indicating a probable liver failure.

LXRβ-/− mice, when fed with a standard diet (Table 2), differ from WT mice in terms of a reduction in the amount of perigonadal fat [41] and in decreased levels of triglycerides in serum and in liver [40] with low serum insulin [41]. Surprisingly, on a diet containing 2% cholesterol (Table 2), the response of LXRβ-/− mice is similar to WT mice; in particular, they maintain the physiological capacity of eliminate excess cholesterol by increasing bile acid synthesis. Indeed, in these mice, the expression of enzymes involved in bile acid metabolism (CYP7A1, CYP7B, CYP8B1, CYP27) is not affected [41]. Interestingly, in LXRβ-/− mice fed with a high fat diet (HFD), the body weight and the amount of the perigonadal fat pad was reduced compared to WT mice [41].
Table 2. Metabolic modifications in LXRβ-/ mice during dietary challenge

<table>
<thead>
<tr>
<th></th>
<th>STANDARD CHOW DIET</th>
<th>2 % CHOLESTEROL</th>
<th>HIGH FAT DIET</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>sex    age (m) ref</td>
<td>sex  age (m) Ref</td>
<td>sex  age (m) ref</td>
</tr>
<tr>
<td>Body Weight</td>
<td>normal MF 12 [41]</td>
<td>-    -   -   -</td>
<td>reduced F - [41]</td>
</tr>
<tr>
<td>Perigonadal Fat</td>
<td>reduced MF 12 [41]</td>
<td>-    -   -   -</td>
<td>reduced F - [41]</td>
</tr>
<tr>
<td>Food Intake</td>
<td>normal - 6 [41]</td>
<td>-    -   -   -</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>O2 consumption</td>
<td>normal - 6 [41]</td>
<td>-    -   -   -</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>Serum Insulin</td>
<td>reduced MF 6-9 [41]</td>
<td>-    -   -   -</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>Hepatic Triglyceride</td>
<td>decreased - 4 [40]</td>
<td>-    -   -   -</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>Hepatic Cholesterol</td>
<td>normal - 4 [40]</td>
<td>normal - 3-4 [38]</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>normal - 4 [40]</td>
<td>normal - 3-4 [38]</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>Serum Triglycerides</td>
<td>normal - 4 [40]</td>
<td>-    -   -   -</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>ALT</td>
<td>-     -   -   [40]</td>
<td>normal - 3-4 [38]</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>LDL</td>
<td>normal - 4 [40]</td>
<td>-    -   -   -</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>HDL</td>
<td>normal - 4 [40]</td>
<td>-    -   -   -</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>VLDL</td>
<td>tendency to be reduced</td>
<td>-    -   -   -</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>Leptin</td>
<td>normal - 12 [41]</td>
<td>-    -   -   -</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>normal - 12 [41]</td>
<td>-    -   -   -</td>
<td>-    -   -   -</td>
</tr>
<tr>
<td>Resistin</td>
<td>normal - 12 [41]</td>
<td>-    -   -   -</td>
<td>-    -   -   -</td>
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</table>
Table 3. Metabolic modifications in LXRα-/-β-/- mice during dietary challenge

<table>
<thead>
<tr>
<th></th>
<th>STANDARD CHOW DIET</th>
<th>WESTERN DIET + CH</th>
<th>WESTERN DIET no CH</th>
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</thead>
<tbody>
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<td></td>
<td>sex</td>
<td>age (m)</td>
<td>Ref</td>
</tr>
<tr>
<td>Body Weight</td>
<td>reduced</td>
<td>M 4-5</td>
<td>[42, 43]</td>
</tr>
<tr>
<td>Perigonadal fat</td>
<td>reduced</td>
<td>MF 12</td>
<td>[41]</td>
</tr>
<tr>
<td>Food Intake</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fat absorption</td>
<td>reduced</td>
<td>M 4-5</td>
<td>[43]</td>
</tr>
<tr>
<td>Serum insulin</td>
<td>reduced</td>
<td>M 4-5</td>
<td>[43]</td>
</tr>
<tr>
<td>Hepatic Triglyceride</td>
<td>reduced</td>
<td>- 4</td>
<td>[40]</td>
</tr>
<tr>
<td>Hepatic Cholesterol</td>
<td>increased</td>
<td>- 4</td>
<td>[40]</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>normal</td>
<td>- 4</td>
<td>[40]</td>
</tr>
<tr>
<td>LDL</td>
<td>increased</td>
<td>- 4</td>
<td>[40]</td>
</tr>
<tr>
<td>HDL</td>
<td>tendency to be reduced</td>
<td>- 4</td>
<td>[40]</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>increased</td>
<td>- 4</td>
<td>[40]</td>
</tr>
<tr>
<td>Serum Triglycerides</td>
<td>reduced</td>
<td>- 4</td>
<td>[40]</td>
</tr>
<tr>
<td>TSH</td>
<td>normal</td>
<td>M 4-5</td>
<td>[43]</td>
</tr>
<tr>
<td>T3</td>
<td>normal</td>
<td>M 4-5</td>
<td>[43]</td>
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<tr>
<td>T4</td>
<td>normal</td>
<td>M 4-5</td>
<td>[43]</td>
</tr>
<tr>
<td>Leptin</td>
<td>normal / lower</td>
<td>- 12</td>
<td>[41, 43]</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>reduced</td>
<td>- 12</td>
<td>[41]</td>
</tr>
<tr>
<td>Resistin</td>
<td>normal</td>
<td>- 12</td>
<td>[41]</td>
</tr>
</tbody>
</table>
These data together represent compelling evidence that LXRα plays a central role in maintaining cholesterol homeostasis in the liver while LXRβ has a different role in fat metabolism, in particular related to a resistance in gaining weight.

The lack of LXRβ isoform is responsible for the lean phenotype also in LXRα-/β-/- mice. On standard diet their body weight is reduced as well as the perigonadal fat pad and the adipocyte size [41-43]. Triglycerides are reduced both in liver and serum as in LXRβ-/- mice while, as in LXRα-/- mice, total cholesterol and LDL-CH are increased, HDL-CH is decreased in serum and hepatic cholesterol is increased. Interestingly, also LXRα-/-β-/- are resistant to obesity when fed a western diet containing 0.2 % cholesterol but they gain weight when only fed an HFD [41].

2.3.3 Neurological phenotype

Given that LXRβ-/- mice maintain their capacity to metabolize dietary cholesterol in the liver, the function of LXRβ has been studied in other organs in which cholesterol plays a physiological key role, especially the central nervous system.

Male LXRβ-/- mice at 7 months of age develop a motor impairment, measured as a reduced ability, compared to WT littermates, to stay on a rotor-rod. Learning ability and muscle strength are not affected. Histopathological analysis of LXRβ-/- spinal cords reveals a loss of large α motor neurons in the latero-ventral horn with increased number of astrocytes and lipid accumulation in association with axonal atrophy [44]. These features, evident only in male mice, are typical of the chronic motor neuron disease amyotrophic lateral sclerosis.
(ALS). In LXRβ-/- mice, accumulation of lipids in motor neurons could be due to a decrease in the expression of transporters like ABCG5, ABCG8 and ABCA1 and could lead to motor neuron degeneration. Interestingly, in the spinal cord of patients affected by ALS, high levels of sphingomyelin, ceramides and cholesterol esters have been demonstrated [45]. Moreover, in the population of Guam, when the diet was based on Cycas, a vegetable rich in β-sitosterol, the incidence of ALS was very high. Mice carrying an SOD1 (Cu/Zn superoxide dismutase 1) mutation, considered an animal model of ALS, show accumulation of phytosterols in the spinal cord [45]. Plant sterols are transported back to the intestinal lumen by ABCG5 and ABCG8 transporters and mice lacking these transporters accumulate plant sterols in the brain [46].

In LXRα-/-β-/- mice, as expected, there is a down-regulation of ABCG5 and ABCG8 transporters. This is accompanied by lipid overload in the brain. The brains of these mice are characterized by obliteration of the lateral ventricles by lipid-laden cells, proliferation of astrocytes, loss of neurons and disorganized myelin sheaths [47].

2.3.4 Embryonic phenotype

Studies from our own laboratory have demonstrated that LXRβ has an important role in the development of the cerebral cortex. At late stage of embryogenesis (E 18.5) and in neonates (P2), LXRβ-/- mice have a smaller brain with a reduction in the number of neurons in the superficial cortical layers. During development, neurons migrate from lower layers to superficial layers. After birth (P2), in LXRβ-/- mice the number of neurons is higher in lower cortical
layers (IV) while in WT mice more neurons are in the upper layer (II-III) indicating a migration defect [48].

2.3.5 Reproductive phenotype

During pregnancy, cholesterol has an important role in modulating membrane activity and stability of oxytocin receptor (OXTR). When activated by its specific hormone, oxytocin, this receptor induces contractile activity in the myometrium during labor. A balanced cholesterol distribution in uterus has a central role in contractile function: depletion increases abnormal contraction of uterine smooth muscle cells in vitro, while cholesterol accumulation in myometrium reduces the amplitude of contractions during labor [49]. LXRβ−/− mice demonstrate a high accumulation of cholesterol esters in longitudinal and circular layers of myometrium, probably due to a reduction in the expression of transporters regulating cholesterol efflux, like ABCA1 and ABCG1, which are under the control of LXRβ. As consequence, uteri of LXRβ−/− mice have an impaired contractile function in response to oxytocin or PGF2α stimulation and these mice show several signs of fetal resorption in the uterine horns, such as unexpulsed pups or death during delivery. Interestingly, this role of LXRβ in cholesterol modulation in uterus seems very specific since LXRα−/− mice do not exhibit any uterine abnormalities [50]. Moreover, another study on female reproductive system [51] has shown that ovarian function is impaired in LXRα−/−β−/− mice; in particular, oocytes lacking both LXRs, fail to resume meiosis after FSH stimulation.

LXRβ also controls cholesterol balance in the male reproductive tract: in the testis of LXRβ−/− mice, there is an accumulation of cholesterol as early as at
2.5 months of age in Sertoli cells that provide structural and nutritional support to
germ cells [52]. There is also a lower proliferation rate of germ cells [53].
Furthermore, although Leydig cells are not affected by lipid accumulation in
LXRβ-/- mice, the secretion rate of testosterone is markedly reduced in LXRα-/-
β-/- mice. No structural or functional alterations are detectable in testis from
LXRα-/- mice indicating a dominant role of LXRβ in these organs. In addition the
epithelial structure in the epididymis is affected in LXRα-/-β-/- mice but not in
single mutant mice. This defect results in sperm head fragility [54].

All the described alterations, both in the female and male reproductive
tract, may together be responsible for the reduced fertility and decreased
number of pups per litter, described in LXRβ-/- mice and LXRα-/-β-/- mice [51].
Furthermore, LXRα-/-β-/- mice become completely infertile by 4-5 months of age
[52, 53] indicating a more severe phenotype in mice lacking both isoforms.

**2.3.6 Immunological phenotype**

By the age of 5-6 months, in LXRβ-/- mice there is splenomegaly and
lymphoadenopathy with expansion of total number of T and B lymphocytes. A
prevalence of B lymphocytes was noticed only in lymph nodes. Moreover,
LXRβ-/- T lymphocytes respond to TCR stimulation with a higher proliferative
rate than that T lymphocytes from LXRα-/- and WT mice. Conversely, in WT
mouse T lymphocytes in cell culture (but not in LXRβ-/- T lymphocytes),
proliferation is inhibited when LXR synthetic agonist, RXR agonist and 22(R)-
hydroxycholesterol are added. In activated LXRβ-/- T lymphocytes in cell culture,
proliferation is increased with an increased fraction of cells in S and G2, M
phase [55].
2.3.7 Cardiovascular phenotype

There are abnormalities in the vascular system in LXRα-/β−/− mice which are most likely due to a combination of both the increase in LDL-CH and of the down-regulation of genes involved in reverse cholesterol transport (ABCG1, ABCA1). In LXRα-/β−/− mice there is accumulation of foam cells with a significant increase in lipid infiltration all around the intima-media layer in the aortic root. Accumulation of lipid in the aorta is less pronounced in single mutant mice [40]. Except for a report that there is lack of neutral lipid accumulation in heart myocytes [50], there is no published information about the cardiac phenotype of LXRβ−/− mice. LXRβ binds to the promoter of renin and LXR agonist treatment leads to an up-regulation of renin mRNA in kidney [56]. Thus it is anticipated that LXRβ−/− mice might be hypotensive but so far no studies on blood pressure in these mice have been published.

2.3.8 Dermatological phenotype

Oxysterols, ligands for LXRs, stimulate keratinocyte differentiation by increasing the expression of epidermal differentiation markers as involucrin, filaggrin, and loricrin, in WT and LXRα−/− mice but not in LXRβ−/− mice [57]. The epidermis of LXRβ−/− mice is thinner with a reduced number of PCNA-positive cells in the basal layer and a slightly decreased expression of differentiation markers. However, in these mice, no macroscopic cutaneous abnormalities or functional impairments are detectable, indicating that LXRβ−/− mice use some compensatory pathways to overcome the consequences of the lack of keratinocyte differentiation [57].
2.3.9 Bone phenotype

LXRβ-/− female mice exhibit increased levels of alkaline phosphatase and osteocalcin in serum, probably indicating an increased osteoblast activity. Besides, the volume of osteoclasts in the trabecular compartment is decreased compared to WT mice [58]. However, no particular alterations have been detected in the cortical or in the trabecular compartment of the femurs of these mice.

2.4 LXRβ genetics in human diseases

The role of genetic mutations or gene polymorphisms of LXRs in human diseases corresponding to the phenotypes described in the transgenic animals is almost completely unexplored; at the moment only three such studies have been published.

According to NCBI database, 4 single nucleotide polymorphisms (SNPs) of LXRβ (chromosome 19) have been identified: LXR1 in intron 5, LXR2 in intron 7, LXR3 in the 3’UTR and LXR4 in intron 2. An association between the risk of developing late-onset (age at onset after 60 years) Alzheimer disease and LXR2 and LXR4 has been shown in an American population of 931 Alzheimer disease patients [59]. Although LXR2 seems to be a silent SNP, LXR4 is likely to be functional, residing in either a coding region or in a splicing junction. Moreover, this association has been confirmed also in a Spanish population of 414 Alzheimer disease patients. In this study there was an increased risk if these SNPs (LXR2, LXR4, LXR1) are associated with a SNP in heme-oxygenase-1 (413 TT) [60].
The third study involves a Swedish population of 559 obese patients. This study revealed that one LXRα (rs2279238) and two LXRβ SNPs (LB44732G>A and rs2695121), in the promoter region and in intron 2, are associated with obesity [61].

More studies are required to confirm that these SNPs have a functional role in the susceptibility of Alzheimer disease and obesity.

2.5 Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by progressive loss of motor neurons in the spinal cord, in the cortex and in the brain stem. The worldwide prevalence of ALS is 4-6 per 100,000 inhabitants with an incidence of 0.5-3 per 100,000 yearly [62]. Approximately 10% of ALS cases are familial (FALS) with a genetic autosomal dominant trait, while the remaining 90% of cases are sporadic (SALS) [63]. In familial cases, the prevalence of affected males is much higher (male:female ratio 7:1) but this gender difference is reduced by increasing age of presentation, reaching a 1:1 ratio in patients in their eighth decade [62, 63]. Typically this disease is fatal within 3-5 years of the onset of symptoms.

Clinically, FALS and SALS are indistinguishable but a distinct manifestation associated with parkinsonism-dementia, called PD Complex (PDC) is seen in the pacific islands of Guam. In the indigenous Chamorro population of this island, the prevalence of ALS is 100 times higher than elsewhere in the world with a more malignant clinical appearance [64]. The ethiopathogenesis of PDC is still unknown but both genetic and environmental factors are thought to be involved. The characteristic pathological finding at
autopsy is the high prevalence of neurofibrillary tangles (NFTs) in patients with PDC. Interestingly, in comparison with control American subjects, healthy Chamorros also have an increase in neurofibrillary tangles [65].

Leucine-rich repeat kinase 2 (LRRK-2), a protein mutated in familial Parkinson disease with unclear function, has been shown to accumulate in these tangles and TDP-43, a transcriptional repressor normally expressed in the cell nucleus, accumulates in glial inclusions [66]. One interesting hypothesis on the role of diet in the etiology of ALS involves the chronic exposure to toxins from Cycas micronesica. This is a palm from which the flour has traditionally been prepared and used as the major source of flour when wheat is scarce. Feeding of monkeys with up to 2 g of cycad flour does not lead to any neurological disease [67]. Thus it is thought that the indigenous Guam population has a genetic predisposition which renders them susceptible to the toxic effects of cycad flour.

Still unknown is also the pathogenesis of the pure sporadic form of ALS. In familial cases a mutation of SOD1 gene has been described [68]. SOD1 is a Cu/Zn-binding superoxide dismutase that catalyzes the dismutation of toxic superoxide anion O$_2^-$ to O$_2$ and H$_2$O$_2$ [69]. The hypothesis is that in FALS patients the activity of SOD1 could be either reduced, leading to an accumulation of toxic superoxide radicals or, more probably, increased leading to excessive levels of H$_2$O$_2$ that can react with some metals like iron and generate highly toxic hydroxyl radicals [70].
2.6 Malabsorption syndrome

Malabsorption syndrome is a clinical condition characterized by a combination of symptoms like weight loss or growth failure in children, steatorrhoea, diarrhea, and anaemia which result from unsuccessful nutrient absorption from the diet. Numerous diseases are responsible for this syndrome and according to their etiology, they can be classified into three groups: (a) alterations of the digestive process due to deficit of enzymes and bile acids such as in chronic pancreatitis, cystic fibrosis, and cholestatic liver diseases; (b) alterations in uptake and transport due to a damage of absorptive surface such as in celiac disease, Crohn’s disease, and autoimmune enteropathy; (c) microbial causes such as bacterial overgrowth and parasitosis [71]. The major cause of defective intraluminal digestion is pancreatic exocrine insufficiency due to chronic pancreatitis and cystic fibrosis. In industrialized countries, the incidence of chronic pancreatitis is between 3.5-10 per 100,000 inhabitants. About 70-80 % of cases are related to long-term alcohol misuse while 10-30 % of cases represent idiopathic pancreatitis for which the etiology is still unknown [72]. A large number of mutations in genes coding for serine protease inhibitor, SPINK1, or the cystic fibrosis transmembrane conductance regulator, CFTR, have been described to be involved not only in the pathogenesis of pancreatitis but also, working in concert with other genetic and environmental factors, in the susceptibility to this disease [73]. Moreover, in humans, it has been shown that genetic polymorphisms of genes regulating the inflammatory response, like heat shock protein 70-2 or tumor necrosis factor α, are associated with an increased risk of acute pancreatitis [74].
3. AIMS

Aim of the thesis is to better understand the role of LXRβ isoform by studying two characteristic phenotypes of LXRβ/- mice: the motor neuron degeneration and the resistance to gain weight.

3.1 Paper I

As described previously [44], by the age of 7 months, LXRβ/- male mice demonstrate a progressive death of big motor neurons in the latero-ventricular horn of the spinal cord.

β-sitosterol, that is structurally similar to cholesterol, has been shown to activate the transcription activity of both LXRs isoforms. Besides, it is known to be a toxic compound for motor neurons and to accumulate together with other phytosterols in the spinal cord of ALS patients. It is also thought to be one of the environmental factors that in concert with unknown genetic predispositions could lead to the ALS-PDC in Guam population.

Aim of this study was to investigate the eventual toxicity of β-sitosterol on LXRβ/- mice with particular attention to the evaluation of:

- Motor coordination
- Intestinal expression of ABCG5, ABCG8 transporters
- Histopathology of spinal cord and substantia nigra, two areas involved in ALS-PCD complex
- Cholesterol levels in brain and serum
3.2 Paper II

As previously discussed, LXRβ/− mice demonstrate a reduction in the size of perigonadal fat pad that is characterized by smaller adipocytes, compared to WT mice. These mice are thinner and are resistant to gain weight when fed with a diet containing high amount of fat. A similar phenotype is described also in LXRα/−β/− mice but not in LXRα/− mice indicating a specific role for LXRβ in controlling body weight.

Malabsorption syndrome is a clinical condition characterized by weight loss or growth failure in children that results from unsuccessful nutrient absorption from the diet. The main cause of malabsorption syndrome is a pancreatic exocrine insufficiency.

Aim of this study is to investigate the pathogenesis of the lean phenotype in LXRβ/− mice with particular attention to the evaluation of:

• Pancreatic exocrine function (assay of serum amylase, lipase, fecal protease)
• Histopathology of the pancreas
• Electron microscopy study of the pancreas

4. NOTES ON METHODOLOGIES

Methods used in this thesis are fully described in the relative section of each paper. They include:

• Immunohistochemistry
• Western blotting
• RT-PCR real time
• Cholesterol assay in brain
• Preabsorption of LXR antibody
• Treatment of mice with LXR agonists
• Evaluation of motor function with rotating rod test
• Amylase and lipase assays
• Total protease assays
• Electron microscopy

5. RESULTS

5.1 Paper I

Evaluation of motor coordination, measured as retention time on a roto-rod, confirmed the motor disability of LXRβ-/− mice already evident from 5 months of age. Administration of β-sitosterol did not affect motor function in WT mice at any age but dramatically worsened the disability of LXRβ-/− mice that at 16 months of age were almost paralyzed.

Indeed the histopathological study of the lateroventricular horn of the spinal cord (L1) showed after treatment a drastic reduction in the number of motor neurons in LXRβ-/− mice. In the few motor neurons left in LXRβ-/− mice, it was possible to localize cytoplasmatic inclusions positive for TDP-43 and ubiquitin, typical of ALS.

Analysis of the substantia nigra demonstrated that dopaminergic neurons of β-sitosterol-treated-LXRβ-/− mice were shrunken with reduced number of projections. Besides, the number of activated microglia was higher in the pars reticulata of vehicle or β-sitosterol treated LXRβ-/− mice. These data demonstrated that when LXRβ signaling is abnormal, ingestion of β-sitosterol
can damage both the spinal cord and the substantia nigra as it happens in ALS-PDC.

To evaluate if a decreased intestinal clearance of β-sitosterol could increase its uptake, mRNA levels of ABCG5/G8 and β-sitosterol serum levels were measured. Interestingly, all these levels were unaffected either by LXRβ deletion or β-sitosterol treatment, indicating a main role for LXRα in controlling these transporters.

Moreover, brain levels of cholesterol were decreased both in WT and in LXRβ-/- mice after β-sitosterol treatment.

5.2 Paper II

Anthropometric measurements showed that both male and female LXRβ-/- mice had significantly lower body weights, weight/length ratios and perigonadal fat pad size than WT mice. Interestingly, the caloric intake monitored for 1 week was not different between the 4 genotypes, whereas a significant increase was detected in LXRβ-/- female mice that despite the augmented caloric intake, did not gain weight.

Evaluation of pancreatic function demonstrates a reduced activity of α-amylase and lipase in serum of both LXRβ-/- male and female mice, compared to WT. Total protease in the gut, considered an indirect index of pancreatic secretion, was evaluated by using Azo-casein as a substrate. A drastic reduction of proteolytic activity in the feces was evident in the transgenic animals of both sexes.

Histopathological analysis of LXRβ-/- pancreas showed a massive inflammatory infiltrate all around large and medium-sized pancreatic ducts that
exhibited a high rate of cell death, without any compensatory proliferation, in the ductal epithelium. Electron microscopic studies of the transgenic pancreas revealed a dilatation of the ducts with dense intra-ductal material, including “scroll-like” structures commonly seen in cystic fibrosis.

With specific antibodies, we studied the expression of LXRβ and the water channel, aquaporin-1 in the ductal epithelium of the pancreas. In wild type mice, ductal epithelial cells expressed LXRβ in the cell nuclei and aquaporin-1 on the plasma membrane. Interestingly, aquaporin-1 was almost undetectable on the luminal surface of pancreatic ductal epithelial cells and in parallel mRNA levels of aquaporin-1 were reduced in LXRβ−/− mice, compared to WT.

To evaluate the possibility that AQP-1 could be a target gene of LXRβ, WT mice were treated with LXR agonist for 7 days: after treatment, levels of AQP-1 mRNA were significantly higher than those in vehicle-treated mice indicating that AQP-1 expression is probably regulated by LXRβ.

6. DISCUSSION

Results of paper I demonstrated that in LXRβ−/− mice, ingestion of β-sitosterol has marked neurodegenerative consequences both in the spinal cord and in the substantia nigra, resembling a phenotype similar to ALS-PDC.

Although LXRs are involved in overall cholesterol homeostasis, blood cholesterol levels were not affected either by LXRβ deletion or β-sitosterol treatment. However, LXRβ−/− mice were characterized by lipid inclusions in motor neurons of the spinal cord and high cholesterol levels in the brain. After β-sitosterol treatment, there was a decrease in brain cholesterol levels while in the
spinal cord it was difficult to evaluate changes in lipid inclusions, because of low number of motor neurons left.

We interpret this data to mean that β-sitosterol, an activator of both LXRα and β, stimulates LXRα in LXRβ-/ mice promoting cholesterol excretion from the brain. Further evidence of an increase in cholesterol elimination from the brain is the high level of brain 24-hydroxycholesterol in β-sitosterol-treated LXRβ-/ mice.

Maintenance of appropriate cholesterol balance in the brain is crucial for many signal pathways like synaptic vesicle turnover, function of calcium channels, neurotransmitter release, signaling of GABA and glutamate. Our studies show that when cholesterol levels are affected in both directions, mice demonstrate a neurological phenotype (table 4).

<table>
<thead>
<tr>
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<th>LXRβ-/- mice</th>
<th>LXRβ-/- mice + β-sitosterol</th>
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<tr>
<td>Blood</td>
<td>no difference vs WT</td>
<td>no difference vs WT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>no differences vs vehicle</td>
</tr>
<tr>
<td>Brain</td>
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<td>lower vs WT (ns)</td>
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<tr>
<td></td>
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<td>lower vs vehicle</td>
</tr>
<tr>
<td>Spinal cord (lipid inclusions)</td>
<td>higher</td>
<td>only few motor neurons left</td>
</tr>
<tr>
<td>Phenotype</td>
<td>ALS</td>
<td>ALS-PDC</td>
</tr>
</tbody>
</table>

**Table 4: summary of the main cholesterol imbalances in LXRβ-/- male mice.**

**Paper II** demonstrates that pancreatic exocrine function is severely affected in LXRβ-/ mice, as shown by low levels of serum amylase and lipase, low levels of fecal total protease, massive infiltration of immune cells all around pancreatic ducts with increased cell death of the ductal epithelium and dense
secretion obstructing the lumen of intralobular ducts. The cause of dense pancreatic secretions seems to be the reduced expression of AQP-1 on the luminal surface of pancreatic ductal epithelial cells. AQP-1 is a water channel protein with a key role in trans-cellular fluid transport. In the digestive system it is expressed in endothelial cells of capillaries, small vessels and lymphatic capillaries of the small intestine [75], in cholangiocytes of liver, bile ducts [76] and gallbladder [77] and in the inter-intralobular pancreatic ducts [78] where it seems to participate in bile and pancreatic juice formation. Interestingly, AQP-1-/mice demonstrate mild growth retardation on standard diet [79] and, when fed with a high-fat diet, they are resistant to weight-gain, develop steatorrhea and have a decreased concentration of amylase and lipase in the pancreatic fluid [80]. It seems that defective secretion of water in the pancreatic ducts leads to a modification in the composition of pancreatic juice that damages the pancreatic epithelia and finally leads to exocrine insufficiency.

Interestingly, AQP-1 is also expressed on the luminal surface of the choroid plexus epithelium [81, 82], the main site of production of cerebrospinal fluid (CSF). This fluid not only provides physical support in the CNS but also facilitates transport of nutrient in the subarachnoid space surrounding the brain and the spinal cord [83]. In mice lacking AQP-1 there is a 25% reduction in CSF production, compared to WT [84]. We speculate that there could be a reduction in AQP-1 expression in the choroid plexus of LXRβ-/ mice. Such a reduction would lead to ionic imbalances and nutrient deficiencies and could be one of the causes of the pathogenesis of neurodegenerative diseases in LXRβ-/ mice.

Several epidemiological studies have examined the role of nutrients as environmental factors that, in concert with genetic predisposition, could
contribute to the pathogenesis of ALS. Interestingly, a premorbid daily intake of n-3 polyunsaturated fatty acids (PUFA) and vitamin E has been shown to be significantly lower in patients with ALS [85, 86] and Parkinson disease [87]. Indeed n-3 PUFA have a strong neuroprotective activity not only by acting as substrate in the synthesis of prostaglandins with anti-inflammatory effects but also by protecting neurons from kainate-induced cell death [88]. Vitamin E is an antioxidant agent that prevents lipid peroxidation and acts as a neuroprotective factor both in humans [89] and in animal models of ALS [90].

During pancreatic exocrine insufficiency, the lack of pancreatic lipolytic enzymes in the intestinal lumen affects the absorption of lipids in particular triglycerides from the diet leading to a reduced uptake of PUFA and vitamins (as vitamin E) that require incorporation into lipid micelles for their absorption. Interestingly, although no primary pancreatic involvement in ALS has been described, it has been shown that patients affected by ALS, have a reduced exocrine pancreatic function in particular after secretion stimulation [91].

We speculate (figure 2) that in LXRβ-/- male mice, the pancreatic exocrine insufficiency, which appears at early age, could lead to a lack of n-3 PUFA and vitamin E. These deficiencies could lead to vulnerability of dopaminergic and motor neurons to oxidative stress.
Figure 2: Hypothesis on the possible interplay between phenotypical alterations in LXRβ/-/ mice

7. CONCLUSIONS AND PERSPECTIVES

The articles in this thesis show a crucial role for LXRβ in maintaining cholesterol homeostasis in central nervous system and water balance in pancreatic function.

Our observation that LXRβ is essential to maintain the physiological response to β-sitosterol administration, leads us to conclude that LXRβ dysfunction could be a genetic predisposition that, in the Guam population, in concert with environmental factors like phytosterols, participates in the pathogenesis of ALS-PDC.

The transcriptional control of AQP-1 by LXRβ which we demonstrated in pancreatic ducts leads to a new perspective on LXRβ function in disease. Lack of LXRβ could be responsible for part of 30% of idiopathic chronic pancreatitis
described in humans. More studies are required to investigate whether AQP-1 is dysregulated in other LXRβ-expressing organs such as the ventral prostate and the lung. Perhaps disruption of fluid secretion may explain the abnormalities of these organs in LXRβ-/- mice.

Further studies are required also to analyze the role of LXRβ in the pathogenesis of ALS focusing on the LXR-inducible zinc-binding metallothionein proteins which are protective against development of ALS [92] and on the possible estrogen-mediated neuroprotection, given the absence of ALS in female LXRβ-/- mice.
8. REFERENCES


9. ACKNOWLEDGMENT

My deepest gratitude is for my supervisor, Jan-Åke Gustafsson for spreading around energy and finding always new challenge, and for my cosupervisor, Margaret Warner for the shining faith in Science, for the smiling support, and for the scientific courage.

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Finally, all my gratitude is for my family and my closest friends for a love that is especially freedom and understanding.

*To all of them, as single drops in the ocean, this thesis is dedicated.*