Chapter 1

Introduction
Cardiac hypertrophy is a clinical finding based on heart morphology and is typically assessed from echocardiographic or magnetic resonance imaging estimates of left ventricular mass. Hypertrophy of the heart can occur irrespective of changes in cardiac function. However, studies indicate that myocardial hypertrophy with normal function typically progresses over time to hypertrophy with worsened function, more precisely defined as decompensated hypertrophy (1,2). Moreover, left ventricular hypertrophy (LVH) is an independent risk factor for heart failure development (3), therefore diagnosing LVH in patients and subsequent therapeutic intervention are imperative for improving clinical outcomes.

The development of pathological cardiac hypertrophy is complex due to a multitude of underlying etiologies. Elevations in hemodynamic load stemming from arterial hypertension or valvular disease, as well as increased neurohormonal activation from catecholamines and vasoactive peptides (angiotensin II, endothelins), inflammatory cytokines, toxins, and cardiac injury from myocardial infarction and obesity, all impose increased wall stress on the myocardium. To compensate, the heart employs adaptive strategies to normalize wall stress and maintain contractile function by inducing cardiac hypertrophy and remodeling, leading to changes in the heart’s shape and size. Cardiomyocyte growth results from accelerated protein synthesis, and increased size is determined by reorganization of the sarcomeric structure. Depending on the hypertrophic trigger and duration of stress, circumferential enlargement occurs when sarcomeres organize in parallel, otherwise known as concentric remodeling, or they may increase in series causing myocytes to lengthen, referred to as eccentric remodeling. The latter is more commonly associated with ischemic injury. Although these changes are initially adaptive, cardiac hypertrophy left untreated may lead to either sudden death due to malignant arrhythmias, with abnormal automaticity and reentry as main causes (4), or ultimately progress to ventricular dilatation and heart failure, one of the leading causes of mortality worldwide.

At the cellular level, pathological hypertrophic growth is characterized by several molecular changes and activation of signal transduction systems. A set of fetal genes are re-induced, such as atrial and B-type natriuretic peptides (ANP and BNP) which function in a paracrine- and autocrine-like manner, regulating cardiovascular, renal, and endocrine homeostasis. They are released into the circulation as potent vasodilators and natriuretic agents, whereas in the heart, they exert anti-hypertrophic and anti-fibrotic effects (5). Contractile proteins are also upregulated such as α-skeletal actin, Acta1, and myosin heavy chain (MHC) β, which diminish contractility. On the other hand, several adult cardiac genes are downregulated, including the fast-contracting αMHC isoform and sarcoendoplasmic reticulum Ca\(^{2+}\) ATPase, SERCA. In addition to re-emergence of the fetal gene program, a plethora of molecular signaling pathways and transcriptional regulators are operative in the pathological sequelae of cardiac hypertrophy. Calcineurin-NFAT and PI3K/Akt/GSK-3-dependent signaling, G
protein-coupled receptors, MAPK and JAK/STAT pathways, MEK and downstream ERK1/2, TGFβ and downstream Smads, Na⁺/H⁺ exchanger, histone deacetylases, components of the renin-angiotensin (RAS) system, nitric oxide, oxidative signals, peroxisome proliferator-activated receptors (PPARs), transcription factors such as GATA4, Nkx-2.5, AP-1, NFAT, NFkB, MyoD, and Tbx5, as well as microRNAs have all been extensively reviewed (6,7).

Multiple cellular processes contribute to cardiac remodeling (Figure 1). Apoptotic myocyte death features less extensively in the early development of cardiac hypertrophy (8), but is hallmark of decompensated hypertrophy and further degeneration of the myocardium (9). Apart from myocyte hypertrophy and apoptosis, cardiac adaptation to stress also involves alterations in other cell types including fibroblasts of the extracellular matrix (ECM), endothelial cells comprising the microcirculation, as well as invasion of inflammatory cells, all of which progress in parallel with declines in cardiac function. Fibroblasts proliferate and are activated to become myofibroblasts, secreting collagens. The accumulation of collagens and glycoproteins among other structural proteins, and changes in regulation of ECM turnover by matrix metalloproteinases and their inhibitors, TIMPs, cause increased deposition and stiffening of the ECM, which collectively reinforce the ECM scaffold to support increased

![Figure 1](image_url)

**Figure 1**
**Mechanisms of pathological ventricular remodeling.** In response to pathophysiological stimuli such as ischemic injury or excessive mechanical load, multiple molecular and cellular processes contribute to ventricular remodeling. These include cardiomyocyte loss through cell death pathways, such as apoptosis. Cardiomyocytes undergo hypertrophy in response to both mechanical and neurohumoral triggers. Accumulation of excess extracellular matrix leads to fibrosis. Metabolic derangements, insulin resistance, and lipotoxicity can occur. Structural changes and alterations in ion transporting processes culminate in a pro-arrhythmic phenotype. Reprinted with permission from: J.S. Burchfield, M. Xie, J.A. Hill, *Pathological ventricular remodeling: mechanisms: part 1 of 2.* Circulation 2013;128(4):388-400.
myocyte mass. Further, hypertrophic myocytes reduce capillary density by decreasing the muscle-to-capillary ratio, and increased interstitial and perivascular fibrosis further restricts adequate perfusion of the myocardium, diminishing the supply of oxygen and nutrients required for myocyte growth and energetics. Capillary growth is enhanced in the early stages of adaptive cardiac hypertrophy, but insufficient neovascularization is prevalent in the chronic stage (10). Lastly, structural changes and alterations in ion transporting processes disrupt electrophysiological signals, culminating in arrhythmia (11).

Pathogenesis of cardiac hypertrophy also co-develops with altered myocardial metabolism, where the heart reverts to increased glucose utilization that is characteristic of the fetal heart. Glucose transporters are increased, whereas PPAR signaling is downregulated to suppress fatty acid oxidation. The switch to glucose is believed to be adaptive primarily because it decreases oxygen consumption per mole of ATP generated, rendering it a more efficient energy source than fatty acids. However, when the heart manifests decompensated hypertrophy and failure as a result of unremitted stress, myocardial metabolism becomes maladaptive. The precise causes and consequences remain unclear, but cardiac insulin resistance (12), impaired capacity for substrate uptake and utilization, lipid accumulation, and mitochondrial dysfunction and energetic insufficiency (13) are postulated to contribute to heart failure transition as the increased energy demands of the failing heart remain largely unmet.

From a clinical perspective, LVH has been identified as a relevant surrogate end point in clinical studies (14,15), and because of this strong association, clinical trials have chosen LVH as a primary end point, for example, the seminal PRIMO trial (16). Cardiac hypertrophy is also a valid target for therapeutic intervention. In the Heart Outcomes Prevention Evaluation (HOPE) trial, the ACE inhibitor ramipril decreased the development and caused regression of hypertrophy independently of its blood pressure-lowering effects, whereas persistence of cardiac hypertrophy predicted adverse outcome (17). In the Losartan Intervention For Endpoint reduction in hypertension (LIFE) trial, patients receiving losartan exhibited significantly reduced hypertrophy and were less likely to suffer a major cardiovascular event (18). Current pharmacological strategies are designed to reduce the load on the heart through antagonism of neurohormonal mechanisms such as renin-angiotensin-aldosterone inhibitors or beta-blockade. In addition, diuretics are prescribed to correct the increases in blood volume, therefore decreasing peripheral vascular resistance and the hemodynamic burden. Although these strategies improve overall morbidity and prognosis, heart failure nonetheless remains elusive. Therefore, new targets with promising therapeutic potential in cardiac pathophysiology need to be identified.

BIOLOGY OF LIVER X RECEPTORS

The oxysterol receptors, liver X receptor (LXR) α and β, belong to the 48-member nuclear
receptor superfamily which bind regulatory regions of target genes and activate gene transcription. LXRα and LXRβ are encoded by the NR1H3 and NR1H2 genes, respectively, and are highly conserved between humans and rodents.

Structure
Similarly to other nuclear receptors, LXRs are comprised of four canonical domains: (1) an amino-terminal ligand-independent activation function domain (AF-1), which may stimulate transcription in the absence of ligand, (2) a central DNA-binding domain containing two zinc fingers, (3) a hinge domain, binding co-repressors in the absence of ligand, and (4) a multifunctional carboxy-terminal domain that mediates receptor dimerization and contains a hydrophobic ligand-dependent transcriptional activation domain (AF-2), recruiting co-activators and stimulating transcription in response to ligand binding (Figure 2) (19,20). Both isoforms share approximately 78% amino acid sequence identity in both their DNA- and ligand-binding domains. The close homology in ligand-binding domains of LXRα and LXRβ poses challenges for developing highly selective agonists (21), yet the ligand-binding domain is very flexible, allowing compounds of highly different structures to bind (21).

Mode of action
Central to nuclear receptor biology is the direct mode of ligand-mediated regulation of the receptor and interaction with the genome. LXRα and LXRβ form obligate heterodimers with the retinoid X receptor (RXR) (22), which is a common partner for other nuclear receptors such as peroxisome proliferator-activated receptor (PPAR), vitamin D receptor (VDR), thyroid hormone receptor (TR), and farnesoid X receptor (FXR). The LXR/RXR complex is a “permissive heterodimer” that can be activated by ligands of either partner. Natural LXR ligands are a specific class of oxidized cholesterol metabolites, or oxysterols (23). The most studied and potent activators include 24(S)-hydroxycholesterol, 22(R)-hydroxycholesterol, and 24(S)-epoxycholesterol, and all activators analyzed are shown to activate LXRα and LXRβ
and with similar potency (24). The ligand for RXR is 9-cis retinoic acid. In the nucleus, the LXR/RXR complex is constitutively bound to liver X receptor response elements, or LXREs, in the DNA which consists of direct repeats (DRs) of a hexameric sequence, 5’-AGGTCA-3’, separated by four nucleotides (DR-4).

LXRs interact with a number of co-regulators that influence its DNA-binding and transcriptional activity. In the inactive state, LXRs recruit co-repressors such as nuclear receptor co-repressor (N-Cor) and silencing mediator for retinoic acid and thyroid hormone receptor (SMRT) (25), which inhibit transcriptional activity of target genes. In response to ligand binding, these co-repressors dissociate and are replaced by co-activators such as Grip1, a p160 co-activator (26), transcription domain-associated protein (TRRAP) (27), RIP140 which is specific for lipogenesis (28), and peroxisome-proliferator-activated receptor γ coactivator 1α (PGC-1α) (29) and PGC-1β (30). In the presence of co-activators, the LXR/RXR complex undergoes a conformational change to facilitate active gene transcription (Figure 3). Ligand-activated LXRs may also repress the transcription of certain genes that do not contain LXREs through antagonizing signal-dependent activation of pro-inflammatory transcription factors such as NFκB, STATs, and AP-1, a mechanism known as transrepression. These initial studies were performed in LPS-stimulated macrophages. It has also been postulated that transrepression is mediated instead via the LXR monomer through protein-protein interactions on gene promoters of inflammatory target genes (31).

![Figure 3](image_url)

**Figure 3**
**Mechanism of transcriptional regulation mediated by LXRs.** LXRs form a heterodimer complex with the retinoid X receptor (RXR), which binds to a LXR response element (LXRE), a direct repeat sequence separated by four nucleotides, in regulatory regions of target genes. In the absence of ligand, co-repressors maintain the complex in a repressed state. Following ligand binding to either LXR or RXR, co-repressors are replaced by co-activators, which results in activation of gene transcription.

**LXR regulation**
LXR activity and DNA binding is regulated by a complex interaction between ligands, co-factors, and posttranslational modifications. These factors may play distinctive roles in determining genomic binding sites, referred to as cistromes (32). LXR signaling may be regulated through changes in receptor expression, and moreover, LXRs are subject to their own regulation. Studies have demonstrated the existence of an autoregulatory loop
in controlling the expression of LXRα (33,34) where LXRα regulates its own promoter region. The human LXRα gene promoter contains three functional LXREs, one of which is activated strongly by both LXRα and LXRβ is reported to be autoregulated in human macrophages (35). Generally, the understanding of how LXRs are transcriptionally regulated is limited, and studies have implicated a role for micro RNAs (miRNAs), a class of highly conserved small, non-coding RNAs that regulate gene expression posttranscriptionally. LXRα has been shown to control its own transcription and expression via interaction with miRNAs: a novel feedback regulation between LXR and miR-206 exists where miR-206 activates LXRα-mediated pathways in cholesterol efflux, and LXRα autoregulates its own expression through miR-206 repression. Interestingly, the activity for miR-206 is cell-specific, acting as a repressor of LXRα signaling in liver cells, but activator in macrophages (36).

Posttranslational modifications involve attachments of small molecules to proteins, and they play an important role in regulating LXR activity, such as phosphorylation (37,38), acetylation and ubiquitination (39), SUMOylation (40), and O-GlcNAcylation (41). SIRT1 has been shown to deacetylate and positively regulate LXRα (39), SUMOylation plays an important role in mediating transrepression (40), and O-GlcNAcylation has been postulated to increase glucose sensing by LXRs (41).

Three different LXRα proteins have been reported (LXRα1-3) due to alternative splicing and differential promoter usage (42), and additional transcripts, LXRα4 and LXRα5, encoding variants in the ligand-binding domain (43) have been identified, suggesting that regulation of LXRα pre-mRNA splicing may be important in normal physiology and disease. Other
nuclear receptors also influence LXR signaling, including those which form heterodimers with RXR and block LXR signaling by competing with LXRs for the common dimeric partner. Also of note, the human LXRα gene contains a PPAR response element, or PPRE (33), and both PPARα and PPARγ agonists have been found to increase LXR expression (44,45).

**LXR expression**

LXRα and LXRβ appear to act in a functionally redundant manner to the same endogenous and synthetic ligands in several cellular responses; however, their tissue distribution differs considerably. LXRβ is expressed ubiquitously, and particularly high levels occur in the brain, whereas LXRα is restricted to tissues mainly involved in lipid metabolism such as the liver and adipose tissue (24). In the heart, both LXR isoforms display comparatively high expression levels with respect to other organs (Figure 4). At the cellular level, LXRs are located in both the cardiomyocyte and non-myocytic fraction, including fibroblasts and endothelial cells (46).

**LXR target genes**

LXRs have been most extensively studied in the liver, adipose tissue, and macrophages, and it is in these organs systems that most of their cognate target genes have been identified. The discovery of numerous gene targets represent a broad spectrum of biological processes subject to LXR regulation, such as modulation of cholesterol and lipid metabolism, glucose homeostasis, inflammation, and immunity. A list of known target genes are presented in the Table (47,48).
### Table. Direct Gene Targets of Liver X Receptors

<table>
<thead>
<tr>
<th>Process</th>
<th>Gene</th>
<th>Role</th>
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<tbody>
<tr>
<td>Cholesterol transport</td>
<td>ABCA1</td>
<td>Efflux from cells to apolipoproteins</td>
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<tr>
<td></td>
<td>ABCG1</td>
<td>Efflux from cell to apolipoproteins; transport</td>
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<td></td>
<td>ABCG4</td>
<td>Cellular transmembrane transport of lipids</td>
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<td></td>
<td>ABCG5</td>
<td>Entero-hepatic sterol absorption and excretion</td>
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<td></td>
<td>ABCG8</td>
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<td></td>
<td>ARL7</td>
<td>Transport</td>
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<td></td>
<td>APOE</td>
<td>Efflux</td>
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<td></td>
<td>IDOL</td>
<td>Lipoprotein receptor degradation</td>
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<tr>
<td></td>
<td>PLTP</td>
<td>Transfer of phospholipids from lipoproteins to HDL</td>
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<td></td>
<td>CETP</td>
<td>Transfer of cholesterol esters from HDL to lipoproteins</td>
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<td></td>
<td>LPL</td>
<td>Triglyceride hydrolysis</td>
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<tr>
<td></td>
<td>APOC1</td>
<td>Cofactor for LPL in triglyceride hydrolysis; inhibits CETP</td>
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<tr>
<td>Cholesterol uptake and modulation</td>
<td>SREBP1C</td>
<td>Fatty acid and triglyceride synthesis</td>
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<td>SCD1</td>
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<td>FASN</td>
<td>Long-chain fatty acid synthesis from acetyl-CoA</td>
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<td></td>
<td>APOC2</td>
<td>Activation of LPL activity</td>
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<td></td>
<td>ANGPTL3</td>
<td>Inhibition of LPL activity</td>
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<td></td>
<td>NR1H3</td>
<td>Autoregulation</td>
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<td>Fatty acid and triglyceride regulation</td>
<td>CYP7A1</td>
<td>Conversion of cholesterol to bile acid</td>
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<td>APOD</td>
<td>Lipid transport</td>
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<td>Adipose metabolism</td>
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<td>Fatty acid synthesis and lipogenesis</td>
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<td>Glucose metabolism</td>
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<td>STAR</td>
<td>Steroid hormone synthesis</td>
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<td>Immune and inflammatory responses</td>
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<td>Inhibition of apoptosis</td>
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<td>MERTK</td>
<td>Phagocytosis</td>
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<td></td>
<td>COX-2*</td>
<td>Inhibition of pro-inflammatory factor</td>
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<td>MMP9*</td>
<td>Extracellular matrix regulation</td>
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<tr>
<td></td>
<td>OPN*</td>
<td>Inhibition of pro-inflammatory cytokine, monocyte chemoattractant</td>
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<tr>
<td>Other</td>
<td>VEGFA</td>
<td>Angiogenesis and neovascularization</td>
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<tr>
<td></td>
<td>RENIN†</td>
<td>Regulation of extracellular volume and vasoconstriction</td>
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<tr>
<td></td>
<td>C-MYC†</td>
<td>Transcription factor activating growth-related hormones</td>
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*Indirectly regulated via NFκB antagonism. †Indirectly regulated via AP-1 antagonism. ‡Response mediated through a cis-acting DNA element (CNRE). ABC, ATP-binding cassette transporter; AIM, apoptosis inhibitor of macrophages; ANGPTL3, angiopoietin-like 3; APO, apolipoprotein; ARL, ADP-ribosylation factor like 7; ARG2, arginase 2; BP, blood pressure; CETP, cholesterol-ester transfer protein; COX-2, cyclooxygenase-2; CYP7A1, cytochrome P450 7A1; FASN, fatty acid synthase; GLUT4, glucose transporter 4; IL-6, interleukin-6; IDOL, inducible degrader of LDL receptor; INOS, inducible nitric oxide synthase; LPL, lipoprotein lipase; MERTK, mer receptor tyrosine kinase; MMP9, matrix metalloproteinase 9; NR1H3, nuclear receptor subfamily 1, group H, member 3; OPN, osteopontin; PLTP, phospholipid transfer protein; SCD, stearoyl CoA desaturase; SREBP1C, sterol-regulatory element-binding protein 1c; STAR, steroidogenic acute regulatory protein; VEGFA, vascular endothelial growth factor A.
AIMS OF THIS THESIS

We have previously demonstrated that pharmacological activation of LXR with the synthetic agonist, T09, attenuated the development of cardiac hypertrophy in a murine model of chronic abdominal aortic constriction, and moreover, this effect was absent in LXRα-null mice (49). Although this finding implicates the potential for a protective role of LXRs in hypertrophic remodeling, there are several caveats. First, T09 is associated with several severe side effects that confound these results, such as hepatic lipogenesis that leads to a fatty liver and elevated circulating triglycerides. Also, T09 is reported to decrease the elevations in mean arterial pressure (MAP) induced by aortic banding, suggesting a regulatory effect on blood pressure (49). Further, LXRs are anti-inflammatory mediators that are also capable of inhibiting inflammatory cell infiltration in susceptible organs (50,51) and the release of cytokines (52). The effect of LXR agonism on the heart may therefore be confounded by such extracardiac factors stemming from systemic LXR activation, and thus not reflective of intrinsic LXR activation within the myocardium. It remains to be determined whether LXR directly affects the heart. Studies employing LXRα knockout mice indicate that LXRα is the isoform conferring cardioprotective effects since neither activated (49) or unactivated (53) LXRβ was sufficient to rescue the hypertrophic or infarcted myocardium (54). To this end, we generated transgenic mice with cardiac-restricted LXRα overexpression in order to investigate the cardiospecificity of LXRα in pathological hypertrophy.

This thesis investigates the heart-specific effects of LXRα activation in pathological cardiac hypertrophy using a genetic and pharmacological approach. The main focus is on hypertrophic-associated remodeling, and we further explore metabolic mechanisms of LXRα.

In chapter 2, we review current evidence regarding the role of both systemic LXR activation and local LXR signaling in the heart, and discuss how LXRs may be a target in heart failure pathogenesis. In chapter 3, we describe generation of a transgenic mouse model with cardiac-specific LXRα overexpression, and also determine whether selective overexpression of LXRα in murine hearts exhibit a cardiac phenotype at baseline, or with aging. We further investigate the cardioprotective potential of constitutive LXRα activation from cardiac hypertrophy, adverse cardiac remodeling, and dysfunction when subjected to various perturbations, including chronic pressure overload and angiotensin II stimulation. The aim of chapter 4 is to test a novel high-affinity LXR agonist, AZ876, in the setting of adverse cardiac remodeling in a murine model of pathological cardiac hypertrophy. First-generation agonists such as T09 and GW3965 are associated with adverse lipogenic side effects, and they are also non-specific in that they indiscriminately activate other nuclear receptors such as farnesoid X receptor (55), pregnane X receptor (56), and retinoic acid receptor signaling (57). This is in contrast to AZ876, which is highly selective for LXRs (58). In chapter 5, we extend upon our observations in chapter 2 that LXRα appears to mediate alterations in
cardiac metabolism, rendering the heart more adaptive to hypertrophic stress. Therefore, we tested the consequences of cardiac LXRα activation in response to a metabolic challenge imposed by high fat diet (HFD)-induced obesity and insulin resistance. Finally, the aim of chapter 6 was to investigate metabolic markers in a clinical setting. Adiponectin is an adipokine which has established functions in lipid metabolism as well as insulin-sensitizing and anti-atherogenic properties. We therefore aimed to describe the relation between adiponectin and left ventricular function and remodeling in post myocardial infarction patients treated with metformin (data from the GIPS-III trial).
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