ROLE OF HYPERCHOLESTEROLEMIA IN OSTEOARTHRITIS DEVELOPMENT

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Submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

Institute of Health and Biomedical Innovation
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2017

Keywords

Articular cartilage chondrocytes, cholesterol, osteoarthritis, oxidative stress, mitochondria, hypercholesterolemia, apolipoprotein E-deficient (ApoE−/−) mice, animal model, subchondral bone

Abstract

The contribution of metabolic factors to the severity of osteoarthritis (OA) is not completely understood. In this study, apolipoprotein E-deficient (ApoE−/−) mice and diet-induced hypercholesterolemia animal models were evaluated to define the potential effects of hypercholesterolemia on the severity of OA. Under baseline conditions, hypercholesterolemic ApoE$^{-/-}$ mice and dietary rat model leads to OA like changes that are mainly characterized by a loss of proteoglycans content, collagen, and aggrecan degradation, synovial inflammation, osteophyte formation, changes to subchondral bone architecture, formation of subchondral bone lesions and eventual progression to cartilage degeneration and joint instability.

Following surgical destabilization/removal of the medial meniscus, the progressive degeneration towards OA was dramatically increased in the cholesterol fed ApoE-/- mice and dietary rat model compared with that in their wild-type littermates. We further show that clinically relevant doses of cholesterol cause mitochondrial dysfunction, reactive oxygen species (ROS) overproduction and increased expression of degenerative and hypertrophy markers that break down cartilage matrix in chondrocytes.

Furthermore, OA changes induced by hypercholesterolemia were attenuated with the treatment of statins (atorvastatin) and mitochondria targeted anti-oxidants (mito-TEMPO and MitoQ). The chondro-protective effect of atorvastatin and mito-TEMPO/MitoQ are largely associated with the suppression of oxidative damage and the restoration of extracellular matrix homeostasis of articular chondrocyte, suggesting their potential therapeutic efficacy.

Taken together, our data show that hypercholesterolemia, which can induce chondrocyte mitochondrial dysfunction, resulting increased ROS production and cell apoptosis, is one of the risk factors for OA progression. This study may provide the basis for development of novel treatments for this OA risk group.

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List of Abbreviations

ABCA1	ATP-binding cassette transporter
ACAN	Aggrecan
ACAT	Acyl coA: Cholesterol acyltransferase
ACCs	Articular cartilage chondrocytes
ADP	Adenosine diphosphate
ALP	Alkaline phosphatase
APAF-1	Apoptotic protease-activating factor-1
ApoE	Apolipoprotein E
ATP	Adenosine triphosphate
BAX	Bcl-2 associate X protein
BMI	Body mass index
BSA	Bovine serum albumin
CC	Calcified cartilage
CD	Control diet
CE	Cholesterol esters
CETP	Plasma lipid transfer proteins
COLII	Type II collagen
COLX	Type X collagen
DAB	Diaminobensidine
DMEM	Dulbecco's modified eagle medium
DNA	Deoxyribonucliec acid
ECM	Extra cellular matrix
EDTA	Ethylene diamine tetra acetic acid

ER	Endoplasmic reticulum
ERK1/2	Extracellular signal-regulated kinases 1 and 2
FBS	Foetal bovine serum
HCD	High cholesterol diet
HDL	High density lipoprotein
IBM	Inner boundary membrane
IL-6	Interleukin 6
JNK	c-Jun amino-terminal kinases
KDa	Kilodaltons
LDL	Low density lipoprotein
LXR	Liver X receptor
MAPKs	Mitogen activated protein kinases
MDA	Malondialdehyde
MMP	Matrix metallo proteinases
MPTP	Mitochondrial permeability transition pores
NADH	Nicotinamide adenine dinucleotide
NCC	Non calcified cartilage
nm	Nanometer
NPC1L1	Niemann-Pick C1 like1
NR1H3	Liver X receptor alpha (LXR-alpha)
OA	Osteoarthritis
OC	Osteocalcin
OCT	Optimal cutting temperature compound
OMM	Outer Mitochondrial Membrane
OMM	Outer mitochondrial membrane

OXPHOS	Oxidative phosphorylation
PBS	Phosphate buffered saline
PFA	Paraformaldehyde
PM	Plasma membrane
PPARs	Peroxisome proliferator-activated receptors
PTP	Membrane transition pores
qRT-PCR	Quantitative reverse transcriptase-polymerase chain reaction
RA	Rheumatoid arthritis
RCT	Reverse cholesterol transport
RNA	Ribonucleic acid
ROS	Reactive oxygen spices
ROS	Reactive oxygen spices
RT-PCR	Reverse transcriptase-polymerase chain reaction
RUNX2	Runt-related transcription factor 2
RXR	Retinoid X receptor
SAA	Serum Amyloid A
SCAP	SREBP cleavage-activating proteins
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
sGAG	Sulphated glycosaminoglycan
SOD	Superoxide dismutase
SREBPs	Sterol regulatory element-binding proteins
TEM	Transmission electron microscopy
TGF-β3	Transforming growth factor beta 3
TM	Tide mark
VLDL	Very low density lipoprotein

μm	Micrometer

List of Conferences and Abstracts

1. Hypercholesterolemia is a danger signal of increasing risk for osteoarthritis. Institute of Health and Biomedical Innovation Post Graduate Student Conference, Nov. 2014, Gold Coast, Australia - **Poster presentation**
2. Hyperlipidemia animal models exhibit and altered cartilage phenotype. Osteoarthritis (OA) Summit, Oct. 2014, Sydney, Australia – **Oral presentation**
3. Role of cholesterol in osteoarthritis development. Matrix Biology Society for Australia and New Zealand **(**MBSANZ), Oct. 2014, Queenscliff, Australia– **Poster presentation**
4. Hyperlipidaemia animal models exhibit and altered cartilage phenotype. Osteoarthritis Research Society International (OARSI) World Congress. April 2015, Seattle, US – **Poster presentation**
5. Hypercholesterolemia is a danger signal for increasing risk for osteoarthritis - role of mitochondrial dysfunction during this process. Institute of Health and Biomedical Innovation Post Graduate Student Conference, Nov. 2015, Brisbane, Australia - **Oral presentation**

List of Papers Submitted

1. Link between lipid, cartilage differentiation and disease, International Journal of Rheumatoid Disease (Accepted)

List of Publications

2. Prasadam, I., Farnaghi, S., Feng, JQ., Gu, W., Perry, S., Crawford, R., & Xiao, Y. (2013). Impact of extracellular matrix derived from osteoarthritis subchondral bone osteoblasts on osteocytes: Role of integrinβ1 and focal adhesion kinase signalling cues, *Arthritis Research and Therapy*, 15(5):R150. doi: 10.1186/ar4333.

3. Farnaghi, S. (2014). Hypercholesterolemia is a metabolic risk factor for osteoarthritis, *Osteoarthritis and Cartilage*, 22, S158 (Conference Paper)

4. Farnaghi, S., Mao, X., Crawford, R., Xiao, Y., & Prasadam, I. (2015). Hypercholesterolemia is a danger signal for increasing risk for osteoarthritis. *Osteoarthritis and Cartilage*, *23*, A296 (Conference paper)

5. Shi, M., Chen, Z., Farnaghi, S., Friis, T., Mao, X., Xiao, Y., & Wu, C. (2016). Copper-doped mesoporous silica nanospheres, a promising immunomodulatory agent for inducing osteogenesis. *Acta biomaterialia*, *30*, 334-344.

6. Farnaghi, S., Prasadam, I., Cai, G., Friis, T., Du, Z., Crawford, R., Mao, X. & Xiao, Y. (2016. Protective effects of mitochondria-targeted antioxidants and statins on cholesterol-induced osteoarthritis, The FASEB Journal, fj-201600600R.

Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

QUT Verified Signature

Signature:

Date: 06/02/2017

Acknowledgements

My PhD years at Queensland University of Technology and this thesis have benefitted from the mentorship of a number of outstanding individuals, both from within the university and outside of it. My heartfelt gratitude and thanks goes out to these individuals, for without their help, this thesis would have not been possible.

First and foremost, I would like to express my sincere gratitude to my principal supervisor, Professor Yin Xiao, for his continuous support during my PhD study and research, for his patience, motivation, enthusiasm, and immense knowledge. My heartfelt thanks go to my associate supervisor, Dr Indira Prasadam, for her constructive criticism and comments from the initial conception to the end of this work. Her support, knowledge, and assistance in the matter of academic concern is greatly appreciated, as it has helped me endure the many challenges during this period. I am also grateful to my associate supervisor, Professor Ross Crawford, for his support during this journey, especially his many stimulating clinical insights, as well as his many and frequent words of encouragement.

Special thanks go to Wei (Willa) Shi for helping me with my histology work. Her organisation and analytical abilities have impressed and inspired me tremendously.

I would like to thank all of my former and current Bone Group colleagues, with whom I have shared moments of both despair and excitement. Their presence during this journey, which at times has felt tremendously solitary, has been very important to me.

I would like to thank professional editor, Kylie Morris, who provided copyediting and proofreading services, according to the guidelines laid out in the university-endorsed guidelines and the Australian Standards for editing research theses.

To my family goes my deepest gratitude for their unflagging love and support throughout my life; this dissertation would simply be impossible without them.

I am indebted to my father for his care and love. I know of no one who is more proud than him of my achievements and who enjoys my success as his own. Deep

gratitude goes to my mother for her love and encouragement to concentrate on my studies. No words can sufficiently describe my gratitude to her. This thesis is dedicated to my parents.

Chapter 1: Introduction

1.1 INTRODUCTION

High fat intakes and inactive lifestyles are becoming most important concerns in today's modern world. They have led to a 'growing prevalence of obesity, hypercholesterolemia, high blood pressure, dyslipidemia, and a condition known as insulin-resistance syndrome or metabolic syndrome'. Metabolic syndrome is well known as ancestors to 'atherosclerosis, cardiovascular disease, and diabetes'. Some studies have reported that most of these conditions may also be associated with an increased risk of osteoarthritis (OA) (Bierma-Zeinstra & Koes, 2007; S. Dahaghin, Bierma-Zeinstra, Koes, Hazes, & Pols, 2007; Martins et al., 2006). To complicate matters further, these risk factors can reportedly interact with each other, worsening the disease rate and state. However, the majority of OA is caused by metabolic risk factors,which can be controlled, treated, or modified, including high blood pressure, obesity, lack of physical activity, cholesterol, and diabetes. Therefore, understanding the mechanisms by which these systemic factors change the course of OA should be an intense area of research to direct patient targeted therapies.

1.1.1. Cholesterol: A new contributor for initiating OA

The body's total cholesterol is derived via dietary intake and the 'cellular cholesterol biosynthetic pathway'. Most cell types, including the chondrocytes, have the capacity for cholesterol biosynthesis. Cholesterol is a vital constituent of cellular membranes, which controls signalling molecules with other intermediates of the cholesterol biosynthetic pathway. It has been reported that cholesterol is essential for 'chondrocyte differentiation and bone formation' (Gentili, Tutolo, Pianezzi, Cancedda, & Descalzi Cancedda, 2005). 'In chondrocytes there is tightly regulated expression of proteins playing a role in cholesterol metabolism'(Gentili et al., 2005; Zerega et al., 2004). For example, the expression of 'Apolipoprotein A-I (ApoA-1) and ATP-binding cassette transporter (ABCA1) is high in differentiating chondrocytes, and drops to the basal level once cells reach hypertrophy'. In contrast, 'hypertrophic chondrocytes express highest level of serum amyloid A (SAA) mRNA' (Gentili et al., 2005; Zerega et al., 2004). These results propose that the synthesis and

intake of cholesterol should be controlled to avoid from hyperlipidemia phenotype and abnormal accumulation of cholesterol in cartilage tissues.

People who are overweight or obese tend to have high cholesterol levels as insulin resistance makes fat metabolism changes. Whether cholesterol levels influence the occurrence of OA is a matter of debate. Epidemiological studies have determined different conclusions regarding the association beween OA and cholesterol. Some found a positive association between elevated serum cholesterol levels and OA (Al-Arfaj, 2003; M. A. Davis, Ettinger, & Neuhaus, 1988; D. J. Hart, D. V. Doyle, & T. D. Spector, 1995; K. Martin et al., 1997; Sturmer et al., 1998), whereas others found no direct correlation (Bagge, Bjelle, Eden, & Svanborg, 1991). Insterestingly, serum cholesterol and triglycerides were linked with the incidence of bone marrow lesions, which are common in OA patients (M. L. Davies-Tuck et al., 2009).

Abnormal cholesterol accumulation occurs when cholesterol influx into the tissue wall (from apoB-containing lipoproteins) exceeds cholesterol efflux. In a more recent study it was demonstrated that cholesterol transport genes such as ApoA-1 and ABCA1 mRNA levels were significantly lower in OA cartilage compared to normal. In addition, the two subtypes of the Liver X receptor (LXR), namely LXRα and LXRβ, mRNA levels were also found to be significantly lower in OA cartilage (A. Tsezou, D. Iliopoulos, K. N. Malizos, & T. Simopoulou, 2010). The differential expression pattern of these cholesterol efflux genes between normal and OA cartilage may signify a causal relationship of cholesterol related mechanisms to development and/or progression of OA. Recently, Gierman and co-workers published a study demonstrating a significant correlation between the development of OA and cholesterol exposure in an animal model, providing the first direct evidence of cholesterol involvement in OA (L. M. Gierman et al., 2013). In their study, they demonstrated that increased dietary cholesterol can cause OA development in a mouse model. They further showed that such an OA development can be inhibited by Atorvastatin, a member of the drug class known as statins (L. M. Gierman et al., 2013).

The next obvious question is how can cholesterol damage the cartilage? The mechanisms behind these observations are not fully understood, but there are number of ways that cholesterol can cause cartilage damage and these hypotheses are summarised below.

1.1.1.1. Potential common mechanism in developing hypercholesterolemia-induced OA and atherosclerosis

Current information suggests that cholesterol-induced OA development can be related to blood vessel dysfunction. This mechanism is similar to the condition leads to atherosclerosis in response to hypercholesterolemia (Conaghan, Vanharanta, & Dieppe, 2005; Katz, Agrawal, & Velasquez, 2010; Saleh et al., 2007). As cholesterol is a 'major constituent of the cell membrane, any alterations could affect fluidity and function of the membrane' so that they result in poor cell behaviour and cholesterol accumulation (N. Wang, Silver, Costet, & Tall, 2000). It is possible that hypercholesterolemia can result in the deposition and oxidation of lipids in tissues, causing damage to the cartilage similar to that seen in diseases like atherosclerosis (Sevin, Yasa, Akcay, Kirkali, & Kerry, 2013). A main contributing factor of the atherosclerotic lesion incidence possibly mirrors the imbalance between cholesterol influx and efflux regulation within the tissue (Schwenke & St Clair, 1993). 'The acquisition and evacuation of cholesterol is known to be mediated by a number of cell surface receptors, including the scavenger receptor class B type I (SR-BI), CD36 and ABCA1' (Ikonen, 2006). The earliest stage in reverse cholesterol transport is associated to ABCA1, 'a transmembrane protein mediating lipid efflux from cells to apolipoproteins (Apo)'. It has been suggested that 'ABCA1 plays a central role in cholesterol homeostasis and alterations in this gene were associated with atherosclerosis' (Rothblat, de la Llera-Moya, Favari, Yancey, & Kellner-Weibel, 2002; Soumian, Albrecht, Davies, & Gibbs, 2005).

'SR-BI, a member of the CD36 superfamily, which is mostly expressed in the steroidogenic tissues and liver, where it facilitates selective cholesteryl ester uptake from HDL' (Krieger, 1999; Marcil, Delvin, Sane, Tremblay, & Levy, 2006). SR-BI is also expressed in macrophages, including tissue macrophages, monocyte-derived macrophages, and macrophages in atherosclerotic lesions (Pagler et al., 2006; Rinninger et al., 2003). SR-BI deficiency is related to de-regulation of cholesterol homeostasis in the arterial wall that results in an increased susceptibility to atherosclerosis (Van Eck et al., 2003). 'CD36 has been well-known as binding and internalizing 'oxidized low density lipoproteins (oxLDL)' as well as a large number of ligands, such as 'anionic phospholipids, long-chain fatty acids and other modified LDL' (Adorni et al., 2012). CD36 identifies 'lipid moieties of oxLDL' and functions as a principal scavenger receptor in the oxLDL uptake (T. Q. Truong, Aubin,

Falstrault, Brodeur, & Brissette, 2010). Moreover, increasing evidence proposes that 'peroxisome proliferator activated receptors (PPARs)' (Barbier et al., 2002; Xia et al., 2012) utilize antiatherogenic effects via increasing cholesterol efflux through initiation of the 'liver X receptor (LXR)–ABCA1 pathway' (Bultel et al., 2008; Di et al., 2012; Hozoji-Inada, Munehira, Nagao, Kioka, & Ueda, 2011; Ouvrier et al., 2009). Therefore, it is possible that the same genes and molecular determinants could affect cartilage cholesterol homeostasis. This hypothesis is further supported by the study showing the expression of some of those genes in the cartilage tissue (A. Tsezou et al., 2010). In another view, Findlay et al. proposed that blood vessel alterations can leads to changed blood flow to the bone that underlies the cartilage in joints. Consequently, the bone and the cartilage are deprived of blood, oxygen, and nutrients that contributes to the whole tissue's degeneration (Findlay, 2007). As cholesterol is known to be a predominant factor that influences altered blood vessel function, it is possible that the above chain of events can lead to OA. However, cartilage and bone homeostasis are complex dynamic systems that involve biology, biomechanics and tissue structure. Morovere, other potentioal mechanisms, which influence this complex system, will disuss under the following sections.

1.1.1.2. Role of inflammation in cholesterol-induced OA

'Obesity and associated metabolic syndromes are related to systemic tissue damage and chronic low-grade inflammation' (Gregor & Hotamisligil, 2011). Continuous lipoproteins accumulation, primarily LDL, in the extracellular matrix of cartilage can trigger the inflammatory process. C-reactive protein (CRP) is a very sensitive marker of inflammation. The presence of inflammation in OA individuals were proposed by a finding demonstrated increased levels of CRP and IL-6 in OA groups. In this respect, the fact that CRP is associated with obesity is noteworthy. A positive association between circulating CRP has been shown in patients with obesity, metabolic syndrome, and OA. Interleukin-6 (IL-6) is a proinflammatory cytokine, which is produced by many cells, including chondrocytes. Studies have also shown increased levels of IL-6 mRNA in the OA specimens and IL-6 has been demonstrated to be the key stimulus for the CRP production. Furthermore, IL-6 appears to be related with 'visceral obesity and insulin resistance'. Recent studies clearly highlight the close link between inflammation and cholesterol homeostasis through mechanisms in which ABCA1 appears to be a major actor. For example, increased intracellular free

cholesterol concentration in ABCA1 KO macrophages is accompanied by enhanced inflammatory response in macrophages (Faggiotto & Ross, 1984; Faggiotto, Ross, & Harker, 1984; H. Li, Cybulsky, Gimbrone, & Libby, 1993).

Another molecule assumed to play a key role in lipid-induced inflammation is the 'transcription factor nuclear factor-κB (NF-κB)'. Its essential role is regulating inflammation and immune responses by activation of different genes, including those that encode cytokines, growth factors, and adhesion molecules. 'NF-κB is redox sensitive and activated by radicals'. A large number of different radicals may be generated by oxidation of lipoproteins, signifying that lipid-induced inflammation in the vascular wall can be mediated by the pro-inflammatory NF-κB activation by radicals drived from oxidized lipids. Indeed, induction of hypercholesterolemia in pigs, rabbits, mice, and many other animals leads to activation of vascular inflammation (Faggiotto & Ross, 1984; Faggiotto et al., 1984; H. Li et al., 1993).

1.1.1.3. Cholesterol activates oxidative stress

Mitochondrial dysfunction is known to be induced by hypercholesterolemia, hyperglycemia, hypertriglyceridemia, and even the process of aging (Lee & Wei, 2012; Puddu, Puddu, Galletti, Cravero, & Muscari, 2005). Numerous studies have shown an association between increased cholesterol oxidation products (oxysterols) and mitochondrion-derived oxidative stress, which typically leads to increased production of mitochondrial reactive oxygen species (mtROS) (Lordan, Mackrill, & O'Brien, 2009). Excessive and continuous accumulation of oxidised cholesterol particles tends to bind to 'sulphate-containing proteoglycans, where they aggregate and become oxidatively modified'. The responsible factors for these alterations are yet to be clarified, but in addition to ROS, different membrane and extracellular tissue-associated enzymes were reported to be involved. There are a number of defence mechanisms such as antioxidant vitamins and enzymes, which inhibit oxidative damage of lipoproteins accumulation, but in circumstances of a constant lipid overload they may finally not function, causing the activation of stress signalling pathways (Oliveira et al., 2005). ROS is typically produced under optimal conditions by mitochondria, and is neutralized by the mitochondrial cellular defence systems. When ROS is overproduced, it can induce deterioration of mitochondrial oxidative stress defences, cause irreversible harm at the cellular and molecular levels in vulnerable

cells, and contribute to pathologic conditions. ROS can also damage DNA, cellular lipids, and proteins, inducing lipid peroxidation, a condition that can more progress mtDNA impairment (Fosslien, 2001). These observations suggest a mechanistic explanation that accumulating free cholesterol causes increased superoxide anion levels, enhances oxidative stress, and activates a transient inflammatory response causing permanent damage to the tissue. It has been shown that increased ROS production, particularly, radicals and superoxide, has been related to cartilage dysfunction in human (Ostalowska et al., 2006) and animal models of disease (Johnson et al., 2004), and there is growing evidence of an association between oxidative stress and cartilage degradation in humans (Afonso, Champy, Mitrovic, Collin, & Lomri, 2007; Regan et al., 2005). Considering the role of cholesterol in triggering an oxidative stress environment, using a mitochondrial targeted anti-oxidant would be a novel therapy to suppress ROS overproduction leading to prevention of OA. However, whether the high cholesterol levels trigger the mitochondria dysfunction in cartilage requires further investigation.

1.1.1.4. Role of cholesterol lowering drugs in preventing OA

The growing list of interconnections between cholesterol and OA indicates that statins that inhibit de-novo cholesterol synthesis may have a disease modifying effect. In one study, Clockaerts et al., demonstrated that the use of statins is associated with a decrease in the overall progression of OA of the knee, but not of the hip in a population based study (Clockaerts et al., 2012). In addition to its effects on lipid metabolism, several anti-inflammatory effects have been ascribed to statins. It has been demonstrated that intra-articular injections of statin during OA progression decrease the infiltration of inflammatory cells and the expression of matrix-degrading enzymes, therefore restraining cartilage degradation (Akasaki, Matsuda, & Iwamoto, 2009). The same research group showed that intra-articular administration of HMG-CoA reductase inhibitor (e.g., statin) moderates cartilage degradation during OA development (Akasaki, Matsuda, Nakayama, et al., 2009). Statins can be capable to decrease the OA damage by several immunomodulatory effects on the joint. However, a few questions remain, and the most important regards to what extent cholesterol should be reduced to nullify any e off-target ffects and to maintain the levels required for cartilage homeostasis.

1.2 HYPOTHESIS

Hypercholesterolemia increases the risk of symptomatic features of OA through changes in mitochondrial related ROS pathways; therefore, lower cholesterol and targeted reduction of ROS may attenuate OA progression in this specific OA phenotype.

1.3 THE SPECIFIC AIMS OF THE THESIS

- ❖ To investigate the cholesterol distribution in cartilage of OA patients.
  - I hypothesised that higher cholesterol distribution may be a characteristic of O A cartilage compared to normal.
  - I also hypothesised that cholesterol regulatory genes may play a role in OA development.
- ❖ To study how abnormal cholesterol challenge alters the chondrocytes phenotype and mitochondrial function.
  - I proposed that cholesterol accumulation could change cartilage hemeostasis.
  - Mitochondrial dysfunction was suggested as one of possible mechanisms by which abnormal cholesterol alters chondrocytes phenotype.
- ❖ To study whether high cholesterol levels could lead to OA development in dietary rat and ApoE$^{-/-}$ mouse models.
  - I suggested that high cholesterol intake could contribute to OA initiation and/or progression in dietary rat and ApoE$^{-/-}$ mouse models.
- ❖ To investigate whether administration of and anti-oxidant Mito-tempo/MitoQ would be a novel alternative therapy to slow down cholesterol-associated OA progression.
  - I hypothesised that using anti-oxidant drugs would be a new theraputical drug to lessen OA progression caused by high cholesterol levels

1.4 THESIS STRUCTURE

This thesis consists of six chapters. **Chapter 1** introduces the topics and **Chapter 2** provides an extended literature review on the topics investigated. The main objective of this study is to build a new concept on a link between abnormal cholesterol levels

and OA pathogenesis at a cellular level. For this purpose, a series of molecular and cell biological methods were used to distinguish the changes in knee joints under a high cholesterol challenge. We conducted two animal model studies to determine how hypercholesterolemia affects the OA phenotype. In these studies, we used both dietary rats and ApoE knockout mice to prove the concept, and to test the therapeutical effect of atorvastatin and mito-TEMPO/MitoQ with respect to OA development. In **Chapter 3,** the methods and materials relating to each experiment are explained.

Chapters 4 specifically presents the results obtained from each experiment. Results from *in-vitro* and *in-vivo* experiments are shown in accordance to each aim of this study. **Chapter 5** provides a summary and draws conclusions of the studies in the former chapters to establish what new insights could been added to the understanding of the risk factors and biology of OA.

Chapter 2: Literature Review

2.1 OSTEOARTHRITIS

Osteoarthritis (OA), a degeradative joint disease, is described by degradation of articular cartilage, alterations in subchondral bone, synovial inflammation, and osteophyte formation. OA is the most common arthritis and may cause severe symptoms such as pain, dysfunction of the joint, and disability (D. T. Felson, Chaisson, C. E., Hill, C. L., Totterman, S. M., Gale, M. E., Skinner, K. M., ... & Gale, D. R, 2001). In people over age of 50, the increase in occurrence of severe OA is exponential (Lawrence, Bremner, & Bier, 1966). OA, can, though, affect young adults (Lowman, 1955). The condition affects approximately three million Australians, or approximately 15% of the population. People with OA are 2 to 3 times more likely to report poor health, very high levels of psychological distress and severe and very severe pain compared to those without the condition. The economic impact of OA on society is also significant. Based on Australian Institute of Health and Welfare (AIHW) disease expenditure data, $1.6 billion was attributed to osteoarthritis in 2008-09 (the most recent year for which data are available). According to the Australian National Hospital Morbidity Database, there were 103,763 hospitalisations in 2012–13 with a principal diagnosis of OA. Joint replacements surgeries, especially for hips and knees, are currently being performed to correct the defect. Moreover, there is no definitive drug for OA patients. The fact that a damaged joint is the common feature of OA patients with different sub-groups based on the risk profile has made developing a drug to slow or stopthe pathogenesis of OA the focus of researchers for decades ("Arthritis research foundation,").

2.1.1 Osteoarthritis pathogenesis

OA is a ‘whole joint’ disorder and its pathologic alterations happen in all tissues, including ‘articular cartilage degradation, osteophyte formation, subchondral bone thickening, synovial inflammation, and degeneration of menisci and ligaments’ (Andriacchi et al., 2004).

2.1.1.1. Cartilage in pathogenesis of OA

Macroscopically, healthy articular cartilage is a white to yellowish tissue covering the joint surface. The synovial fluid creates it smooth looking and delivers its low frictional properties. Macroscopically, OA cartilage is typically yellowish or brownish, usually soft, with presence of chipping and tiny cracks. The surface displays 'roughening in the early stages and obvious fibrillation and matrix loss in the later stages, up to the subchondral bone plate is visible'. These alterations can be observed and classified radiographically and can be studied in more detail on the histologic level. Cartilaginous outgrowths can frequently be found at the margins of joints (chondro-osteophytes). In severe cases, there is a total cartilage loss between the bones of the joints, creating high friction between the bones, leading to pain and limitation of joint mobility.

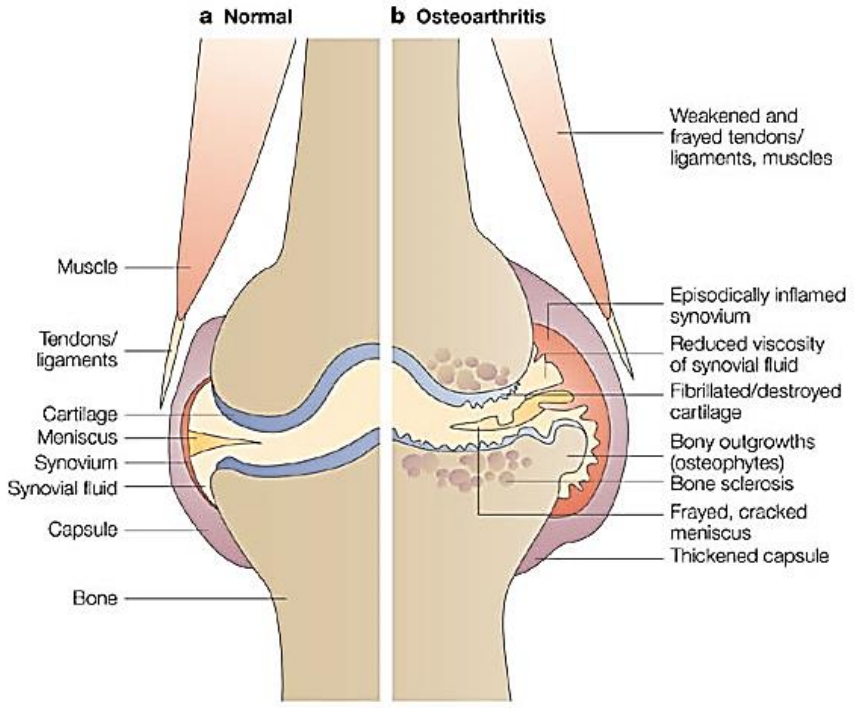

Figure 2.1: Normal versus OA articular structures. (a) Normal tissue without any cartilage fissures, synovial inflammation, and subchondral bone thickening. **(b)** OA tissue with central degenerate lesion and 'fibrillated' cartilage, as well as remodelling of bone and degeneration of meniscus (Heike A. Wieland, 2005).

Microscopically, the structure of articular cartilage is clearly classified as having four zones, the superficial zone, middle zone, deep zone, and calcified zone, which have different cell structures and matrix organizations (Lyons, Stoddart, McClure, & McClure, 2005; A. R. Poole et al., 2001). The majority of the superficial layer consists of collagen fibres and a few flattened chondrocytes arranged in a meshwork pattern. The middle layer contains chondrocytes that are larger, and the collagen fibres in this layer are randomly oriented. In the deep stratum, chondrocytes are arranged in vertical columns separated by collagenous fibrils. The last layer is called calcified cartilage as it contains partially mineralized matrix and hypertrophic chondrocytes and this zone shares many similarities with chondrocytes present in the hypertrophic zone of growth plate cartilage (H. J. Mankin, Buckwalter, J.A, 2000; Sun & Kandel, 1999). The calcified zone is separated from the deep zone of cartilage by the tidemark. The structure of the tidemark consists of 8-10 layers of collagen fibrils arranged as a thick bundle (Figure 2.2) (Doulabi, 2014; Lyons et al., 2005; A. R. Poole et al., 2001). Normal cartilage consists of evenly stained collagen and proteoglycan rich extracellular matrix with distributed cartilage cells. Chondrocytes present less than 5% of the total volume of cartilage but are obviously important for the preservation of the tissue structure (Aigner & Schmitz, 2011).

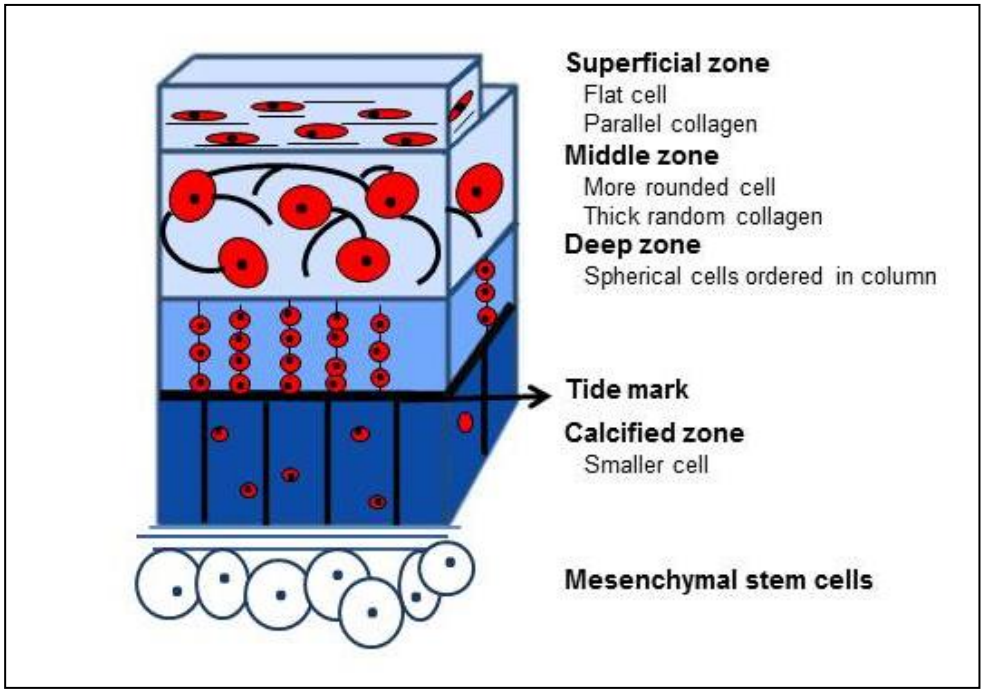

Figure 2.2: Modified microscopic healthy cartilage illustrating the zonal architecture and distribution of chondrocytes (Li & Aspden, 1997).

The microscopic changes that occur in OA cartilage are hallmark features of the disease. The surface indications are roughening in the early with obvious fissures and cracking, as well as matrix loss in the later stages until the subchondral bone plate becomes observable. In addition to the total destruction of the matrix, the degradation of matrix molecules also significantly contributes to preceding processesand final loss of the particular matrix regions (loss of Safranin O staining replicating the proteoglycans loss in affected cartilage). Destabilization of molecular structures also occurs in addition to the degradation of molecular components. For example, disruption of the collagen network leads to microscopically and eventually, macroscopically evident matrix damage (D. B. Burr & Radin, 2003; Hattori, Ikeuchi, Morita, & Takakura, 2005; B. Li & Aspden, 1997).

Generally, the grading of OA cartilage degradation is relatively difficult as all patients have, to at least some extent, distinct symptoms, morphologic changes, and histories. Apart from articular cartilage, several other tissues contribute to this process; however, the cartilage degradation has traditionally been contributes most to the scoring of OA severity (Figure 2.3). Overall, the joint destruction process can be assessed during the OA pathogenesis and the grading system most frequently used is 'the histochemical-histologic grading system' by Mankin and coworkers in 1971 (Table 2.1).

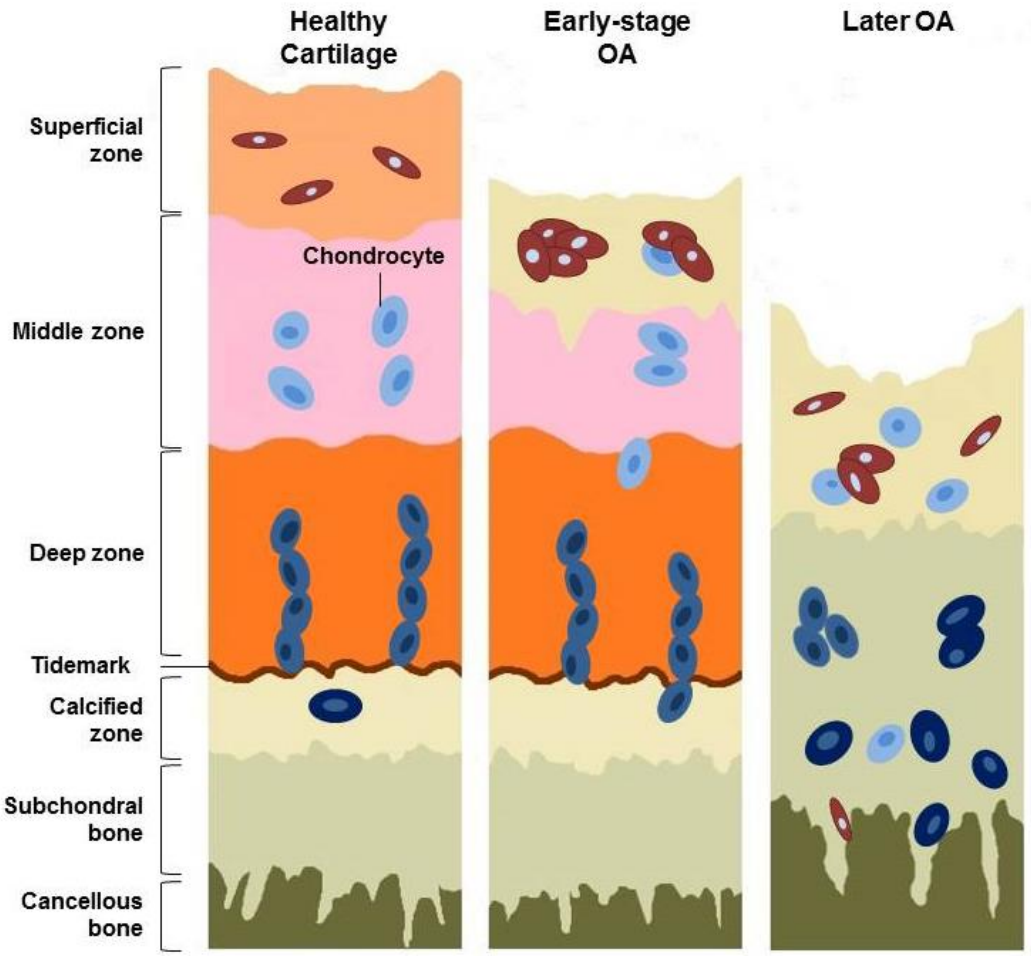

Figure 2.3: Modified histological scoring of cartilage during OA pathogenesis (Jiang & Tuan, 2015).

Table 2.1: Grading of OA cartilage according to the Mankin score (H. J. Mankin, Dorfman, Lippiello, & ZARINS, 1971).

Feature	Score	Histologic feature
Cartilage structure	0	Normal
	1	Superficial fibrillation
	2	Pannus and superficial fibrillation
	3	Fissures to the middle zone
	4	Fissures to the deep zone
	5	Fissures to the calcified zone
Chondrocytes	0	Normal
	1	Diffuse hypercellularity
	2	Cell clusters
	3	Hypocellularity
Safranin-O staining	0	Normal
	1	Slight reduction
	2	Moderate reduction
	3	Severe reduction
	4	No staining
	5	Total disorganization
Tidemark	0	Intact
	1	Tidemark penetrated by vessels

Articular cartilage is a flexible load bearing tissue that covers the surface of long bones at joint site (Freeman, 1973). Articular cartilage is an 'aneural, avascular, and alymphatic structure consisting of only one cell type called chondrocytes' (Buckwalter, 1997), which have different morphologies extending from more flattened at the surface to rounder and larger in the deeper zones (M. B. Goldring, 2012). Chondrocytes are specific spheroidal cells in charge of synthesizing and maintaining the matrix infrastructure (F. Chen, Frenkel, & Di Cesare, 1999). Articular cartilage contains principal building components such as water, collagen, and proteoglycans. Proteoglycans are responsible to maintain the electrolyte and fluid balance in the articular cartilage.

At the molecular level, chondrocytes in normal adult cartilage are stable and differentiated cells, which maintain tissue homeostasis by synthesizing very low levels of extracellular matrix components to preserve the structural integrity of the cartilage matrix. Normal chondrocytes produce different collagen types: mainly type II, type XI, and type IX, generating a 'fibrillar collagen network' that lures the large

aggregating proteoglycan, aggrecan, along with several other molecules such as small proteoglycans. On the other hand, two other collagen types, type X and type VI are not produced by all chondrocytes. Collagen type X is only produced by the hypertrophic chondrocytes in 'growth-plate cartilage or in articular cartilage near the tidemark and through the calcified cartilage' (M. B. Goldring, 2012).

At a molecular level in OA, the "activated" chondrocytes, described by 'cell proliferation, cluster formation, and increased production of both matrix proteins and matrix degrading enzymes' (Sandell & Aigner, 2001). Furthermore, OA chondrocytes are also capable of synthesizing typical terminal differentiated markers that point towards the fact that OA articular chondrocytes are undergoing differentiation towards hypertrophic phenotype (Iannone & Lapadula, 2008; O. Pullig, Weseloh, Ronneberger, Kakonen, & Swoboda, 2000). In agreement to this phenotype, it has been reported that OA chondrocytes express typical hypertrophic differentiation related markers, such as type X collagen (COLX) and runt-related transcription factor 2 (RUNX2) (Iwamoto et al., 2003; O. Pullig, Weseloh, Gauer, & Swoboda, 2000; O. Pullig, G. Weseloh, D. Ronneberger, et al., 2000). Given that chondrocyte hypertrophy is a feature of OA cartilage, it is likely that these phenotypic changes can increase the expression of degenerative enzymes in cartilage (Cheung, 2005).

Chondrocytes mainly produce these degradative enzymes in response to stimuli such as oxidative stress, injury, mechanical stress, cell-matrix interactions, and alterations in growth factor responses and matrices (Mary B Goldring & Marcu, 2009). Among proteinases that damage cartilage collagens and proteoglycans in joint disorder, aggrecanases and matrix metalloproteinases (MMPs) have been highlighted as they damage native collagens and proteoglycans (Cawston & Wilson, 2006; Rengel, Ospelt, & Gay, 2007). These consist of the collagenases MMP-13 and aggrecanases ADAMTS (ADAM with thrombospondin-1 domains)-5 as the primary degrading enzymes in cartilage (Arner, 2002; Glasson et al., 2005; G. Murphy & Nagase, 2008). Therefore, a shift in the phenotypic changes of articular cartilage to a chondrocyte hypertrophic phenotype can be destructive to the structure, health, and integrity of the cartilage, resulting in its degradation.

2.1.1.2. Subchondral bone in the pathogenesis of OA

Periarticular bone can be divided into two anatomic areas that consist of the subchondral bone plate and the subchondral trabecular bone. The subchondral bone plate contains of cortical bone, which is fairly nonporous and poorly vascularized. Subchondral bone is defined from the overlying articular cartilage by the zone of calcified cartilage (Mary B. Goldring & Goldring, 2010). The cell-mediated processes of remodelling and modelling are responsible for the maintenance of properties and architecture of the subchondral bone. Subchondral bone consists of different cell types, including osteoclasts, osteoblasts, and osteocytes. Osteoclasts mediate the remodelling cycle through bone resorption. Following the resorptive course, a bone formation phase is initiated by mediating osteoblasts, and the formation and resorption phases are regulated to preserve the bone mass in physiological conditions, while the architecture and shape of the bone might be altered (David B Burr, 2004; R. Martin, 2007). Apart from the impact of bone shape and architecture, the mechanical properties of bone are furthermore effected by the composition and organization of the organic phase of bone and the content and chemistry of the mineral phase (J. Day et al., 2001; Meunier & Boivin, 1997).

Modifications in the periarticular and subchondral bone that take place in OA may also play a role in cartilage pathology (Ruocco et al., 2005). However, it is unclear whether alterations in this tissue occur prior to alterations in the cartilage or whether subchondral bone modifications are secondary progressions after changes in the biomechanical properties of the overlying cartilage. The strong link between both is demonstrated by the fact that the cartilage marker and the bone marker were increased along with each other in patients with OA (H. L. Quasnichka, J. M. Anderson-MacKenzie, & A. J. Bailey, 2006; Helen L Quasnichka, Janet M Anderson-MacKenzie, & Allen J Bailey, 2006; Yamada, Healey, Amiel, Lotz, & Coutts, 2002). Radin and coworkers suggested that the integrity of the articular cartilage is dependent on the mechanical properties of the underlying bone (Radin & Rose, 1986). However, strong evidence for a key role of bone remodelling in the etiology has not been established. Several studies along with a previous study in our group have documented an increase of subchondral bone turnover and formation of bone spurs accompanied by specific architectural changes in the subchondral trabecular bone of OA joints (David B Burr, 1998; Goker, Sumner, Hurwitz, & Block, 2000; Karsdal et al., 2013;

Pastoureau, Leduc, Chomel, & De Ceuninck, 2003; Pelletier et al., 2004). Densification of subchondral bone in OA is a late event that includes only the calcified cartilage and subchondral plate; the subchondral cancellous bone underneath the subchondral plate may have lower bone density.. However, it has been reported that both early-stage bone loss and elevated remodelling, and the late-stage subchondral densification and reduced remodelling are significant features of the pathogenic procedure results in OA (David B. Burr & Gallant, 2012; Hunter & Spector, 2003; Lane, 2002). "The interrelated changes to biochemical and mechanical properties of the subchondral bone were consequently a challenge for delineating the molecular mechanisms underlying OA initiation"(Hayami et al., 2006). Recently, subchondral and trabecular bone mechanical loading in regards to muscle and ligament strenght has been proposed as a new pathway contributes to OA pathogenesis. Egloff et al. showed that muscle weakness resulted in significantly higher OA scores in the patellofemoral joint leading to significant cartilage damage in all four compartments of the rabbit knee (Egloff et al., 2014). In another study, peripheral quantitative computed tomography measurments reported great changes in bone mineral density after anterior cruciate ligament (ACL) reconstruction in trabecular bone wich may be associated with greater OA severity (Mundermann et al., 2015).

It has been radiographically demonstrated that the osteophyte formation and subchondral sclerosis may occur in OA joints of patients before alterations in thickness of articular cartilage measured as 'joint space narrowing' (Figure 2.4). These radiographic features are temporally separated, mainly as the bone's rich blood supply allows it to quickly react to changes in the joint, while a physical cartilage loss that happens at a late stage of the disease is required in advance to joint space narrowing (J. Buckland-Wright, Lynch, & Dave, 2000; J. C. Buckland-Wright, Macfarlane, D. G., & Lynch, J. A. , 1992; S. R. Goldring, 2012; Hudelmaier, Wirth, Nevitt, & Eckstein, 2013).

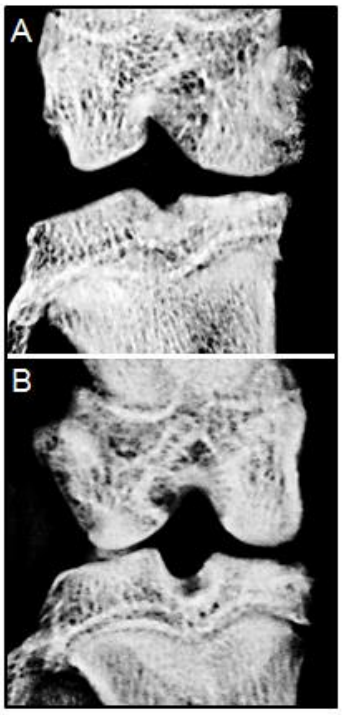

Figure 2.4: Radiograph of a normal versus OA rat knee. (A) Normal knee **(B)** OA knee showing joint space loss and an increase in subchondral sclerosis. The articular surfaces look flattened (C. Buckland-Wright, 2004).

At cellular level, it has been shown that the function of subchondral osteoblasts in production of inflammatory markers may be altered in OA patients compared to normal group (Massicotte et al., 2002). It has been reported that OA subchondral osteoblasts express higher levels of alkaline phosphatase (ALP) compared tothose from normal people (L. H. Truong, Kuliwaba, Tsangari, & Fazzalari, 2006). Similarly, osteocalcin (OC) expression was elevated at the protein level in OA subchondral osteoblasts (Gevers et al., 1989; Hilal, Martel-Pelletier, Pelletier, Ranger, & Lajeunesse, 1998). Moreover, it has been reported that the growth factors such as insulin-like growth factor (IGF)-1,2 and transforming growth factor (TGF)-β levels are upregulated in OA subchondral osteoblasts (Hilal et al., 2001). In a study, the levels of ALP, Transglutaminases, C-terminal type 1 procolagen propeptide, OC, interleukin (IL)-6,8 and TGF-β1 were reported to be significantly elevated in sclerotic OA subchondral osteoblasts compared to non-sclerotic osteoblasts (Sanchez et al., 2008). These findings together suggest that the OA subchondral osteoblasts have increased osteogenic capabilities and produce abnormal cytokine and growth factors compared

to normal osteoblasts. In addition, it has been suggested that this abnormal osteoblasts' behaviour can affect the overlying cartilage properties (Lajeunesse & Reboul, 2003). Indeed, the hypothesis that there is an interaction between subchondral bone osteoblasts changes and articular cartilage chondrocytes phenotype has been well-demonstrated previously in our group study (Prasadam, Crawford, & Xiao, 2010; Indira Prasadam et al., 2010).

2.1.1.3. Synovial membrane in the pathogenesis of OA

The synovial membrane, or synovium, is a membranous structure that lies between articular capsule and joint cavity. The main function of synovium is to provide nutrients to cartilage and produce lubricants (synovial fluid) that are essential for normal joint movement (Firestein, Budd, O'Dell, Gabriel, & McInnes, 2012). The normal synovial membrane consists of two distinct structures: (1) intimal lining layer, comprised of synovial lining cells; (2) Synovial sublining, which consists of connective tissue containing blood vessels, fibroblasts, adipocytes, and a certain number of monocytes/macrophages and mast cells (M. Smith et al., 2003).

In contrast to rheumatoid arthritis (RA), OA has long been described as a non-inflammatory disease due to the absence of neutrophils (a marker of classical inflammation) in the synovial fluid, especially in hand and knee OA (Vlad, Neogi, Aliabadi, Fontes, & Felson, 2011). Despite this, there is increasing evidence that patients with OA have varying degrees of acute or chronic synovial inflammation, both in early and late OA; and synovitis has been recognized as a common feature in OA patients (Sandell & Aigner, 2001). The common features of chronic inflammatory arthritis include inflammatory cell infiltration and thickening of the lining layer, which are caused by hyperplasia of the lining cells with a dense cellular infiltrate mainly consisting of lymphocytes and monocytes/macrophages through to a synovial membrane (D. Loeuille et al., 2005; M. D. Smith, Triantafillou, Parker, Youssef, & Coleman, 1997). Histological studies have shown that, in some cases, OA synovial inflammatory infiltrates of mononuclear cells were indistinguishable from infiltration observed in RA (Goldenberg, Egan, & Cohen, 1982; Lindblad & Hedfors, 1987; Revell, Mayston, Lalor, & Mapp, 1988). Synovitis and synovial inflammation can be defined histologically by the pattern of synovial changes (Figure 2.5). It seems the accumulation of monocytes/macrophage induces hyperplasia of the lining cells and the

subsequence development of synovial membrane inflammation. The presence of increased numbers of macrophages in synovium (human, OA patients), infra-patella fat pad (bovine model), and subchondral bone (human, OA patients) are detected both in early and late OA (Bastiaansen-Jenniskens et al., 2012; Benito, Veale, FitzGerald, van den Berg, & Bresnihan, 2005; Lisignoli et al., 1999). Bondeson and colleagues tested synovial cells from human synovial specimens and suggested that inflammation of the synovial membrane is one of the main contributors to cartilage matrix destruction, and it is driven by the release of cytokines through activated synovial macrophages (J. Bondeson, 2010).

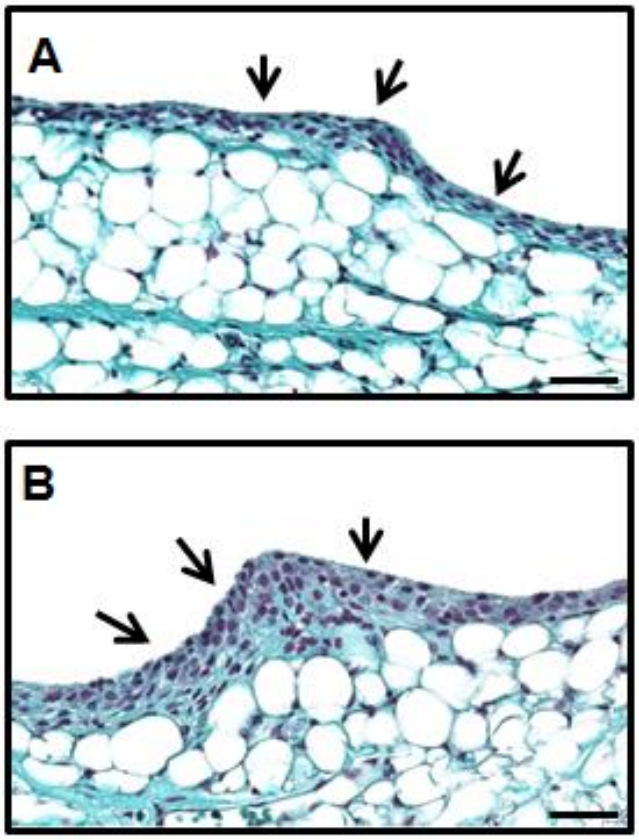

Figure 2.5: Microscopic features of the normal versus OA synovial membrane in mouse. (A) Normal synovial membrane composed of 1-4 layers of synovial lining cells, infiltration is moderate. **(B)** Hyperplasia of the lining cells with a dense cellular infiltrate (OA) (Damien Loeuille et al., 2005; Neuhold et al., 2001). The arrows point to the synovial membrane. Scale bar = 50 μm.

2.1.2 Animal models of OA

Animal model methods provide one pathway to investigate OA pathophysiology, develop 'disease-modifying' therapeutic agents and biologic markers for diagnosing and/or prognosis of the disease. OA is a heterogeneous disorder causing reduced joint function and pain because of a structurally damaged joint. Therefore, it is difficult to determine an optimal model system for such a heterogeneous condition and various models engaging different species are currently in use. These consist of spontaneous and induced (surgically, enzymatically/chemically (injection), mechanically, and genetically) models (A. Bendele, 2001). In all animal models, surgical methods of OA induction work through a combination of joint destabilization, altered articular surface contact forces, and intra-articular inflammation. These methods create models intended to represent post-traumatic OA. The surgical models include meniscectomy, anterior cruciate ligament (ACL) transection, and osteotomy. 'Intra-articular injection of mono-ido-acetate (MIA)' is another method of OA induction that act by stimulating intra-articular inflammation, direct matrix damage, and chondrocyte toxicity. These methods are useful for studying matrix degeneration, but are limited in that the bulk death of chondrocytes is not representative of either spontaneous or post-traumatic OA progression (Teeple, Jay, Elsaid, & Fleming, 2013). Mechanically-induced OA is commonly used, and is related to the possibility of inducing lesions of various severities by techniques that include the combinations of ligament transection and menisectomy in mouse knee joints and is suitable for detecting early-stage modifications in whole joint tissues (Cohen-Solal, Funck-Brentano, & Hay, 2013).

2.1.3 Different subtypes/phenotypes of OA

OA is a "heterogeneous" disorder with several risk factors and consists of various phenotypes that ultimately lead to mutual radiographic and clinical manifestations (Castañeda, Roman-Blas, Largo, & Herrero-Beaumont, 2013). Several categorizations have been performed based on the stage of disease, the main underlying mechanisms, involved joints, clinically relevant patient characteristics, and degree of inflammation (Haynes, Hume, & Smith, 2002; Herrero-Beaumont, Roman-Blas, Castañeda, & Jimenez, 2009; MacGregor, Li, Spector, & Williams, 2009; Scanzello et al., 2009). It has been proposed that the various patients with knee OA indeed consist of different phenotypes or subgroups (Driban, Sitler, Barbe, &

Balasubramanian, 2010; D. T. Felson, 2010). Recognizing different phenotypes of OA can be very important for treatment and prevention from disease development.

Classifying OA joints as primary and secondary OA has been disputed recently, as OA is "multifactorial", with intrinsic and extrinsic causes (K. D. Brandt, Dieppe, & Radin, 2008). Considering the significance of causative factors, it has been suggested that primary OA can be classified into three subsets: (1) type 1: genetically determined OA, (2) type 2: hormone-dependent OA, and (3) type 3: aging related OA (Herrero-Beaumont et al., 2009). Trauma and congenital or developmental diseases induce secondary OA, although inappropriate biomechanics has been regularly linked to primary and secondary OA. (Altman, 1986; Roach, Aigner, Soder, Haag, & Welkerling, 2007). Moreover, the extra-articular contributing factors (e.g., obesity, overloading of joints, etc.) also play a role through the OA progression (Figure 2.6). Apart from mechanical overload, changed adipokine levels also contribute to the OA development in overweight people. (D. T. Felson et al., 1997; Iannone & Lapadula, 2003). Recently, metabolic OA has been suggested as a new OA phenotype (Sellam & Berenbaum, 2013; Velasquez & Katz, 2010; Qi Zhuo, Wei Yang, Jiying Chen, & Yan Wang, 2012). The metabolic syndrome (MetS) frequently happens in the general aged population, and is related to numerous metabolic and non-metabolic abnormalities that possibly give rise to cardiovascular risk (Lakka, 2002). The worldwide prevalence of MetS ranges from <10% to 84%, depending on composition (sex, age, race, and ethnicity) and the region of the people studied, and the syndrome definition (Desroches & Lamarche, 2007; Kolovou, Anagnostopoulou, Salpea, & Mikhailidis, 2007). Generally, the International Diabetes Federation (IDF) estimates that one-quarter of the world's adult population has the MetS (IDF). However, E Bonora et al. reported that approximately one-third of examined individuals, who were randomly recruited from the general population, turned out to be affected by the syndrome when they applied the World Health Organization (WHO) criteria (Bonora et al., 2003).

Among different OA phenotypes, metabolic syndrome can be easily modified. However, little research has been undertaken to address the mechanism underlying these changes. Therefore, more evidence supporting the mechanism by which these systemic factors alter the course of OA could be useful in directing OA patient targeted therapies.

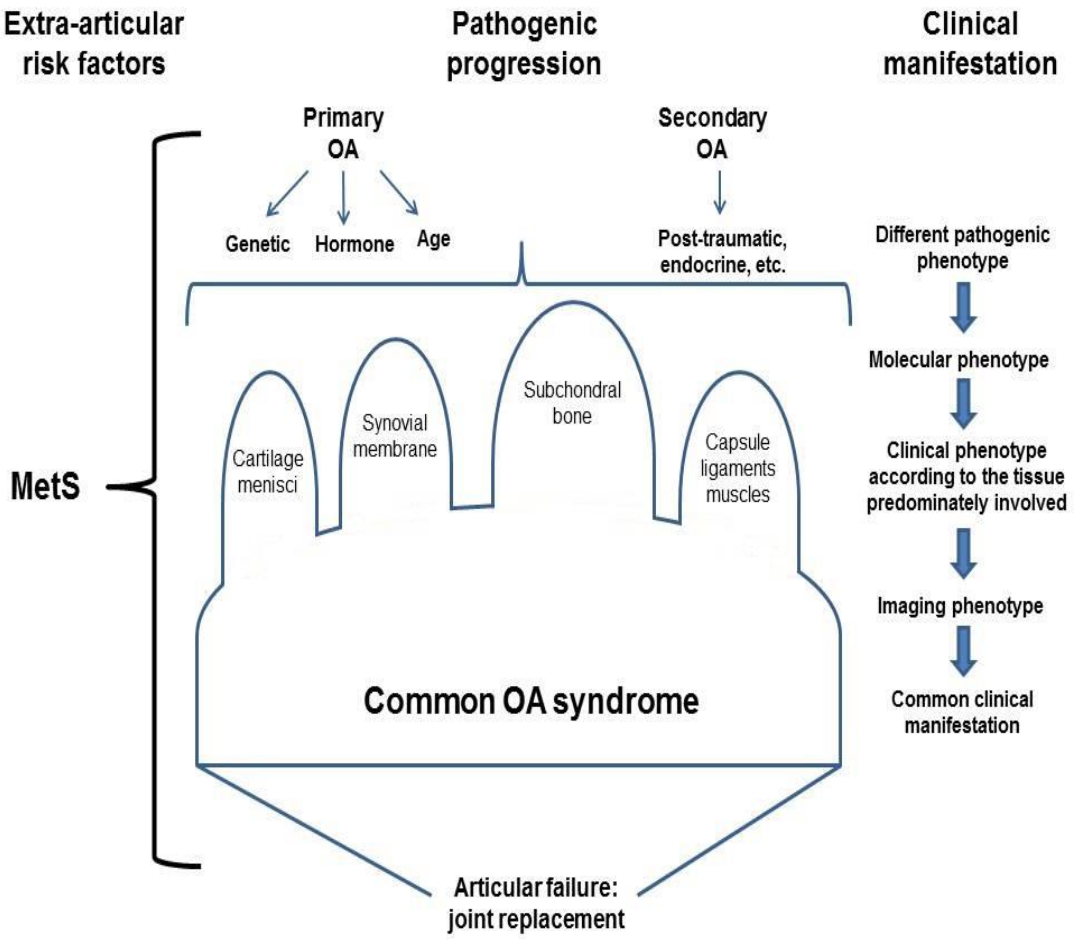

Figure 2.6: Adopted pathogenic and clinically integrated view of OA progression (Castañeda et al., 2013).

2.2 OSTEOARTHRITIS AND METABOLIC SYNDROME

A growing body of evidence suggests that OA is not simply a disease related to traditional well-known risk factors of joints, but rather a "metabolic disorder" (Puenpatom & Victor, 2009). Although the metabolic syndrome definition is rather undefined, as many criteria have been suggested, it is seen as a combination of conditions that enhance the cardiovascular risk, including hypertension, dyslipidemia, insulin resistance or diabetes, and obesity (Alshehri, 2010). The link between metabolic syndrome and OA may be looked from two different perspectives. OA can be related to either metabolic syndrome or each of its components (glucose, lipids, hypertension).

Among the metabolic syndrome components, obesity has long been documented as a governing condition for OA development. The effect of obesity on OA is found mainly in weight-bearing joints, such as the knee and hip, and has to some extent been linked to mechanical trauma related to excess body weight. Though, there is also evidence of a metabolic link between OA and obesity in non-weight–bearing joints, suggesting that factors other than weight play an important role (Felson & Chaisson, 1997; Grotle, Hagen, Natvig, Dahl, & Kvien, 2008; Woolf, Breedveld, & Kvien, 2006).

2.2.1 Obesity and incidence of OA in humans

Obesity is considered one of the most important risk factors for OA in knee(s). A systemic review and meta-analysis by Blagojevic et al. examined 36 papers reporting on body mass index (BMI) and reported that all demonstrated obesity and being overweight to be risk factors for knee OA (Blagojevic, Jinks, Jeffery, & Jordan, 2010). To summarize, many longitudinal studies have shown a strong association between obesity, defined as a BMI above 30, and radiographic knee OA, e.g., in the Framingham Study (D. T. Felson et al., 1997), the Chingford Study (Spector, Hart, & Doyle, 1994), the Baltimore Longitudinal Study of Aging (M. Hochberg et al., 1995), the John Hopkins Precursors Study (Gelber et al., 1999), and in longitudinal studies in UK (C. Cooper et al., 2000) and the Netherlands (Reijman et al., 2007). More recently, three large population based studies that documented the link between obesity and knee OA were published (M. Grotle, Hagen, Natvig, Dahl, & Kvien, 2008; L. S. Lohmander, de Verdier, M. G., Rollof, J., Nilsson, P. M., & Engström, G, 2009; Toivanen et al., 2010). Another recent study also found that there was a greater risk for knee OA with increasing duration of being overweight (Holliday et al., 2011). Thus, the World Health Organization (WHO) initiative on counteracting obesity also accepts OA as a consequence of obesity (Woolf, Breedveld, & Kvien, 2006). However, the link between obesity and OA in hip(s) and hand(s) are still debated (M. Grotle et al., 2008; Woolf et al., 2006) and a weaker association with hip OA has been reported (C. Cooper et al., 1998; M. Grotle, Hagen, K. B., Natvig, B., Dahl, F. A., & Kvien, T. K, 2008b). In another study, however, obesity was associated with more OA severity not only at the knee, but also at the hip, with earlier and more frequent hip replacement surgery in patients who had high body mass index (BMI) values (L. S. Lohmander, de Verdier, M. G., Rollof, J., Nilsson, P. M., & Engström, G, 2009). A strong link

between obesity and radiographic hip OA has been demonstrated in some cross-sectional studies (C. Cooper et al., 1998; Hartz et al., 1986; Heliövaara et al., 1993; Tepper & Hochberg, 1993; Vingård, Alfredsson, & Malchau, 1997), as well as in longitudinal studies (Järvholm, Lewold, Malchau, & Vingård, 2005; Oliveria, 1999; Vingård, 1991). In large longitudinal studies of Gelber et al. (Gelber et al., 1999) and Reijman et al. (Reijman et al., 2007) high BMI was related to hip OA. Moreover, large cross-sectional studies have not found a strong relationship between hand OA and obesity in either males or females (Bagge et al., 1991; M. A. Davis, Neuhaus, J. M., Ettinger, W. H., & Mueller, W. H, 1990; M. C. Hochberg, Lethbridge-Cejku, Scott, Plato, & Tobin, 1993), although some data have recognized that obese patients have a higher chance of developing hand OA (Carman, Sowers, Hawthorne, & Weissfeld, 1994; Oliveria, 1999).

Table 2.2: Summary of literature studying the association between obesity and OA in humans.

References	Study design	Results
D. T. Felson & Chaisson (1997)	Cohort	High body mass was associated with increased risk of radiographic OA and weight loss is directly associated with the decreased risk of OA development.
Spector et al. (1994)	Cohort	Obesity was an important factor correlated to OA incidence, 47% of women with high BMI had OA, compared with 10% in low BMI group.
M. Hochberg et al. (1995)	Cohort	Women and men in the highest MBI group had a significantly increased chance of developing both definite and bilateral knee OA.
Gelber et al. (1999)	Cohort	The incidence of knee, but not hip, OA was highly related to BMI index

		evaluated at ages 20 to 29 years and 30 to 39 years.
G. M. Cooper (2000)	Cohort	The risk of incident radiographic knee OA was significantly higher among individuals with higher BMI index after adjusting for age and sex.
Reijman et al. (2007)	Cohort	A high BMI was linked to incidence of knee OA, but not with incidence of hip OA. Similarly, a high BMI was correlated with knee OA progression. A significant link between hip OA progression and BMI was not found.
L. S. Lohmander, de Verdier, Rollof, Nilsson, & Engström (2009)	Cohort	All measures of being overweight were related with the incidence of knee OA, with the strongest relative risk gradient detected for BMI. The incidence of hip OA revealed smaller but significant differences between normal weight and obesity.
Toivanen et al. (2010)	Cohort	The risk of developing knee OA was significantly correlated with BMI after adjustment for age and gender and other variables, and compared with the normal BMI group (BMI < 25.0).
M. Grotle et al. (2008)	Cohort	After adjustment for age, gender, work status etc., a high BMI (> 30) was strongly related to knee OA and a dose-response association was found for this relationship. In addition, obesity was strongly associated with hand OA, but not with hip OA. There was no

		statistically significant interface effect between BMI and gender, age, or the other variables.
Holliday et al. (2011)	Case-control	BMI was related to knee and hip OA. Those who became obese earlier in adulthood displayed higher risks of lower limb OA.
C. Cooper et al. (1998)	Case-control	Obesity and previous hip injury were independent risk factors for hip OA among men and women.
M. C. Hochberg et al. (1993)	Cohort	Age was strongly correlated with hip OA; though, other sociodemographic factors, obesity, and fat distribution were not related to hip OA.
Vingård et al. (1997)	Case-control	The relative chance of hip OA incidence resulting in total hip replacement after the age of 50 was associated with an increased BMI.
Oliveria (1999)	Case-control	The body weight was a risk factor for the hand, hip, and knee OA incidence.
Järvholm et al. (2005)	Cohort	The relative risk for hip OA was more than two times greater in individuals with a BMI of 20-24 than in people with a BMI 17-19.

2.2.2 *In vivo* association between obesity and OA

Although obesity is noticeably linked to clinical OA, only a few animal models have been used to study the link between OA and obesity (Griffin & Guilak, 2008). These animals are usually rodent models that are either developed with high-fat diet or are overweight/obese inbred strains that spontaneously develop OA. Obese mouse

models on a high-fat diet, specifically the C57BL/6J strain, have been widely used as animal models to study human obesity (S. Collins, Martin, Surwit, & Robidoux, 2004). C57BL/6J on a high-fat diet showed more severe phenotypical changes similar to OA at an earlier age than C57BL/6J mice on a low-fat control diet (Griffin et al., 2010; Silberberg, 1950b, 1960; Sokoloff, Mickelsen, Silverstein, Jay, & Yamamoto, 1960). During these animal model studies, the prevalence of OA is comparable in males and females (Silberberg, 1950b), and interestingly a lard-based fat diet develop more severe OA than vegetable-based fat diet (Silberberg, 1960; Sokoloff et al., 1960). High-fat diet is also able to initiate OA-like changes in rats (Silberberg, 1960; Sokoloff et al., 1960). The STR/Ort mouse and the Hartley guinea pig, which are inbred strains of spontaneous OA, may be considered obese animal models of OA in comparision to other mouse and guinea pig strains, respectively (Mason et al., 2001; Sokoloff et al., 1960). Remarkably, a recent study has demonstrated that STR/ort mice have three chromosomal locations linked with body weight or fatty acid metabolism using quantitative trait studies (Jaeger et al., 2008). In another study, body weight control in the Hartley guinea pig by food restriction significantly decreased the severity of OA in the knee joints (A. M. Bendele & Hulman, 1991). It has been reported that C57BL/6J mice on high-fat or low-fat fed diets with significantly increased body weights compared to diet-matched mice showed OA-like changes, confirming an association between OA and body weight. However, mice on a low-fat diet who did not develop OA and mice on high-fat diet whodeveloped OA had comparable body weights, signifying that weight alone did not contribute to high-fat diet-induced OA (Silberberg, 1950a).

All of the studies using these animal models of OA have suggested that body weight is a factor that facilitates the progress of spontaneous knee OA. However, recent studies in obese mice have reported that leptin-impaired mice, which developed morbidity obesity, did not exhibit knee OA (Griffin, Huebner, Kraus, & Guilak, 2009). Moreover, mice on extremely high-fat diet (60% fat measured by kilocalories) developed knee OA, which was moderately improved by exercise, without any weight loss (Griffin, Huebner, Kraus, Yan, & Guilak, 2012).

This evidence suggests that adiposity (or weight) alone may not be a contributing factor for joint degradation, but that inter-relationships amongst diet, joint loading and

local or systemic inflammation may play a role in OA development (Guilak et al., 2004).

Table 2.3: Summary of literature studying the association between obesity and OA *in vivo* using animal models.

Authors	Animal model	Results
Silberberg (1950b)	C57BL/6J mice	The OA incidence in C57BL/6J mice fed a high-fat diet increased significantly two-fold with increasing age compared to animals fed a lower-fat control diet.
Silberberg (1960)	C57BL/6J mice	In male C57BL mice, a stock diet enriched with 25% cottonseed oil did not adversely influence the course of OA compared with animals fed a stock diet enriched with 25% lard.
Sokoloff et al. (1960)	DBA/2JN C57L/HeN, STR/N mice Osborne-Mendel rats	The highest fat content diet induced OA in two strains of mice and in the rat. Weight-gain induced with lower fat diets did not raise the OA incidence. DBA/2JN mice did not develop OA when fed the 60% vegetable fat diet.
Griffin et al. (2010)	C57BL/6J mice	Dietary-induced obesity in mice was associated with increased knee OA changes and impaired musculoskeletal force generation

		compared with controls. In addition, a high-fat diet caused symptomatic OA phenotype.
Jaeger et al. (2008)	STR/ort × C57BL/6J mice	Elevated weight, serum cartilage oligomeric matrix protein (COMP) levels and knee OA changes in the F2 generation compared to C57BL/6 parental animals confirm Mendelian inheritance. Quantitative trait analyses revealed three weight-, one serum COMP- and one OA-locus.
A. M. Bendele & Hulman (1991)	guinea pigs	The severity of the OA lesions was decreased by 40%, in combination with a 28% body weight reduction, in the diet-restricted group. These results point out that body weight in guinea pigs, as in humans, is a significant risk factor for knee OA development.
Griffin et al. (2009)	leptin deficient (*ob/ob*) and leptin receptor deficient (*db/db*) mice	Adiposity was increased approximately 10-fold in *ob/ob* and *db/db* mice compared to controls, but it was not related with more knee OA occurrence. In addition, serum cytokine levels were not altered in *ob/ob* and *db/db* mice compared to controls. Leptin impairment was correlated with decreased

		subchondral bone thickness and increased trabecular bone volume in the tibia.
Griffin et al. (2012)	C57BL/6J mice	Feeding mice with a very high-fat diet elevated knee OA scores. Wheel-running exercise decreased OA progression in the medial femur of obese mice.

2.2.3 How does obesity influence OA development?

The pathophysiology of obesity-induced OA is dependent on many different elements. The association between mechanical loading and the condition of the joint was highlighted in many *in vivo* studies, and proposed that the normal mechanical regulation of chondrocyte activity might be adversely affected by the presence of cytokines and pro-inflammatory mediators in the joint. Structural joint damage can occur due to mechanical factors, such as increased forces about the joint, decreased muscle strength, and changed biomechanics throughout daily activities (King et al., 2008; Runhaar, Koes, Clockaerts, & Bierma-Zeinstra, 2011), and metabolic elements, such as obesity also raise the risk of OA development in joints like the hands (M. Grotle, Hagen, K. B., Natvig, B., Dahl, F. A., & Kvien, T. K, 2008a; Oliveria, 1999; A. J. Teichtahl, Wluka, A. E., Wang, Y., Hanna, F., English, D. R., Giles, G. G., & Cicuttini, F. M, 2009).

2.2.3.1. Biomechanical mechanism

One obvious mechanism that obesity would contribute to the pathogenesis of OA is increased load and force on the weight-bearing joints as a consequence of obesity through additional mechanical load (D. T. Felson, 1996). These excess forces increase stress in articular cartilage and induce its final breakdown, which consecutively causes proliferation of periarticular bone and results in reduced joint space.

A recent study by Moyer et al. designated this linkage, presenting a link between dynamic knee joint loading and body weight (Moyer, Birmingham, Chesworth, Kean, & Giffin, 2010). Clinical and animal findings have demonstrated that excess loads can modify the configuration, structure, and mechanical properties of cartilage, resulting in the primary OA (Maly, 2005; Mündermann, 2005; Rejeski, Craven, Ettinger, McFarlane, & Shumaker, 1996; M. R. Sowers & Karvonen-Gutierrez, 2010). Gushue and colleagues completed a gait study and remarked that their "overweight group of children showed a significantly higher peak internal knee abduction moment in early stance" and that "these data suggest that overweight children may not be able to compensate for alterations in the frontal plane during gait, which may lead to increased medial compartment joint loads" (Gushue, Houck, & Lerner, 2005). In another study, a strong link between body weight and peak values of compressive forces, abduction moments , resultant forces, and the medial rotation moments of the knee has been found; each pound of weight loss leads to a four-fold decrease in load per step through regular activities (Messier, Gutekunst, Davis, & DeVita, 2005).

Recently, mechanoreceptors were found to exist on the surface of chondrocytes (A. J. Teichtahl, Wang, Wluka, & Cicuttini, 2008). Moreover, two studies also demonstrated that weight gain elevates the mechanoreceptors activation within the cartilage '(stretch-activated channels, a-5B1 integrin, CD44 on chondrocytes)' (Guilak et al., 2004; Pottie et al., 2006). It has been hypothesised that the mechanical stimulation of these mechanoreceptors might increase the expression of growth factors, cytokines, and metalloproteinases, generating mediators such as nitrous oxide and prostaglandins (Guilak et al., 2004). Although it has not been well-studied, these series of actions may result in extra oxidative stress, inflammation, and the onset of cartilage degradation (Pottie et al., 2006).

Another potential biomechanical mechanism that plays a role in the pathogenesis of obesity-induced OA involves muscle strength. Biomechanically, muscles and muscle forces strongly contribute in the load diffusion across the joint (M. R. Sowers & Karvonen-Gutierrez, 2010). It has been reported that obesity may lead to muscles weakness, which will possibly cause more stress on the articular cartilage, activating growing degradation of the joint (Segal, Zimmerman, Brubaker, & Torner, 2011). Amin et al. suggested that patients with stronger quadriceps have less degradative cartilage at the lateral compartment of the patellofemoral joint, along with less pain

and improved physical knee function at follow-up (Amin, 2009). Moreover, animal studies of muscle weakness suggested when muscle weakness was combined with a local inflammation there was a trend towards an increased rate of cartilage damage compared to muscle weakness alone (Egloff et al., 2014). Andersone et al. reported that non-invasive knee injury do not result in increased levels muscle weekness markers in injured mice compared to sham mice (Anderson et al., 2016). On the other hand, recent papers show that knee OA patients have the same (Meireles et al., 2016), or lower loading that healthy matched controls (Adouni and Shirazi-Adl, 2014; Wellsandt et al., 2016).

2.2.3.2. Systemic inflammatory mechanism

Another mechanism that plays a role in the pathophysiology of obesity-induced OA is systemic inflammation. Although clinically untreated RA has been well-known to stimulate the body's inflammatory system, it has recently been proposed that untreated OA also shows clinical and joint tissue inflammatory response. Adipose tissue, which was believed to beonly an energy store, is an endocrine organ that is able to secrete cytokines and cytokine-like molecules termed adipokines (Pottie et al., 2006; M. R. Sowers & Karvonen-Gutierrez, 2010). Therefore, finding that stimulated macrophages inside adipose tissue release cytokines and adipokines influences other cells and tissues through the body led to new characterization of obesity as a mild, chronic inflammatory disorder.

Twospecific adipokines, leptin and adiponectin, are believed to be the most important molecules playing a role in the association of obesity and OA, andhave been the focus of more recent studies (Weisberg et al., 2006; Weisberg et al., 2003; Xu, 2003). It has been suggested that dysregulated adipokines levels in obese individuals might be mostly key mediators, as a biochemical environment results from obesity makes chondrocytes unable to respond to such challenges. As an example, chondrocytes from obese OA patients have been demonstrated to respond differently to leptin levels compared with normal or overweight patients (Stéphane Pallu, 2010). In another study, it has been shown that people with OA exhibit strongly increased levels of leptin and adiponectin than controls (de Boer et al., 2012); however, notable weight loss in obese individuals with knee OA showed reduced circulating levels of leptin, but an increase in adiponectin levels (Richette et al., 2011).

These findings suggest that the very high level of leptin in obese individuals have directed researchers to propose a leptin resistance syndrome as a similar concept to insulin resistance. Consistently, body mass is directly related to the level of leptin in OA people. Moreover, it has been identified that leptin and its receptor, which is present in human chondrocytes, osteophytes (Dumond et al., 2003; Gegout, Francin, Mainard, & Presle, 2008), synovium, and infrapatellar fat pad (Presle et al., 2006), might influence growth factor metabolism and regulation. Analysis of cartilage, subchondral bone, and osteophytes evidence that increased leptin expression is highly linked to the grade of cartilage degradation (Dumond et al., 2003; T. Simopoulou et al., 2007) and it has been reported that leptin and proinflammatory cytokines have a synergistic effect (Dumond et al., 2003). The direct pro-inflammatory and catabolic role of leptin in cartilage homeostasis was further confirmed when chondrocytes from OA patients, which stimulated with leptin, showed increased IL-1b, MMP-9, and MMP-13 levels (T. Simopoulou et al., 2007), suggesting leptin or its receptor as a new therapeutic target. Leptin may also contribute to the OA development through peripheral and central mechanisms that regulate bone mass (Ducy et al., 2000; Kishida, 2005). In *ob/ob* mice, leptin deficiency lead to a mosaic bone mass phenotype with increased bone mass in the axial skeleton and decreased bone mass in the appendicular skeleton (Hamrick, Pennington, Newton, Xie, & Isales, 2004). In another animals study, the lack of the leptin gene, as perceived in *ob/ob* mice, elevated hypertrophic chondrocyte apoptosis and weakened endochondral ossification (Kishida, 2005).

While there is growing evidence highlighting the association between leptin and OA, the functional role of adiponectin and joint damage, with both reported anti-inflammatory and pro-inflammatory properties has been less investigated (Gomez et al., 2011; Tilg & Moschen, 2006). It has been reported that adiponectin induces metalloproteinase 1 expression in synovial fibroblasts in OA patients, signifying a potential catabolic character in cartilage homeostasis (Pottie et al., 2006). Conversely, another study has reported a possible protective role against OA for adiponectin (M. R. Sowers & Karvonen-Gutierrez, 2010). Therefore, further research will be needed to clarify the roles of these adiponectin in the OA pathogenesis.

2.2.3.3. Metabolic–related mechanism

Apart from adipocytokines, obesity can cause other systemic impacts associated with OA, such as roles for dysregulated glucose and lipid metabolism (Manuel T. Velasquez 2010). Consequential metabolic alterations from insulin resistance and elevated glucose levels are closely associated with the production of pro-inflammatory cytokine, which is a typical feature of a chronic inflammation (Tamakoshi et al., 2003). Moreover, the generation of advanced glycation end products (AGEs), i.e. substances that can be a factor in speeding up oxidative damage to cells and in changing their normal behaviour under metabolic syndrome can be linked with changes in the mechanical properties of the cartilage extracellular matrix, increased collagen stiffness, and reduced proteoglycan synthesis, thus probably leading to cartilage degradation (DeGroot, 2004). Remarkably, chondrocytes express the functional receptor for AGEs, which induces production of pro-inflammatory cytokines via stimulation with ligands (Loeser et al., 2005).

These data propose that systemic metabolic change is a potential mechanism playing a part between obesity and OA. In a review study Sowers & Karvonen-Gutierrez have summerized supporting evidence of metabolic relationship between OA and obesity (M. R. Sowers & Karvonen-Gutierrez, 2010). A recent study reported that mid-aged women who had BMI $\geq$30 kg/m^2 (characterized as obese) and had two or more cardiovascular risk factors had a more than six times chance of having knee OA compared to non-obese females without cardiometabolic clustering. Cardiometabolic risk factor clustering includes the prevalence of two or more of the following factors: 'HDL-c, LDL-c, triglycerides, blood pressure, waist: hip ratio, glucose, and C-reactive protein'. (M. Sowers et al., 2009).

In a massive cohort study of the general US population, the incidence of metabolic syndrome was higher in OA patients even after adjustment for BMI and age (Puenpatom RA, 2009). A Japanese ROAD study found that the prevalence of having more metabolic-syndrome components was related to an accelerating risk of knee OA (Yoshimura et al., 2012). In another study, the incidence of obesity and at least two other metabolic-syndrome components in women was linked to an greater risk of knee OA compared to obesity itself (M. Sowers et al., 2009). The same synergistic results for hand OA were reported for accumulation of hypertension and diabetes over obesity (S. Dahaghin, Bierma-Zeinstra, S. M., Koes, B. W., Hazes, J. M., & Pols, H. A, 2007).

Radiological evidence has suggested that hypertension, hypercholesterolemia, and blood glucose are related to the incidence of knee OA independent of obesity supporting the conception that consequential systemic and metabolic component contribute to OA etiology (DEBORAH J Hart, DAVID V Doyle, & Tim D Spector, 1995). Interestingly, it has been demonstrated that each of the components of metabolic syndrome may similarly be linked with OA (Qi Zhuo et al., 2012), although available data are rather inconsistent (Qi Zhuo et al., 2012). Recently, Berenbaum et al., reviewed papers studying the role of diabetes in the initiation of OA and showedthere were strong relationships between diabetes and OA from *in vivo* and epidemiological studies. However, they suggested more *in vitro* and *in vivo* experimental models were needed to obtain a better understating of the underlying mechanism (Berenbaum, 2012). Back to the last century, the correlation between lipid abnormalities and OA was raised by the presence of increased levels of serum cholesterol in OA patients (DEBORAH J Hart et al., 1995; T. Stürmer et al., 1998). The levels of total fatty acids and arachidonic acid from OA cartilages have been shown to increase with greater severity of cartilage damage (Lippiello, Walsh, & Fienhold, 1991). Moreover, in OA cartilage, decreased expression of genes regulating for cholesterol uptake was detected (Aspasia Tsezou, Dimitrios Iliopoulos, Konstantinos N Malizos, & Theodora Simopoulou, 2010); althought; more *in vitro* and *in vivo* models are required to establish this correlation. As metabolic disorders have recently been regarded as new risk factors for OA, it is important to study the effect of each component of the syndrome (glucose, lipids, and hypertension) on joint tissues and cells. The most important concern resulting from current data is that few studies have investigated the effect of each MetS component independent of obesity. Such a component is hypercholesterolemia, which has been shown as possibly existing in non-obese individuals with normal BMI (Giltay et al., 1998; Vikram et al., 2003). Although hypercholesterolemia's role in the pathogenesis of different diseases is well-known, more confirmatory *in vivo* evidence is required to establish the role as a risk factor in the development of OA and for potential treatment options.

Table 2.4: Summary of literatures studying association between MetS and OA.

Authors	Study design	Results
M. Sowers et al. (2009)	Cohort	The knee OA prevalence in non-obese women without cardiometabolic clustering was 4.7%, compared to 12.8% in obese women without cardiometabolic clustering and 23.2% in obese women with cardiometabolic clustering. Non-obese women without cardiometabolic clustering were less likely to perceive themselves as limited compared to women in all other obesity/cardiometabolic groups.
Puenpatom RA (2009)	Cohort	Each of the five cardiovascular risk factors that comprise MetS was more prevalent in the OA population versus the population without OA. Metabolic syndrome was more prevalent in subjects with OA regardless of sex or race. The association between OA and MetS was greater in younger subjects and diminished with increasing age.
Yoshimura et al. (2012)	Cohort	Logistic regression analyses after adjusting for possible risk factors showed that the knee OA occurrence strongly increased according to the number of MetS

		components present. In addition, progression of knee OA significantly increased based on the number of MetS components present.
S. Dahaghin, Bierma-Zeinstra, S. M., Koes, B. W., Hazes, J. M., & Pols, H. A (2007)	Cohort	The association between diabetes and hand OA was only present in people aged 55–62 years, but was absent in the total population or in other age groups. The association of hypertension with hand OA was weak, and disappeared when BMI was adjusted. There was no significant association between total/HDL cholesterol ratio and hand OA. The concurrent presence of overweight, diabetes and hypertension resulted in an even higher prevalence of hand OA compared with people without these conditions; this occurrence was higher in the youngers.
DEBORAH J Hart et al. (1995)	Cohort	There were significant associations between bilateral knee OA and hypertension and both high and moderately elevated serum cholesterol. No correlation was found with elevated triglyceride or

		HDL levels or with current systolic blood pressure.
Berenbaum (2012)	Review paper	A number of epidemiological and experimental data support the hypothesis that diabetes might be a self-governing risk factor for OA bringing on the conception of a diabetes-induced OA phenotype.

2.3 CHOLESTEROL

Cholesterol is required by all mammalian cells for growth and maintenance and it functions as a stabilizing component of the bilaminar membranes of cells. It is also a component of the myelin sheath of nerves, a modified plasma membrane (PM), and is present in all plasma lipoproteins. The cholesterol in tissues and plasma may be in free (unesterified) or esterified form; however, most of the total cholesterol in the animal body is present as free cholesterol (Myant, 1990). Cholesterol also plays a role in intracellular transport, cell signalling and nerve transmission within the cell membrane (Hanukoglu, 1992; Simons & Toomre, 2000).

There are the two potential sources of cholesterol in the organism: diet and synthesis. From a dietary perspective, cholesterol is noticeably found in animal fats, which are complex combinations of triglycerides. Therefore, all foods with animal fat contain cholesterol with different ranges (Christie, 2010). The cholesterol present in a specific tissue has either been synthesised *de novo* in the cells of that tissue, or was derived from circulating lipoprotein cholesterol. Approximately 20–25% of total daily cholesterol production takes place in the liver, while the reproductive organs, adrenal glands, and the intestines are other sites of high synthesis rates.The liver converts carbohydrates and fatty acids into triglycerides, which it packages into lipoproteins to deliver to tissue. The liver uses dietry source of sterol for lipoprotein synthesis, when that is available. When dietary cholesterol is inadequate, the liver synthesizes its own cholesterol (Clayton, 1998).

2.3.1 Cholesterol biosynthesis

All cells produce cholesterol with corresponding manufacture rates for their use, differing by cell type and organ function. Cholesterol is derived from acetyl-CoA through the isoprenoid pathway in endoplasmic reticulum (ER) (Horton, Goldstein, & Brown, 2002). Most cellular cholesterol is produced from *de novo* synthesis in extrahepatic tissues (Dietschy & Turley, 2001), while hepatocytes (liver cells) find most of their cholesterol by receptor-mediated uptake of plasma lipoproteins including low-density lipoprotein (LDL). Following internalization of LDL by the LDL receptor, it is transported to lysosomes through the endocytic pathway, where hydrolysis of the core cholesteryl esters (CE) occurs. 'Cellular cholesterol levels are also controlled by a cycle of cholesterol esterification mediated by acyl-CoA:cholesterol acyltransferase (ACAT) and hydrolysis of the CE, by cholesterol metabolism to bile acids and oxysterols, and by cholesterol efflux' (Liscum, 2008).

Figure 2.7 provides more detail regarding the cholesterol biosynthetic pathway. Sterols are manufactured from the two-carbon building block, acetyl-CoA. The acetyl-CoA and acetoacetyl-CoA, which are converted to each other by soluble enzyme acetoacetyl-CoA thiolase, condensed later by 3-hydroxy-3-methylglutaryl (HMG)-CoA synthase to procedure HMG-CoA. Using two molecules of NADPH, HMG-CoA reductase catalyses the reduction of HMG-CoA to mevalonate. HMG-CoA reductase is the rate-determining enzyme of the cholesterol biosynthetic pathway and, like HMG-CoA synthase, is tightly controlled by the source of cholesterol. This is the regulated, rate-limiting and irreversible step in cholesterol synthesis and is the site of action for the statin drugs (Panini, 1993; Vance & Vance, 2002).

Mevalonate is decarboxylated to isopentenyl pyrophosphate, which is a main metabolite for several biological responses. Three molecules of isopentenyl pyrophosphate condense to make farnesyl pyrophosphate via the action of geranyl transferase. Two molecules of farnesyl pyrophosphate then condense to generate squalene by means of squalene synthase. Oxidosqualene cyclase then mediates squalene for the production of lanosterol. Lastly, cholesterol results from lanosterol through a series of oxidations, reductions, and demethylations (Kim, 2001; Vance & Vance, 2002). Apart from endogenous cholesterol biosynthesis, the exogenous source of cholesterol that comes from external dietary intake is another important proportion of circulating lipoprotein cholesterol.

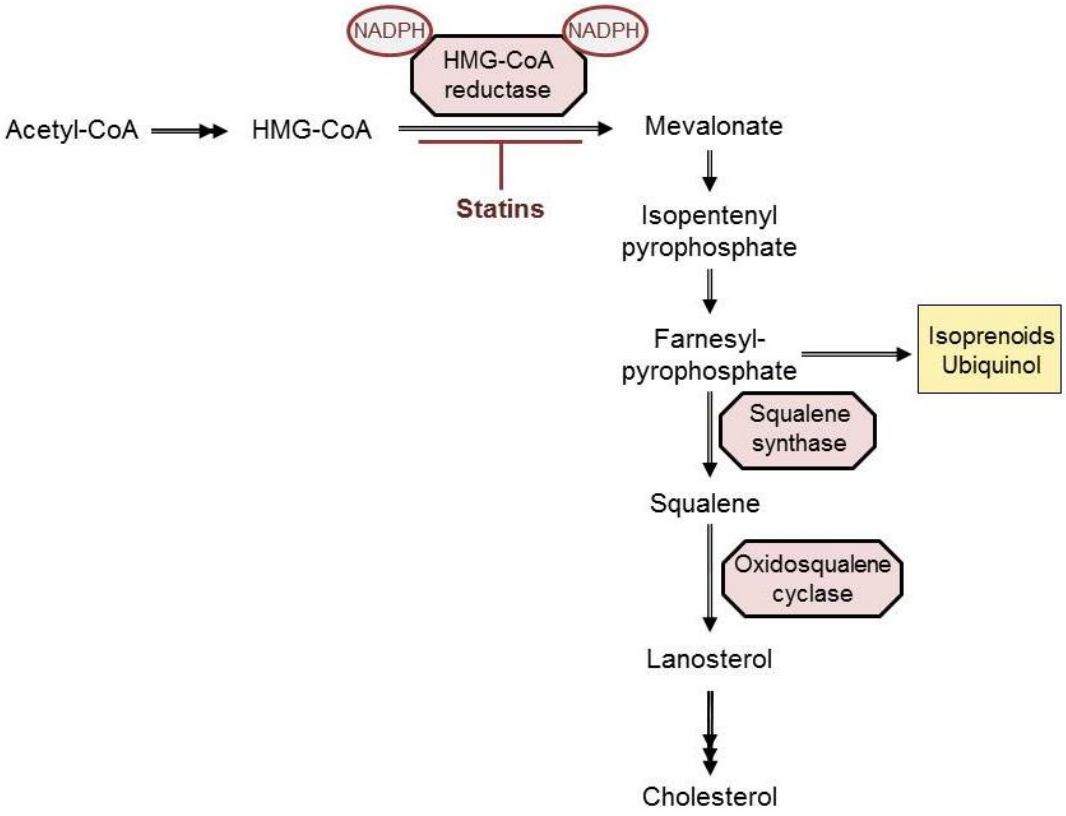

Figure 2.7: Pathway of cholesterol biosyntheisis from Acyl-colA.

2.3.2 Absorption of dietary cholesterol

Dietary cholesterol is absorbed from bile salt in the proximal parts of the small intestine. During the past few years, the role that main proteins play in dietary cholesterol uptake has been recognized. It has been found that protein Niemann-Pick C1-Like 1 **(**NPC1L1) is one of the key proteins that contributes to this process (Aiello, Brees, & Francone, 2003). The NPC1L1 protein is critical for intestinal uptake of cholesterol and is localized to the brush-border membrane of enterocytes (H. R. Davis, Zhu, L. J., Hoos, L. M., Tetzloff, G., Maguire, M., Liu, J., . . . & Lund, E. G, 2004).

Recent research also proposes that the cholesterol-lowering drug ezetimibe targets NPC1L1 protein (Garcia-Calvo et al., 2005; L. Yu et al., 2006). However, it is not clear whether the NPC1L1 plays a direct role as a typical cholesterol transporter via the plasma membrane or indirectly contributes to the process. Moreover, the family of ATP-binding cassette (ABC) transporters, i.e. cholesterol-to-bile half-transporters ABCG5 and ABCG8 (sterolin-1 and sterolin-2) form a functional components in

controlling sterol absorption (Graf et al., 2002; Turley & Dietschy, 2003). The ABCG5 and ABCG8's indirect role in dietary cholesterol absorption is to inhibit dietary cholesterol uptake by enterocytes, which triggers hepatic sterol excretion into the bile, and thus controls the bile-acid/sterol ratio, most likely to stimulate the secretion of absorbed sterols from the intestinal epithelium back into the gut lumen (D. Y. Hui & Howles, 2005). Therefore, it is important for the body to take different regulatory mechanisms to maintain a normal circulating cholesterol level.

2.3.3 Cholesterol regulation

The level of cholesterol synthesis in the liver is largely controlled by cholesterol intake, which is transported to cells throughout the body by lipoproteins. When the dietary intake of cholesterol is high, synthesis is reduced; and when the dietary intake is low, synthesis is increased (Goldstein & Brown, 2001). Conversely, cholesterol synthesised in other tissues is not controlled by such a mechanism, and therefore requires other regulatory molecules to maintain normal cholesterol levels.

First of all, when cholesterol levels are increased in other tissues, there is a rapid response of esterification of excess cholesterol by an endoplasmic reticulum (ER) enzyme, acyl CoA:cholesterol acyltransferase (ACAT) (Chang, 1997). The ACAT activity as a cholesterol homeostatic sensor controlled by cholesterol levels (Tabas, 2002). This regulation happens at the following levels: ACAT is allosterically regulated by cholesterol (Zhang et al., 2003), and accumulation of cholesterol in cells stimulates more prompt efflux of sterol from the plasma membrane (Wüstner, Mondal, Tabas, & Maxfield, 2005), which might promote the delivery rate to ACAT in the ER (Lange, Ye, & Steck, 2004).

Many other genes contribute to cholesterol metabolism at the next level, which are controlled by SREBP (sterol regulatory element-binding proteins) (Brown & Goldstein, 1999). At the high cholesterol levels, SREBP and SCAP (SREBP cleavage-activating protein) are reserved in the ER by binding to a resident ER protein, INSIG at the low cholesterol levels, the SREBP–SCAP complex leaves the ER, and SREBP is cleaved by two proteases. After this proteolytic leavage, the cytosolic domain of SREBP is released and transported into the nucleus, which then controls several genes transcription such as the LDL receptor and HMG-CoA reductase, the cholesterol

synthesis rate-limiting enzyme. Therefore, this process controls both the cholesterol synthesis and its absorption via lipoproteins.

Cholesterol efflux within cells is a third mechanism of cholesterol regulation. Although the molecular mechanisms involved in cholesterol export have been investigated over the years, there are still important knowledge gaps regarding the function and regulation of these systems. High-density lipoproteins (HDLs) and one of their related apolipoproteins, ApoA-I are the most important extracellular molecules for cholesterol uptake (Tall, Costet, & Wang, 2002), whereas the ABC transporters deliver cholesterol and phospholipids to apolipoproteins, and any dysregulation during this transport results in different human disorders (Pohl, Devaux, & Herrmann, 2005). Moreover, it has been suggested that the lipid-bilayer properties of cell membrane effect cholesterol efflux, but the exact mechanisms undergoing this influence is not clear. In a study, expression of caveolin, the coat protein of caveolae, has been associated with cholesterol efflux and it has been suggested that these cholesterol-rich domains in membranes might be a cholesterol efflux site (Fielding & Fielding, 2001).

In most peripheral tissues, cholesterol metabolism is a minor biological pathway. Most cells transform a small fraction of cholesterol to oxysterols (Schroepfer, 2000), which are key intracellular signalling molecules. For instance, excess cellular cholesterol activates the nuclear liver X receptor/retinoid X receptors (LXR/RXR), possibly using oxysterol intermediates, which initiates a ‘reverse cholesterol transport’ (RCT) system, including both efflux of cellular cholesterol and delivery of the effluxed cholesterol to the liver for bile discharge (Costet et al., 2003). Precisely, activation of LXR/RXR results in the induction of the efflux receptors ABCA1 (ATP-binding cassette transporter sub-family A member 1) and ABCG1(ATP-binding cassette sub-family G member 1), the plasma lipid transfer proteins (CETP), the cholesterol-to-bile transporter ABCG5/G8 and the efflux enhancer apolipoprotein E (Chawla, Repa, Evans, & Mangelsdorf, 2001; Oram, 2002; Tall et al., 2002). Inside the liver, cholesterol is discharged into the bile both as free cholesterol and after alteration to bile acids.

To maintain biophysical properties of the cell membrane, not only cholesterol regulation, but also the degree of the acyl chains unsaturation in phospholipids play a key role. The fatty acids are required for integration into phospholipids and their levels are regulated by many factors, such as their extracellular availability (e.g., via dietary

sources), as well as a network of metabolic regulatory systems. In addition to the SREBP role in cholesterol synthesis, it has important impacts on proteins levels contributing to fatty-acid synthesis and metabolism (Horton et al., 2002). Moreover, SREBP, along with other transcriptional regulators of fatty-acid metabolism including the LXRs, hepatocyte nuclear factor, and the peroxisome proliferator-activated receptors (PPARs) may all be regulated by polyunsaturated fatty acids (Horton et al., 2002; Sampath & Ntambi, 2005). This regulatory system impacts the fatty acids levels within the cell, as well as their unsaturation, which is able to change the saturation of the fatty acids integrated into phospholipids in addition to cellular cholesterol:phospholipid ratio. Although this procedure facilitates the proper maintenance of cell membrane biophysical properties, it is not clear if membrane bilayer properties themselves directly contribute to the regulation of these pathways or not (Horton et al., 2002; Sampath & Ntambi, 2005).

2.3.4 Cholesterol transport

2.3.4.1. Intracellular cholesterol transport from the plasma membrane (PM) to the ER and from the ER to the PM

Newly synthesised cholesterol has to move away from the ER to function. Therefore, it travels through a concentration gradient to get to the PM. One route from the ER to the PM is vesicular transport through the Golgi. Although vesicular transport via the Golgi can transport some newly synthesised cholesterol to the PM, it is not the main pathway (DeGrella & Simoni, 1982; Urbani & Simoni, 1990), as cholesterol transport from the ER to the PM is likely non-vesicular. Moreover, there are some adjacent physical membrane sites between the ER and the PM mediating this transport (Maxfield & Wüstner, 2002; Prinz, 2002). However, regardless of involvement of these sites, cytosolic transfer proteins such as the caveolin family are possibly involved (Puglielli et al., 1996).

While cholesterol obviously moves from the PM to the ER using a more diverse method than newly synthesized cholesterol exiting the ER (Field, Born, Murthy, & Mathur, 1998), the mechanism underpinning cholesterol transport from the PM to the ER is not clear. Cholesterol might undertake vesicular transport to endosomes to get into the ER for esterification. Apart from endosomes, reversing vesicular transport via the Golgi has been associated in PM-to-ER transfer as well (Neufeld, 1998); however,

PM to ER cholesterol transport is also mainly non-vesicular. Accordingly, cholesterol PM is believed to undergo a minimum of two routes for PM to ER transport: (1) a vesicular pathway through endosomes and/or Golgi; and (2) an substitute non-vesicular pathway (Wojtanik & Liscum, 2003).

It is thought that transport from the PM to the ER needs to be tightly regulated as some excess free cholesterol can remain on the large surface area of the ER and synthesize phospholipids (Blanchette-Mackie, 2000; Tabas, 2002). It has been reported that excess PM cholesterol triggers an apoptotic response in mouse macrophages (Kellner-Weibel, Geng, & Rothblat, 1999); however, a recent study highly associated ER cholesterol to the apoptotic pathway (Feng et al., 2003). This association reveals a novel research area and highlights the significance of cholesterol regulation and transport from and to the ER.

2.3.4.2. Cholesterol transport in plasma

In plasma, cholesterol, regardless of its source, is transported within lipoproteins with water-soluble outward-facing and lipid-soluble inward-facing surfaces. Different forms of lipoproteins in the blood are identified based on their intensity: they are chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) (Brown, Kovanen, & Goldstein, 1981).

Lipoprotein particles have some apolipoproteins that govern the start and end points of cholesterol transport to particular tissues by binding to specific receptors on cell membranes. Chylomicrons as the lowest density molecules in cholesterol transport have apolipoprotein E, apolipoprotein B-48 and apolipoprotein C (Havel, 1986). The role of chylomicrons is to carry fatty acids from the intestine to other tissues to produce energy and fats. Unexploited cholesterol resides in more cholesterol-rich chylomicrons and the liver then transports them from these lipoproteins to the bloodstream (Ai, Tanaka, & Schaefer, 2014).

VLDL particles are formed in the liver and have apolipoprotein E and apolipoprotein B100. On the blood vessel wall, lipoprotein lipase degrades VLDL molecules. The main cholesterol carriers in the blood are LDL molecules, which are identified by LDL receptors in peripheral tissues (Brown & Goldstein, 1986; Sugii, Reid, Ohgami, Du, & Chang, 2003). LDL receptors play a role during cholesterol

absorption as well, and its synthesis rate is controlled by the similar protein that regulates the *de novo* cholesterol synthesis, SREBP. When a cell has a high amount of cholesterol, the synthesis of LDL receptors is blocked to inhibit new cholesterol uptake by LDL particles. On the other hand, synthesis of LDL receptors continues if the cholesterol levels in the cells are not sufficient (Havel, 1986). Dysregulation of this process makes LDL particles appear in the blood without their receptor. After these LDL particles are oxidized, macrophages take them up leading to inflamed particles that form foam cells. Foam cells are regularly trapped in the blood vessels walls and involve formation of atherosclerotic plaque (Meir & Leitersdorf, 2004; Stemme et al., 1995).

It is well-known that HDL particles play a role in a RCT process in which cholesterol is transported back to the liver for excretion or for other tissues that produce hormones (Lewis & Rader, 2005; von Eckardstein et al., 1995). Moreover, high levels of HDL molecules have been linked with better health condition, while low HDL levels are correlated with development of atheromatous disease (Gordon et al., 1989; Vakkilainen et al., 2003). Therefore, it is not surprising that dysregulation in cholesterol metabolism and transport plays a remarkable pathogenic role in human diseases, particularly hypercholesterolemia.

2.3.5 Hypercholesterolemia

Hypercholesterolemia, also called dyslipidemia, is the existence of high levels of cholesterol in the blood. Elevated plasma triglyceride level in association with hypercholesterolemia is common and contributes an increased risk of heart disease. Typically such patients have elevated VLDL and LDL cholesterol levels and decreased HDL cholesterol levels (Sarwar, 2007). The normal range for total blood cholesterol in humans is between 140 to 200 mg per decilitre (mg/dL) of blood and above 240 mg/dl is classified as a high cholesterol level in blood (Daniels & Greer, 2008), while a normal cholesterol range in rats is 40-130 mg/dL and in mice it varies between strains and gender (30-100 mg/dL) (Pritchett & Taft, 2006). Hypercholesterolemia is usually caused by a combination of genetic and environmental factors. Obesity and dietary intake are typical examples of environmental factors that induce hypercholesterolemia. However, hypercholesterolemia, in some cases such as in familial hypercholesterolemia, is

totally in control of genetic irregularities where one or more mutations in the autosomal dominant genes exists (Grundy Md, 1998). This condition affects approximately 1 in 500 people in most countries (Liyanage, Burnett, Hooper, & van Bockxmeer, 2011). While hypercholesterolemia itself is not symptomatic, a longstanding increase of serum cholesterol can cause atherosclerosis. A number of clinical trials with HMG-CoA reductase inhibitors (statins) have revealed significant risk reduction through LDL cholesterol lowering in patients with heart disease, mainly via increased LDL-receptor activity (Baigent et al., 2011; S. J. Nicholls et al., 2007; Nissen et al., 2006). The role of hypercholesterolemia, specifically in the development of coronary artery disease, has been well documented, and many studies have shown its deteriorating impact on atherosclerosis development (Göran K Hansson, 2005; Peter Libby, Ridker, & Maseri, 2002). However, its role in OA development as a metabolic syndromes component has not been fully investigated.

2.3.6 Epidemiological link between hypercholesterolemia and OA

Epidemiologic studies have developed different conclusions regarding the association between OA and cholesterol. Some have found a positive link between OA and increased serum cholesterol levels (Al-Arfaj, 2003; M. A. Davis et al., 1988; D. J. Hart et al., 1995; K. Martin et al., 1997; Sturmer et al., 1998), whereas others have found a negative correlation (Bagge et al., 1991). Many studies have proposed that lipids may contribute to the pathogenesis of OA. In early population studies, a positive correlation between hand OA and high serum cholesterol levels was reported (Kellgren, 1961). Many years later, the Chingford study found a link between hypercholesterolemia and knee OA in women and this link was stronger for bilateral knee OA independent of BMI (DEBORAH J Hart et al., 1995). The hypothesis that hypercholesterolemia is a metabolic risk factor for OA was further supported by the Ulm study, whereas a cross-sectional study of OA demonstrated a link between radiological OA in hand joints and high serum cholesterol levels (T. Stürmer, Sun, Y., Sauerland, S., Zeissig, I., Günther, K. P., Puhl, W., & Brenner, H, 1998). In another epidemiological study, Davies-tuck et al. showed total cholesterol and triglycerides are associated with the development of new bone marrow lesions in asymptomatic middle-aged women (Miranda L Davies-Tuck et al., 2009). Lastly, Oliviero et al. demonstrated that even Apolipoprotein A1, a lipid transport protein that contains a

main component of HDL, is significantly increased in the serum of the OA group compared to healthy people (F. Oliviero, Sfriso, P., Baldo, G., Dayer, J. M., Giunco, S., Scanu, A., ... & Punzi, L, 2009), although it is dramatically reduced in the synovial fluid of OA group compared to RA individuals.

2.3.7 Cholesterol regulation in healthy and OA chondrocytes

In chondrocytes, the expression of proteins involved in cholesterol metabolism is strongly regulated. For example, the expression of Apolipoprotein A-I (ApoA-1) and ATP-binding cassette transporter (ABCA1) is high in differentiating chondrocytes, and reduces to a basal level when cells reach hypertrophy. In contrast, hypertrophic chondrocytes express the maximal level of Serum Amyloid A (SAA) mRNA (Gentili et al., 2005; Zerega et al., 2004). This suggests that the synthesis and use of cholesterol should be strongly controlled to inhibit hyperlipidemia and abnormal accumulation of cholesterol within cartilage tissues, which may lead to cartilage damage. This is further supported by a proteomic analysis study showing that several lipid metabolism-related proteins are diversely expressed in OA cartilage compared to normal (Iliopoulos, Malizos, Oikonomou, & Tsezou, 2008; Wu et al., 2007). Furthermore, Tsezou et al. detected oxidized low-density lipoprotein (Ox-LDL) in the synovial fluid and its receptor, lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1) in cartilage of weight-bearing and non-weight-bearing joints; however, LOX-1 was not expressed in normal cartilage (T Simopoulou, Malizos, & Tsezou, 2007). These findings demonstrated that chondrocytes are definitely able to internalize lipids.

Abnormal cholesterol accumulation occurs when cholesterol influx into the tissue wall (from apoB-containing lipoproteins) exceeds cholesterol efflux. A more recent study demonstrated that cholesterol transporting genes such as ApoA-1 and ABCA1 mRNA levels were significantly lower in OA cartilage compared to normal. Additionally, in the two subtypes of the Liver X receptor (LXR), LXRα and LXRβ, mRNA levels were also found to be significantly lower in OA cartilage (A. Tsezou et al., 2010). Interestingly, the same group also showed a central role of up-regulated SREBP-2 in the pathogenesis of OA, as well as a potentially involving mechanism (Kostopoulou et al., 2012). The differential expression pattern of these cholesterol

efflux genes between normal and OA cartilage may signify a causal relationship of cholesterol related mechanisms to the development and/or progression of OA.

2.3.8 *In-vivo* link between hypercholesterolemia and OA

Although the association of hypercholesterolemia with coronary heart disease has been frequently reported (H Mabuchi, 1989), whether cholesterol levels influence the occurrence of OA is a matter of debate. A few researchers have recently studied the possible link between plasma high cholesterol levels and OA *in-vivo*. A study by Gierman et al. found an association between metabolic stress and spontaneous OA development in mice. A high-fat diet induced cartilage damage independent of body weight, and this effect might be eliminated by decreasing cholesterol levels (L. M. Gierman et al., 2012). Two years later, this group also showed that dietary cholesterol played a role in the development of OA in an ApolipoproteinE*3Leiden mice. To better study the particular role of cholesterol in OA development, they used the drug ezetimibe, which decreases intestinal cholesterol absorption, and consequently, decreases serum cholesterol levels in animals fed a cholesterol-rich diet. In that study, ezetimibe therapy did not reduce OA development, even when fed a cholesterol-rich diet, suggesting that the processes independent of cholesterol exposure also contribute to the OA development (L. Gierman et al., 2014). In both studies, the authors proposed that metabolic stress-induced inflammation may be more likely caused by cholesterol-induced cartilage damage than mechanical overload, as anti-inflammatory medication suppressed the OA progression. They demonstrated that OA and atherosclerosis are both linked to the MetS and that the cell types that play a role in the atherosclerosis development (such as macrophages) (Peter Libby, Lichtman, & Hansson, 2013) also contribute to OA pathology (Jan Bondeson, Wainwright, Lauder, Amos, & Hughes, 2006; Haywood et al., 2003).

One of the hallmarks of hypercholesterolemia is elevated LDL levels (Koskinen et al., 2012). Contribution of LDL in OA pathology was evidenced in a mice animal model of the disease. Recently, Koskinen et al. found that elevated levels of LDL (by LDLR deficiency or a cholesterol-rich diet), in an inflammatory OA model induced pathology. In mice with high LDL levels, synovial production of S100A8 and TGF-β signalling were increased signifying synovial activation. Additionally, it has been found that enhanced levels of LDL caused more ectopic bone formation, both at the

bone margins and in the collateral ligaments (de Munter et al., 2013). Even though they suggested a definite role for oxLDL, the major emphasis of that study was the consequential impacts of elevated LDL levels on OA development. They suggested, that future experimental studies testing the effect of oxLDL or oxLDLRs on joint health will possibly provide evidence of oxLDL contribution. In another study, Triantaphyllidou et al., also showed that HDL (lipoprotein that carries cholesterol) metabolism was predisposed to OA (Triantaphyllidou et al., 2013). Despite the fact that the above studies have demonstrated important and complex interactions between cholesterol and OA, the mechanisms remain unclear. To better understand the mechanism involved in this interaction, further investigation to address the following potential issues is required.

2.3.9 Hypercholesterolemia has common molecular mechanisms in developing OA and atherosclerosis

Current information suggests that OA development can be associated with changed blood vessel function, similar to that observed in atherosclerosis (Conaghan et al., 2005; Katz et al., 2010; Saleh et al., 2007). As cholesterol is a major constituent of the cell membrane, any alterations could affect fluidity and function of the membrane so that they result in poor cell behaviour and cholesterol accumulation (N. Wang et al., 2000). It is possible that hypercholesterolemia can result in the deposition and oxidation of lipids in tissues, causing damage to the cartilage similar to that seen in diseases such as atherosclerosis (Sevin et al., 2013). A main contributing factor in the atherosclerotic lesion incidence is the possible imbalance between cholesterol influx and efflux regulation within the tissue (Schwenke & St Clair, 1993). The regulation of cholesterol is known to be mediated by a number of cell surface receptors, such as scavenger receptor class B type I (SR-BI), CD36 and ABCA1 (Ikonen, 2006). The first stage in reverse cholesterol transport is related to ABCA1, a transmembrane protein mediating lipid efflux from cells to apolipoproteins (Apo). It has been well-established that ABCA1 plays a key role in cholesterol regulation and alterations in this gene have been linked to atherosclerosis (Rothblat et al., 2002; Soumian et al., 2005). SR-BI, a member of the CD36 superfamily, is mostly expressed in the steroidogenic tissues and liver, where it facilitates selective cholesteryl ester uptake from HDL (Krieger, 1999; Marcil et al., 2006). SR-BI is also expressed in

macrophages, including tissue macrophages, monocyte-derived macrophages, and macrophages in atherosclerotic lesions (Pagler et al., 2006; Rinninger et al., 2003). SRBI deficiency is associated with de-regulation of cholesterol homeostasis in the arterial wall leading to an increased susceptibility to atherosclerosis (Van Eck et al., 2003). CD36 has been known to bind and internalize oxLDL, but also a wide range of different ligands such as anionic phospholipids, apoptotic cells, long-chain fatty acids and other modified LDL (Adorni et al., 2012). CD36 distinguishes lipid moieties of oxLDL and function as the main scavenger receptor in oxLDL uptake (T. Q. Truong et al., 2010). Moreover, increasing evidence proposes that peroxisome proliferator activated receptors (PPARs) (Barbier et al., 2002; Xia et al., 2012) apply antiatherogenic effects by increasing cholesterol efflux via activation of LXR–ABCA1 pathway (Bultel et al., 2008; Di et al., 2012; Hozoji-Inada et al., 2011; Ouvrier et al., 2009). Therefore, it is possible that the same genes and molecular determinants could affect cartilage cholesterol homeostasis. This hypothesis is further supported by the study showing the expression of some of those genes in the cartilage tissue (A. Tsezou et al., 2010). Yet, further studies are required to support such a hypothesis. In another view, Findlay et al. proposed that blood vessel alterations can cause changed blood flow to the bone that underlies the cartilage in joints. As a results, the bone and cartilage are deprived of blood, oxygen, and nutrients, which induce the whole tissue to degenerate (Findlay, 2007). As cholesterol is known to be a predominant factor that influences altered blood vessel function, it is possible that the above chain of events can lead to OA.

2.3.10 The role of inflammation in hypercholesterolemia-induced OA

Obesity and related metabolic syndromes are associated with chronic low-grade inflammation and systemic tissue damage (Gregor & Hotamisligil, 2011). Continuous accumulation of lipoproteins, primarily LDL, in the extracellular matrix of cartilage can trigger the inflammatory process. C-reactive protein (CRP) is a very sensitive marker of inflammation. The presence of inflammation in OA individuals were proposed by a finding demonstrated increased levels of CRP and IL-6 in OA groups. In this respect, the fact that CRP is associated with obesity is noteworthy. A positive association between circulating CRP has been demonstrated in patients with obesity, metabolic syndrome and OA. Interleukin-6 (IL-6) is a proinflammatory cytokine

produced by many cells, including chondrocytes. Expression studies have also shown increased levels of IL-6 mRNA in the OA specimens. IL-6 has in fact been shown to be the main stimulus for the CRP production. Moreover, IL-6 seems to be associated with visceral obesity and insulin resistance. Recent studies clearly highlight the close link between inflammation and cholesterol homeostasis through mechanisms in which ABCA1 appears to be a major factor. For example, increased intracellular free cholesterol concentration in ABCA1 KO macrophages is accompanied by enhanced inflammatory response in macrophages.

Another molecule assumed to play a viatal role in lipid-induced inflammation is the 'transcription factor nuclear factor-κB (NF-κB)'. Its essential role is regulating inflammation and immune responses by activation of different genes, including those that encode growth factors, adhesion molecules, and cytokines. 'NF-κB is redox sensitive and activated by radicals'. A large number of different radicals may be generated by oxidation of lipoproteins, signifying that lipid-induced inflammation in the vascular wall can be mediated by the pro-inflammatory NF-κBactivation by radicals drived from oxidized lipids. Indeed, induction of hypercholesterolemia in mice, rabbits, pigs, and many other animals leads to activation of vascular inflammation (Faggiotto & Ross, 1984; Faggiotto et al., 1984; H. Li et al., 1993).

2.3.11 Statins' role in preventing OA

The growing list of interconnections between cholesterol and OA indicate that statins that inhibit *de-novo* cholesterol synthesis may have a disease modifying effect. Unfortunately, statins have not yet been evaluated in OA in a placebo-controlled trial with solid end points. In one study Clockaerts et al. demonstrated that the use of statins was related to a decrease in OA progression in the knee, but not of the hip in a population based study (Clockaerts et al., 2012) . In addition to its effects on lipid metabolism, several anti-inflammatory effects have been ascribed to statins. Leung et al. found that simvastatin significantly restrained not only developing, but also established collagen-induced arthritis in a mouse model (Leung et al., 2003). It has been demonstrated that the intra-articular injections of statin during OA development decreased the infiltration of inflammatory cells and the expression of matrix-degrading enzymes, hence, controlling cartilage degradation (Akasaki, Matsuda, & Iwamoto, 2009). The same authors, in another study, showed that intra-articular administration

of HMG-CoA reductase inhibitor (e.g. statin) decreases inflammatory cell infiltration and matrix-degrading enzyme expression, therefore, preventive cartilage degradation (Akasaki, Matsuda, Nakayama, et al., 2009). Simopoulou et al. suggested that *in vitro* loading of atorvastatin in OA cultured chondrocytes may have potential chondroprotective effects, mostly by reducing cartilage degradation protein MMP13 (Theodora Simopoulou, Malizos, Poultsides, & Tsezou, 2010). Furthermore, it has been demonstrated that mevastatin upregulates the mRNA levels of bone morphogenetic protein-2, collagen type II and aggrecan, along with enhancing proteoglycan synthesis in rat chondrocytes (Hatano, Maruo, Bolander, & Sarkar, 2003), while Yudoh and colleague's findings proposed that statins may be capable of inhibiting the catabolic stress-induced chondrocyte disability and aging detected in articular cartilage of STR/OrtCrlj mice that spontaneously develop OA (Yudoh & Karasawa, 2010). By virtue of the various immunomodulatory and chondroprotective functions exerted by statins, they may be able to reduce the damage. However, a few questions remain, and the most important uncertainty is to what extent cholesterol should be reduced to nullify any off-target effects and to maintain the levels required for cartilage homeostasis.

2.3.12 Cholesterol triggers mitochondrial function

It has been shown that in some pathological states, mitochondria are enriched with high levels of cholesterol, suggesting that cholesterol play a role in mitochondrial-induced pathological conditions (Echegoyen et al., 1993; Rouslin, MacGee, Gupte, Wesselman, & Epps, 1982; W. Yu et al., 2005). Regardless of the physiological role of cholesterol in mitochondria, the mechanisms underlying trasnport to this organelle are not fully studied. It has been observed that the relative amount of fatty acids chains and the interactions between phospholipid and cholesterol, as important constituents of biological membranes, may influence permeability and physical resistance of membranes, including mitochondrial membrane (Phillips, 1972). Recent observations have reported the activity of cholesterol-transporting polypeptides, including the steroidogenic acute regulatory protein (StAR) that contribute to the mitochondrial intermembrane trafficking of cholesterol (Hall et al., 2005; Soccio & Breslow, 2003).

There is growing evidence documening that cholesterol enrichment in mitochondria can impair specific mitochondrial components accounting, in part, for

the mitochondrial dysfunction described in cells (Colell et al., 2003; Crain, Clark, & Harvey, 1983; Feo, Canuto, Garcea, & Gabriel, 1975). The mechanisms by which free cholesterol overloading triggers these events may be conducted by direct membrane effects or by activation of death-promoting molecules or both (W. Wang, Yang, & Huang, 2007; Yao & Tabas, 2001). This may be of potential relevance as recent strategies target mitochondria in different cholesterol-associated disease therapy (Galluzzi, Larochette, Zamzami, & Kroemer, 2006; W. Yu et al., 2005). these theraputical approaches trigger alterations in the mitochondrial apoptotic pathways (Yao & Tabas, 2001), mitochondrial membrane permeability (MMP) and mitochondrial membrane potential and ATP synthesis levels (Bosch et al., 2011) which contribute to different cholesterol-induced pathogenesis.

This thesis aims to determine whether high levels of cholesterol mimic human hypercholesteremic can trigger the changes of cartilage cell's mitochondrial function and morphology.

2.4 MITOCHONDRIA

Mitochondria are small membrane-enclosed bean-shape organelles that are present in large numbers in cells of all eukaryotic organisms. They have their own maternally inherited, ~16-kb extra-chromosomal DNA, the mitochondrial genome (mtDNA), implying that mitochondrial function is regulated by both nuclear and mitochondrial transcripts (Lanza & Nair, 2010). Mitochondria are of great importance in cellular metabolism, survival, differentiation, and homeostasis. Mitochondria not only contribute to energy production, but also to other cellular activity, such as calcium buffering, signal cascade, apoptosis induction, and so forth (M. T. Lin & Beal, 2006).

2.4.1 Mitochondrial structure

Mitochondria were one of the first subcellular organelles studied using electron microscopy. Sjostrand and Palade were the first researchers who found that mitochondria contained more than one membrane in the early 1950s; however, their initial three-dimensional models of mitochondria structure were not the same. Sjostrand (Sjöstrand, 1956) identified that mitochondria have a third membrane apart from the outer and inner boundary membranes (IBM), forming septa separating the

matrix into several parts. In Palade's model (Palade, 1952), mitochondria also had outer and IBM, but the inner membrane coiled inward to shape baffles that he called cristae. The current mitochondrial structure in textbooks is based on Palade's model, in which the mitochondrial boundary is identified with the smooth outer membrane and much larger constant closed inner membrane accommodated inside the space determined by the outer membrane generating wide folds to form cristae. The inner and outer membranes in the "baffle" model consist of two internal compartments: the intermembrane space between the two membranes and the matrix within the inner membrane. The space inside the cristae folds is not basically defined as a distinct compartment; however, it is extended through intermembrane space interacting via broad openings. This mitochondrial structure is the most currently accepted model (Frey & Mannella, 2000; Perkins & Frey, 2000) (Figure 2.8). The inner membrane possesses several enzymes performing as a barrier, which is poorly permeable to many molecules (Mitchell, 1979). However, there are some transporters in this membrane permitting the entrance of endogenous compounds such as pyruvic acid, fatty acid, and glutathione. The mitochondrial membranes may lose their structural and functional integrity in some pathophysiological conditions, specifically when the mitochondrial permeability transition pores (MPTP) are opened. Apart from numerous drugs and toxic composites, high levels of few endogenous products (e.g., calcium, fatty acids, and bile salts) may open MPTP (Bernardi et al., 2006).

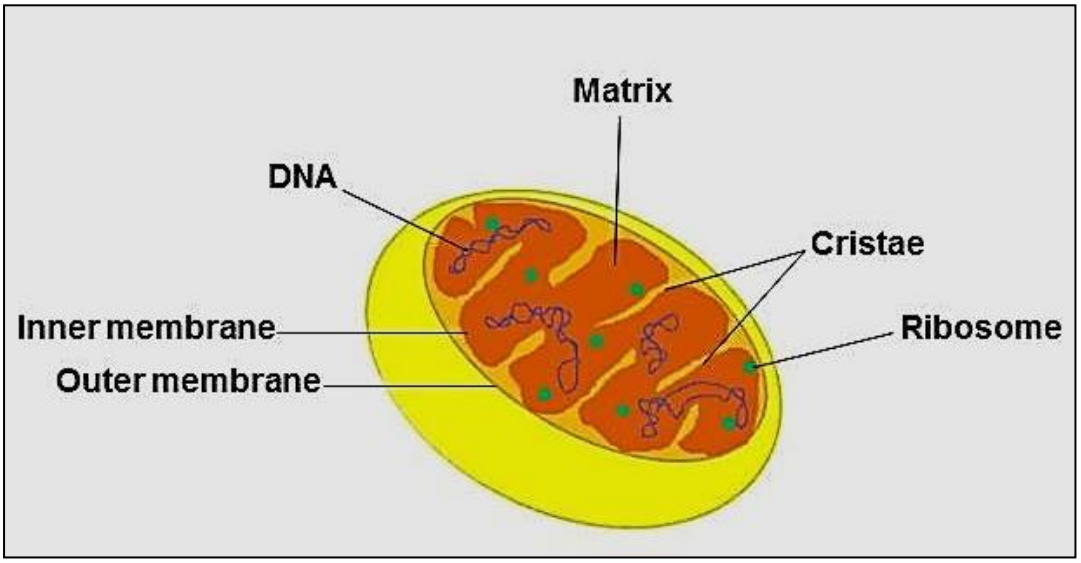

Figure 2.8: Mitochondria structure. A schematic mitochondrial structure.

The mitochondria have relatively different shapes and sizes, with a remarkable difference in the cristae number and structure. Though it is known that the cristae function increases the inner membrane surface area, the cristae shape still remains undefined even after viewing its cross sections under the electron microscope. Recently, advanced 3-dimensional images of outer and inner mitochondrial membranes and cristae have been obtained using electron tomography, but the importance of new discoveries (cristae junctions) require great elucidation. The number and morphology of mitochondria as dynamic organelles may alter inside a cell through development, the cell cycle, and several toxic challenges. Visualizing living cells labelled by key fluorescent probes including Rhodamine 123 and JC-1 that precisely label mitochondria further supported the concept that mitochondria are extremely dynamic organelles with many shape alterations (Bereiter-Hahn & Vöth, 1994; L. B. Chen, 1988; Rizzuto, Simpson, Brini, & Pozzan, 1992).

A new interesting field of study is related to mitochondrial dynamics shape and the distribution of mitochondria within specific cells (Okamoto & Shaw, 2005). It has been found that mitochondria have to constantly fiss and fuse with each other in many in many organisms and their interface with the cytoskeleton reassures their distribution in differentiating and dividing cells along with their ideal localization within cells (Chan, 2006; H. Chen et al., 2003; T. J. Collins & Bootman, 2003).

2.4.2 Mitochondrial function

Mitochondria are unique organelles as they contain their own DNA called mitochondrial DND (mtDNA). Several copies of the mitochondrial genome exist in each mitochondrion (G. M. Cooper, 2000). Mitochondria play multi-factorial roles within the cell. In addition to their central bioenergetic task of adenosine triphosphate (ATP) regeneration, the organelles are the main source of both reactive oxygen species (ROS) and of the cell's anti-oxidant defences and mediate apoptosis (Cadenas & Davies, 2000; Gibson, 2005; J. T. Liu et al., 2010), while the properties of the Ca2+ transport pathways in the inner mitochondrial membrane are such that the organelles can avidly accumulate the cations under conditions of elevated local cytoplasmic Ca2+ (D. Nicholls & Åkerman, 1982).

Mitochondrial oxidative phosphorylation (OXPHOS) produces ATP via a series of reactions where macronutrients are oxidized, oxygen is converted to water, and adenosine diphosphate (ADP) is phosphorylated to ATP to form energy for cells. Carbon substrates come into the tricarboxylic acid cycle and are then oxidized to make decreasing counterparts in the form of NADH and $FADH_2$, providing electron drift through respiratory chain complexes I (NADH dehydrogenase) and II (succinate dehydrogenase). Electrons flow from complexes I and II and come together in complex III (ubiquinone-cytochrome *c* reductase), in addition to electrons transported in from electron transferring flavoproteins (beta oxidation), via the mobile electron carrier coenzyme Q. Cytochrome c as a next mobile electron carrier shuttles electrons into complex IV (cytochrome *c* oxidase) where they are eventually shifted to oxygen, generating water. A proton gradient through the inner mitochondrial membrane is formed due to electron flow through complexes I, III, and IV. Complex V (ATP synthase) phosphorylates ADP to ATP using the potential energy of this gradient (Hebert, Lanza, & Nair, 2010; Lanza & Nair, 2010).

ROS are formed at complex I and complex III in addition to the OXPHOS process producing ATP. ROS are very sensitive molecules that can damage nucleic acids, lipids, and proteins by overproduction. mtDNA is believed that greatly endangered of oxidative damage caused by ROS. The mitochondrial-induced aging concept suggests that ROS plays a key role in oxidative damage to mitochondria and mtDNA results in aging (Harman, 1972; Linnane, Ozawa, Marzuki, & Tanaka, 1989). The main idea of this theory is that a brutal mitochondrial damage cycle leading to dysfunction induces the aging procedure.

The ROS generated through OXPHOS can damage mtDNA, lipids, and proteins. Therefore, damaged and mutated mtDNA can result in mutations that may occur within the cell as a result of unrepaired oxidative damage may cause dysfunctional proteins formation. These proteins themselves lead to more generation of ROS that will worsen this vicious cycle (Gibson, 2005). A growing body of data proposes that the production of ROS mainly depends on the mitochondrial membrane potential ($\Delta\psi$). While membrane potential is high, mitochondrial redox potential is high, permitting more potential for electrons backflow over complexes I and III (Balaban, Nemoto, & Finkel, 2005). It has been demonstrated that low mitochondrial membrane potential and mitochondrial depolarization are in charge of pro-apoptotic proteins release from

the mitochondria inducing apoptosis (Bossy-Wetzel, Newmeyer, & Green, 1998; Kroemer, Zamzami, & Susin, 1997).

2.4.3 The role of mitochondria in apoptosis

Programmed cell death, orapoptosis, is a cellular self-destruction mechanism palys a role in different biological events, including tissue homeostasis, the removal of unwanted cells, and developmental sculpturing. Excessive or insufficient apoptosis contributes to numerous diseases and is involved in the growth and regression of tumours (Kerr, Wyllie, & Currie, 1972; Saikumar et al., 1999). Mitochondrial dysfunction, which is induced by DNA damage and other genotoxic factors, results in an irreparable event, apoptotic cell death (Green, 1998). The mitochondria perform at the core of the apoptotic pathway providing many essential factors, such as those that induce caspase, a group of cysteine proteases that can cleave many cellular substrates to dismantle cell contents (Salvesen & Dixit, 1997), activation, and chromosome fragmentation (Adams, 2003). Mitochondrial fragmentation during apoptosis was associated with the loss of the mitochondrial membrane potential ($\Delta\Psi m$) that was considered an irreparable point in the death cascade (Zamzami et al., 1995).

In most, but definitely not all apoptotic pathways, mitochondria play an early role releasing cytochrome c from the intermembrane space and possibly the intracristal space as a result of one or more different signals such as presence of oxidants, high [Ca2+], ceramide and pro-apoptotic proteins. Cytochrome c release activates apoptosome (Acehan et al., 2002) assembly from ATP, apoptotic protease-activating factor-1 (Apaf-1) and procaspase-9, which triggers effector caspase-3 and caspase-7, resulting in oligonucleosomal DNA fragmentation (Baliga & Kumar, 2003; Bao & Shi, 2007).

Release of cytochrome *c* from the intermembrane space requires large openings in the outer membrane to allow the cytochrome *c* to pass. Two mechanisms have been suggested to influence cytochrome *c* release. The first is dependent on the swelling of the mitochondrial matrix to an extent that its external pressure breaks the outer membrane discharging all of the intermembrane space substances (Bernardi, 1996). Some stimuli, including high [Ca^{+}] and/or oxidants, can open a high conductance channel in the inner membrane of isolated mitochondria leading to loss of the

electrochemical proton gradient, and the hyperosmolarity of the matrix makes it expand to the point that it can rupture the outer membrane (Green, 1998). However, the findings that cytochrome *c* is able to leave mitochondria before loss of the inner mitochondrial membrane potential proposes another mechanism, the generation of enough large pore in the outer membrane to permit cytochrome *c* to move into the cytosol (Pavlov et al., 2001). The membrane permeability transition pore (PTP) is regulated by pro-apoptoic and anti-apoptotic Bcl-2 family proteins including Bax, Bak, Bcl-2 and Bcl-xL (Harris & Thompson, 2000; Kelekar & Thompson, 1998). Through early steps of apoptosis, the pro-apoptotic proteins Bax translocates to the outer mitochondrial membrane (OMM) and, almost immediately after translocation, gather into submitochondrial punctate foci (Nechushtan, Smith, Lamensdorf, Yoon, & Youle, 2001). Moreover, Bak co-localizes with Bax in these foci. It has been demonstrated that Bax and Bak triggers cell death through mitochondrial outer membrane permeabilization (MOMP) that results in small pro-apoptotic molecules release including cytochrome *c* (X. Liu, Kim, Yang, Jemmerson, & Wang, 1996), second mitochondria-derived activator of caspase/direct IAP (inhibitor-of apoptosis) binding protein with low pI (Smac/Diablo) (Du, Fang, Li, Li, & Wang, 2000), Omi/HtrA2 (Hegde et al., 2002), apoptosis- inducing factor (AIF) (Susin et al., 1999), and endonuclease G (EndoG) (L. Y. Li, Luo, & Wang, 2001) from the mitochondrial intermembrane space to the cytosol and consequent induction of the caspase-activating pathways in apoptotic cell death pathways (Donovan & Cotter, 2004) (Figure 2.9).

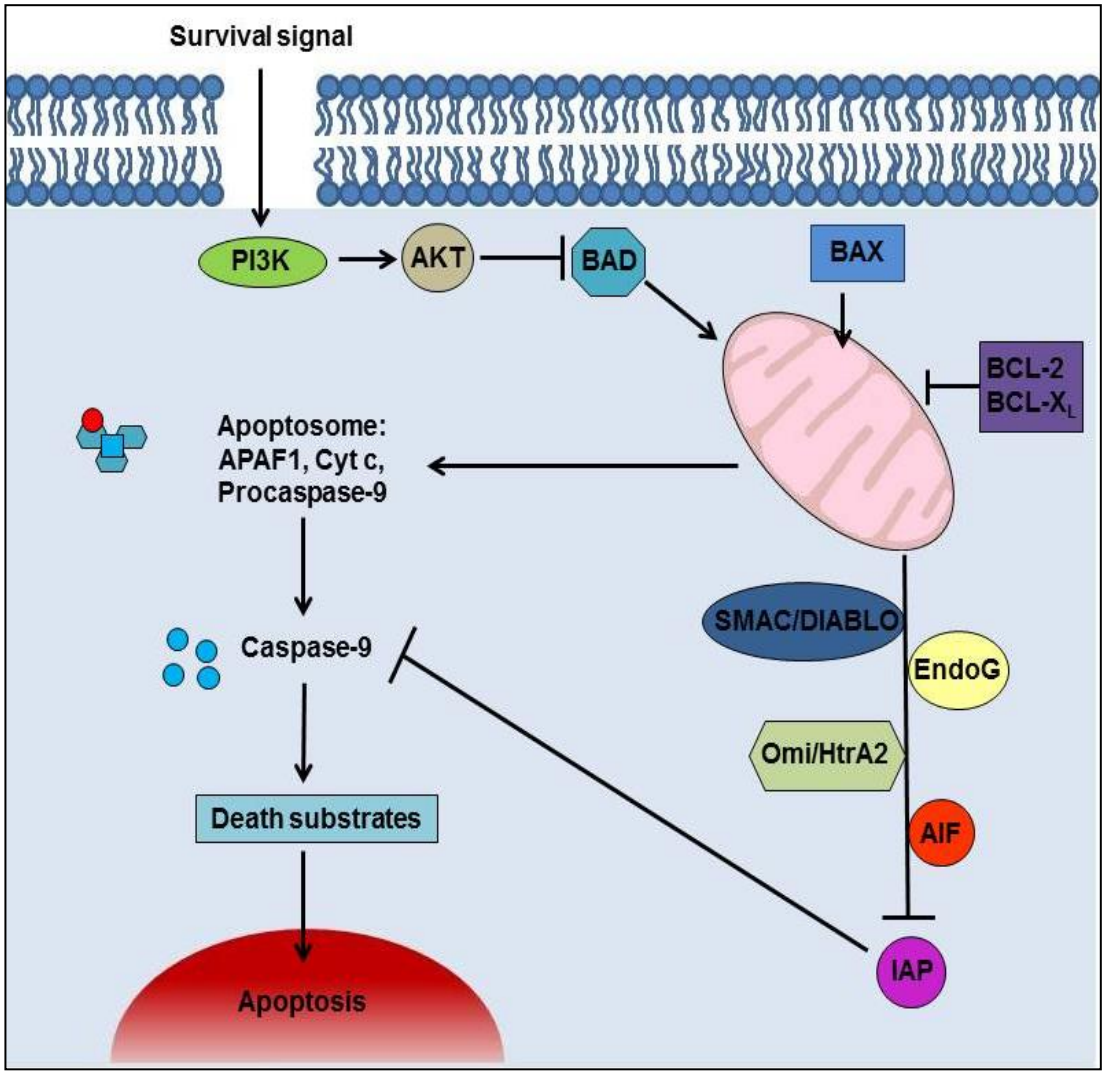

Figure 2.9: Modified apoptosis signalling through mitochondria: Stimuli can start apoptosis via the mitochondrial pathway. After stimulation of mitochondria, cytochrome c (Cyt c) releases into the cytosol and forms apoptosome by binding to apoptotic protease activating factor 1 (APAF1). Initiator caspase-9 is stimulated at apoptosome and conducts apoptosis. Anti-apoptotic proteins such as the anti-apoptotic BCL2 family members and inhibitors of apoptosis proteins (IAPs), which are controlled by SMAC/DIABLO (second mitochondria-derived activator of caspase/direct IAP binding protein with low pI) are able to inhibit mitochondria apoptosis at different levels. An alternative way is via survival signals, such as cytokines and growth factors, that trigger the phosphatidylinositol 3-kinase (PI3K) pathway (Igney & Krammer, 2002).

2.4.4 Mitochondrial dysfunction in the course of OA progression

ROS, a major by-product of OXPHOS, can play a positive role as signalling molecules. Mitochondrial oxidative pathways produce ROS to synthesise ATP for energy to maintain normal cartilage homeostasis when subject to physiological mechanical strains, with the signal cascade stimulated by mechanical distortion of the mitochondria (Brouillette et al., 2014; Montagne et al., 2014; Wolff et al., 2013). However, overproduction of ROS can induce oxidative damage in the form of lipid peroxidation and mtDNA damage. It is possible that mitochondrial dysfunction results in a signal cascade associated with programmed cell death, or apoptosis. The damage to mitochondrial membrane phospholipids, DNA, or proteins can differently influence mitochondrial function, such as increased permeability of mitochondrial membranes, decreased activity of the mitochondrial respiratory chain complex, loss of the mitochondrial components (e.g., cytochrome c) and dissipation of mitochondrial membrane potential ($\Delta\psi m$). Apoptosis is a physiological procedure, not only to maintain both embryogenic and adult tissue homeostasis, but it also contributes to articular cartilage degradative disorders such as OA.

It has been shown that the protein expressions of BCL-2 and BAX were increased in cartilage from OA patients. BCL-2 and BAX are two factors that play a vital role in mitochondria-related apoptosis. Increased synthesis or translocation of BAX, a pro-apoptotic protein, can cause the mitochondrial membrane potential depolarization, increasing the cytochrome c discharge. Interestingly, Li et al. used an ingenuity pathways analysis (IPA) to identify mitochondria-related pathways involved in the pathophysiology of OA. They presented the first evidence displaying that canonical pathways involved in oxidative phosphorylation, pyruvate metabolism and apoptosis signalling are associated with the mitochondrial dysfunction found in OA patients, in addition to evidence for the underlying mechanism of mitochondrial dysfunction in the cartilage of OA patients. In their study, genes and pathways that are related to apoptosis pathways were significantly up-regulated in OA, which can lead to extreme chondrocyte apoptosis and abnormal expression of the apoptosis-related genes in articular cartilage (C. Li et al., 2012).

Cytochrome *c* has recently been recognized as a main mediator in apoptotic pathways and is generally used as a marker of the mitochondrial apoptotic procedure in the cells. When Cytochrome *c* releases from mitochondria into the cytoplasm

activates caspase-9 and 3, and eventually results in cell apoptosis. Liu et al. reported that OA articular chondrocytes demonstrated significantly decreased complex II, III, IV and V activities, loss of mitochondrial membrane potential, but showed an increased mitochondrial mass compared to normal chondrocytes. Moreover, findings from cultured OA chondrocytes revealed a decrease in ATP levels and an increase in percentage of cells with de-energized mitochondria. Moreover, it has been observed that a release of mitochondrial cytochrome *c* and stimulation of capase-9 and 3 occurred in OA samples. Thus, chondrocytes from OA patients showed more positive apoptotic cells than the healthy controls (J. T. Liu et al., 2010).

More recently, Gavriilidis et al. proposed that mitochondrial dysfunction in OA is related to superoxide dismutase 2 (SOD2) down-regulation . SOD2, a subfamily of superoxide dismutase (SOD), acts as a defence against ROS. Increased levels of ROS may elevate levels of mtDNA damage, lipid peroxidation, as well as activation of signalling pathways, all of which may intensify cartilage degradation, cleavage of collagen, and hyaluronan. All SODs are expressed at lower levels in OA cartilage comparing normal control cartilage at both the messenger RNA (mRNA) and protein level (Gavriilidis, Miwa, von Zglinicki, Taylor, & Young, 2013), which suggests that decreases in the SOD2 levels in OA human articular chondrocytes may induce an increase in mitochondrial superoxide levels, and additionally, mitochondrial dysfunction.

2.4.5 Cholesterol triggers oxidative stress

Mitochondrial dysfunction is known to be induced by hypercholesterolemia, hyperglycemia, hypertriglyceridemia, and even the process of aging (Lee & Wei, 2012; Puddu et al., 2005). Numerous studies havefound a link between increased cholesterol oxidation products (oxysterols) and mitochondrion-derived oxidative stress, which typically leads to overproduction of mitochondrial reactive oxygen species (mtROS) (Lordan et al., 2009). Excessive and continuous accumulations of oxidised cholesterol particles tend to bind to ‘sulphate-containing proteoglycans, where they aggregate and become oxidatively modified’ (Eva Hurt-Camejo & 1997).The factors in charge of these changes are not fully known, but apart from ROS, different membrane and extracellular tissue-associated enzymes were reported to be involved. There are numerous defence systems, such as antioxidant vitamins and

enzymes that inhibit oxidative damage of accumulated lipoproteins, but in conditions of a constant lipid overload these defence mechanism may ultimately not function, causing the activation of stress signalling pathways (Oliveira et al., 2005). ROS is typically produced under optimal conditions by mitochondria and are neutralized by the mitochondrial cellular defence systems. When ROS is overproduced, it induces deterioration of mitochondrial oxidative stress defences, causes irreversible harm at the cellular and molecular levels in vulnerable cells, and contribute to pathologic conditions via c-Jun N-terminal kinase (JNK) pathway activation (Kamata, 2005). ROS may also harm DNA, cellular lipids, and proteins, increasing lipid peroxidation, a condition that may more induces mtDNA damage (Fosslien, 2001). It has been shown that increased ROS production, particularly, radicals and superoxide, has been related to cartilage dysfunction in human (Ostalowska et al., 2006) and animal models of disease (Johnson et al., 2004), and there is growing evidence of an association between oxidative stress and cartilage degradation in humans (Afonso et al., 2007; Regan et al., 2005). These observations led to our hypothesis that accumulation free cholesterol increases superoxide anion levels, induces oxidative stress, and activates a transient inflammatory response causing permanent damage to the joint tissues. However, whether the high cholesterol levels trigger the mitochondrial dysfunction in cartilage needs further investigation.

Thus, the study objectives are as below:

1. To test the effect of excess cholesterol on primary chondrocytes *in vitro* using a 3D pellets culture method and determine how cholesterol may challenge chondrocytes under this condition.

- This objective help author to find out if high cholesterol levels challenge cartilage andchondrocytes function and phenotype or not.
- Chondrocytes 3D pellet culture is well-stablished method mimcs cartilage condition and used to define how cholestreol change OA-like marker in chondrocytes.

2. To study the link between hypercholesterolemia and osteoarthritis using two different animal models ($ApoE^{-/-}$ mice and dietary-induced hypercholesterolemia [DIHC] rats) mimicking the human hypercholesterolemia condition.

3. Using these two animal models will help to investigate possible association between hypercholesterolemia and OA. ApoE$^{-/-}$ mouse is a well-stablished model to study hypercholesterolemia-related condition. Moreover, using dietary rat model will help to test the effect of dietary high cholestreol intake on OA.To study the effect of high levels of cholesterol on chondrocytes mitochondrial function both *in vitro*.

- Using 3D culture model of chondrocytes, we are going to investigate effet of high cholesterol levels on chondrocytes' mitochondrial function. Different well-stablished functional methods will be used to study chondrcoytes' mitochondrial changes under cholestreol challenge

4. Study the effect of cholesterol-lowering drug and mitochondrial protector (anti-oxidants) on cartilage using *in vivo* (ApoE$^{-/-}$ mice and DIHC rats) and *in vitro* models.

We are going to use statin as a positive cholesterol-lowering drug in our *in vivo* and *in vitro* models. New anti-oxidant drug will be used to protect mitpochondrial from oxidative damge caused by high cholesterol levels. Following anti-oxidants drug administration, I will investigate joint biology to figure out the theraputical effect of this drug..

Chapter 3: Research Design

3.1 ETHICS

Ethics approval for human cartilage collection was granted by Queensland University of Technology and Prince Charles Hospital Human Research Ethics Committees (approval ethics number: HREC/14/QPCH/186) and informed consent was given by all patients involved.

Ethics approval for the rats' study was granted by Queensland University of Technology, University Animal Ethics Committee (UAEC) (approval ethics number: 1300000283) and the $ApoE^{-/-}$ mice study was approved by the Xiangya Hospital, the Central South University of China (approval ethics number: 2013-053).

3.2 METHODOLOGY

3.2.1 Articular cartilage sample collection

OA cartilage (n= 24) were sourced from the main defective area of the medial compartment cartilage showing degenerative changes (Figure 3.1). The average age of OA patients used in this study was 65.20 ± 15.4. Normal cartilageswere obtained from OA patients (n=24), where smooth knee tissue was available. To eliminate early OA symptoms, samples showing any evidence of cartilage changes were excluded for normal cartilage collection. These macroscopic changes included softening of the hyaline articular cartilage, thinning and fibrous dislocation, ulcerations of the cartilage, and light sclerosis of the subchondral bone. At the microscopic level, cartilage was classified according to the Mankin score (H. J. Mankin et al., 1971) based on saffranin-O and H&E histology staining, where a score of 0-4 (Grade1) indicated normal cartilage and a score over 10 (Grade 4) indicated advanced degenerative OA cartilage. Due to limition of OA research in accessing normal human cartilage from healthy donors, Grad 1 cartilage is used for control group.

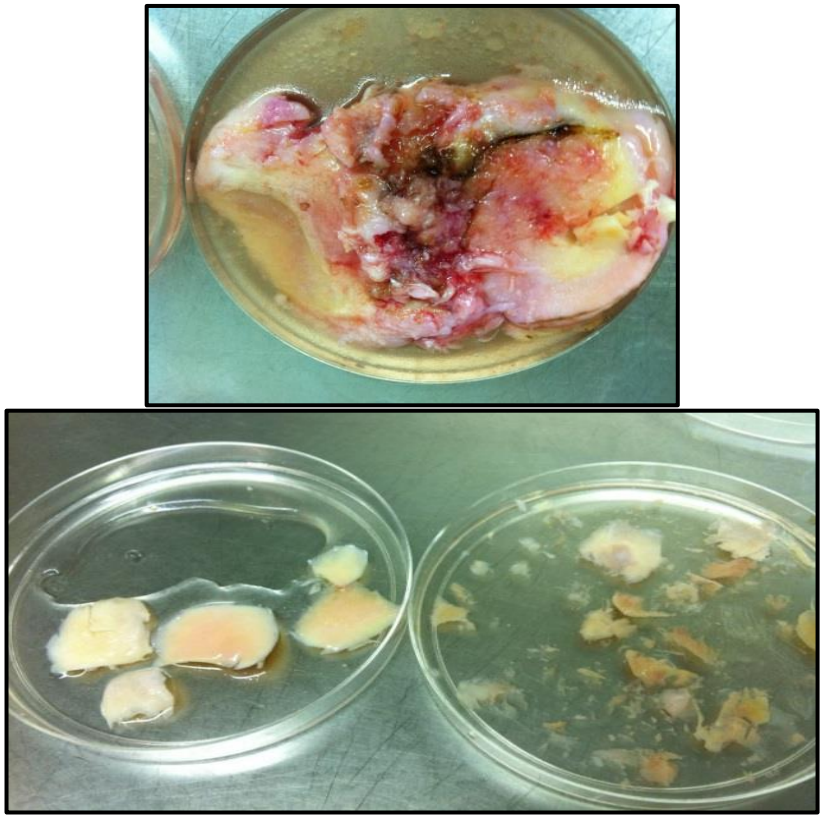

Figure 3.1: Tibia plateau collected from patients undergoing knee replacement. The right panel represents OA cartilage pieces with a rough degenerated surface and normal cartilage with a smooth surface under gross examination.

3.2.2 Primary articular cartilage chondrocytes (ACCs) isolation

Chondrocytes from the cartilage tissues were isolated following a method described previously with minor modifications (Schnabel et al., 2002). Briefly, cartilage was dissected into small pieces using a sterile scalpel, and washed several times with 1X PBS (phosphate buffered saline). Chondrocytes were released by digesting the tissues in 0.2% collagenase type II mixed in high glucose DMEM at 37°C for 16 hrs. The cell suspension was filtered (70µm mesh) and centrifuged at 1000g for 10 min and resuspended in complete Dulbecco's Modified Eagle Medium (DMEM, Gibco-Invitrogen,VIC, Australia) containing 10% fetal bovine serum (FBS, Thermo Scientific, VIC, Australia) and 50u/ml penicillin and 50µg/ml streptomycin (Gibco-Invitrogen, VIC, Australia) and plated at a density of 2500 cells/cm^2. Only early passage ACCs, showing a strong expression of type II collagen (COL2) and aggrecan (ACAN) were used for subsequent experiments.

3.2.3 Cell culture

The obtained primary ACCs from the protocol described above were seeded into the tissue culture flasks containing DMEM supplemented with 10% FBS and 1% penicillin/streptomycin and incubated at 37°C in humidified CO_2 incubator. The cells were passaged at approximately 80% confluence by trypsinization and expanded through a maximum of two passages for this study.

3.2.4 Chondrocyte pellet culture

Cell culture systems known to preserve the chondrocyte phenotype were used in the co-culture studies. Chondrocytes pellet culture was performed as described previously in our research group (Prasadam et al., 2010). Two x 10^5 cells of ACCs were trypsinized and resuspended in a serum free chondrogenic media (serum-free medium-high glucose DMEM) (Invitrogen, Mt Waverley, VIC, Australia) supplemented with 10 ng/mL transforming growth factor-β3 (Bio Scientific, Gymea, NSW, Australia), 10nM dexamethasone, 50 mg/mL ascorbic acid, 10 mg/mL sodium pyruvate, 10 mg/mL proline, and an insulin+transferrin+selenium supplement (final concentration: 10 µg/mL insulin, 5.5 µg/mL transferrin, 5 ng/mL sodium selenite, 0.5 mg/mL bovine serum albumin and 4.7 µg/mL linoleic acid) and centrifuged at 600 *g* for 20 minutes to form a pellet. Pellets were allowed to differentiate for two weeks in 3-dimensional conditions in 15 mL Falcon tubes, in a chondrogenic medium, which was replenished every two to three days. After two weeks of chondrogenesis ACCs pellets (Figure 3.2) were processed for histological analysis, immunofluorescence staining, western blotting and qRT-PCR.

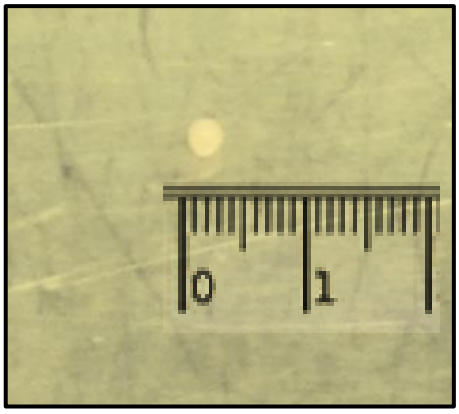

Figure 3.2: ACCs in 3-D pellet chondrogenic culture.

3.2.5 Bovine explant culture

Bovine tibia cartilage has previously been validated as a model relevant to human disease (W. Hui, Barksby, H. E., Young, D. A., Cawston, T. E., Mckie, N., & Rowan, A. D, 2005) and was cultured as previously described (Morgan, 2006). Three bovine cartilage explants were harvested from the proximal femoral condyle of joint of three one-year-old animals from local butchery and animals were not killed for the purposes of this study. 6mm discs of bovine knee cartilage (Figure 3.3) were taken using a biopsy punch. Cartilage discs were washed twice with PBS and stabilized for 48 hours in Dulbecco's Modified Eagle Medium (DMEM, Gibco-Invitrogen, VIC, Australia) supplemented with 10% fetal bovine serum (FBS, Thermo Scientific, VIC, Australia), and 1% (v/v) penicillin/streptomycin (Gibco-Invitrogen, VIC, Australia) at 37◦C in a humidified CO_2 incubator. The medium was then removed and replaced with serum-free medium supplemented with cholesterol and without cholesterol as a control for seven days. The medium was changed every three days and supernate was collected each time and kept at -80◦c to quantify Sulfated glycosaminoglycan (sGAG) content during the bovine explant culture. Bovine cartilage discs release proteoglycans into a culture medium when they loos their cellular GAG. Therefore, storing supernate from bovine explant culture would enable us to quantify the sGAG content of bovine explant cartilage under cholesterol challenge.

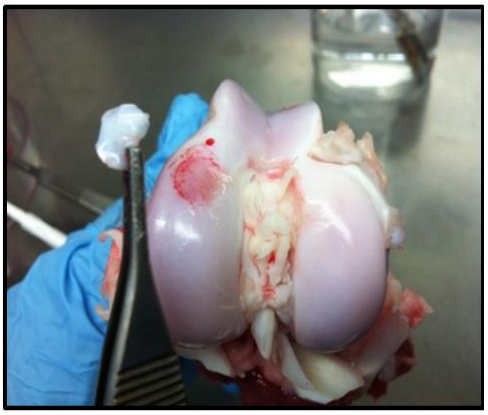

Figure 3.3: 6mm discs of bovine cartilage explant.

3.2.6 Cholesterol, atorvastatin, and mito-TEMPO loading in vitro

Cholesterol was delivered to chondrocytes by using Chol:MβCD complex (Sigma-Aldrich, #C495, NSW, Australia). Cyclodextrins (CD) have been used extensively as drug delivery vehicles and chemically modified β-CDS, such as methylated β- cyclodextrins (MβCD) have been made to form soluble inclusion complexes with cholesterol, thereby enhancing its solubility in aqueous solution. *In vitro*, β-CDs have a high affinity for cholesterol as compared to other lipids, which may make this compound quite effective in modifying cholesterol metabolism. This method has been widely used to successfully deliver cholesterol in a variety of cell types (Choi & Toyoda, 1998; Christian, Haynes, Phillips, & Rothblat, 1997; Pucadyil & Chattopadhyay, 2004).

Human articular chondrocytes (ACCs) were isolated and differentiated in 3D pellets as described above. The pellets were treated with different doses of Chol:MβCD (5,10,30µg/ml) in 0.2% (w/v) BSA for 14 days. Pellets incubated with 0.2% BSA without Chol:MβCD treatment served as controls. Similarly, cartilage disks were harvested from the femoropatellar grooves of one year old bovine calf knee joints (obtained from a local butcher) as previously described and treated with Chol:MβCD complex for seven days as described above.

Mito-TEMPO (Sigma-Aldrich, #SML0737, NSW, Australia) treatment was performed following cholesterol loading in ACCs pellets. Mito-TEMPO treatment (10µM) was performed for 14 days in cholesterol-stimulated (30µg/ml) ACCs pellets

after pre-incubation for one hour at the dose of 5μM. Media was changed every three days and stored in -80◦c to perform sGAG assay. The same method was used to treat bovine explants by mito-TEMPO for seven days. Media was changed every three days and stored in -80 ◦c to be used for sGAG assay. Chol:MβCD treatment in both ACCs pellet and bovine discs served as controls.

3.2.7 Sulfated glycosaminoglycan (sGAG) assay

sGAG assay was performed based on a protocol provided in the Blyscan Sulphated Glycosaminoglycan assay kit (Biocolor, life science assays, UK). Supernant from chondrocytes pellet culture and bovine explant cultured were collected every three days and immediately stored at -80◦c. On the day of the experiment, supernant was thawed and sGAG were precipitated, drained, and recovered. sGAG content was measured at the wavelength of 595 nm by a microplate spectrophotometer (Benchmark Plus, Tacoma, Washington, USA). sGAG content was determined by measuring sulphated glycosaminoglycan content compared to a chondroitin sulphate standard and expressed as μg/ml since each ml of condition media contained similar amounts of bovine cartilage and ACCs pellets.

3.2.8 Histological Haematoxylin and Eosin (H&E) staining

The knee samples were fixed in 4% paraformaldehyde (PFA) and decalcified in 10% Ethylenediaminetetraacetic acid (EDTA) for three-four weeks. Decalcified samples were then embedded in paraffin and 5 μm thick serial sections were cut from paraffin blocks with a microtome. Selected slides from each sample were stained with H&E (HD Scientific, Wetherill Park, NSW, Austalia). Briefly, following dewaxing and hydration, Maye's haematoxylin was used to stain the cell nucleus. After dehydration in increasing alcohol concentrations, the cell plasma and extracellular matrix were stained with eosin. Images of the stained slides were captured using Axion software under a light microscope (Carl Zeiss Microimaging GmbH, Gottingen, Germany).

3.2.9 Histological Safranin O staining

The knee and chondrocytes 3D pellet cultured samples were fixed in 4% paraformaldehyde (PFA) and decalcified in 10% Ethylenediaminetetraacetic acid (EDTA) for three-four weeks. Decalcified samples were then embedded in paraffin and 5 μm thick serial sections were cut from paraffin blocks with a microtome. Selected slides from each sample were stained with safranin O staining method. Briefly, following dewaxing of sections in xylene (6 minutes) and hydration in a descending ethanol series (100%, 90% and 70%) for 3 minutes in each, Weigert's iron haematoxylin working solution (Sigma-Aldrich, NSW, Australia) was applied for nuclei staining for two minutes, fast green solution for cytoplasm (five minutes), and Safranin O solution for cartilage staining for 10 minutes. After dehydration in increasing alcohol concentration, slides were mounted in Depex mounting medium. Images of the stained slides were then captured using Axion software under a light microscope (Carl Zeiss Microimaging GmbH, Gottingen, Germany), and OA severity in the tibia plateau was evaluated according to a modified Mankin's histological grading system (Mankin's score, 0-14) (H. J. Mankin et al., 1971). This scoring system is mainly focused on the Safranin O staining intensity, chondrocytes' cellularity, and cartilage structure integrity. Accordingly, 0 points implies normal healthy cartilage, whereas 14 points represents the most severe cartilage lesions.

3.2.10 Histological Alcian blue staining

The chondrocytes 3D pellet cultures were fixed in 4% paraformaldehyde (PFA), embedded in paraffin and 5 μm thick serial sections were cut from paraffin blocks using a microtome. Selected slides from each sample were stained with alcian blue (Sigma-Aldrich, NSW, Australia). Briefly, following dewaxing of sections in xylene (6 minutes) and hydration in a descending ethanol series (100%, 90% and 70%) for 3 minutes in each, alcian blue and fast red solutions were applied for Glycosaminoglycans (GAGs) and nuclei staining respectively. After dehydration in increasing alcohol concentration, slides were mounted in Depex mounting medium. Images of the stained slides were then captured using Axion software under a light microscope (Carl Zeiss Microimaging GmbH, Gottingen, Germany).

3.2.11 OCT embedding and Cryostat microtome

The fresh cartilage tissue samples were collected from patients undergoing knee replacement. Cartilage pieces classified to normal and OA using gross view examination of macroscopic changes included softening of the hyaline articular cartilage, thinning and fibrous dislocation, ulcerations of the cartilage, and light sclerosis of the subchondral bone. Then, cartilage pieces were frozen in liquid nitrogen and embedded in optimal cutting temperature compound (OCT). A frozen block of tissue was sectioned using cryostat microtome. Frozen sections were collected by touching the glass slide directly onto each section. The slides were then kept at room temperature to air dry. Slides were then fixed by dipping the slides into acetone five times. Fixed slides were stained with Oil Red O staining to compare lipid distribution between normal and OA cartilage.

3.2.12 Histological Oil Red O staining

Serial 5μm sections were incubated with Oil Red O solution for 10-20 minutes at room temperature. Stained slides were washed very gently by dipping two-four times in distilled water, followed by air drying. Finally, slides were mounted in Depex mounting medium. Images of the stained slides were then captured using Axion software under a light microscope (Carl Zeiss Microimaging GmbH, Gottingen, Germany).

3.2.13 Histochemical staining for cholesterol

Cellular cholesterol staining was performed based on a protocol provided in the cholesterol assay kit (Sapphire Bioscience Pty. Ltd). Wells of a 96-well plate were seeded with $2x10^4$ cells/well. Cells were left overnight to attach. The next day, culture medium from the wells was removed and cells were fixed with cell-based assay fixative solution and stained with Filipin III solution, incubating in the dark for 30-60 minutes. Samples were analysed using a fluorescent microscope (Carl Zeiss Microimaging GmbH, Gottingen, Germany) under an excitation of 340-380 nm and emission of 385-470 nm.

3.2.14 Immunohistochemistry

Tissue slices were dewaxed and hydrated. Endogenous peroxidases were blocked by incubation in 0.3% peroxide for 15 minutes. Non-specific proteins were removed with the incubation of 10% swine serum for one hour. Sections were then incubated with an optimal dilution of primary antibody overnight at 4^0C, followed by incubation with a biotinylated swine-anti-mouse, rabbit, goat antibody (DAKO Multilink, CA, USA) for 15 minutes, and then incubated with horseradish perioxidase-conjugated avidin-biotin complex for 15 minutes. Antibody complexes were visualized by the addition of a buffered diaminobenzidine (DAB) substrate for three minutes. Sections were counterstained with Mayer's haematoxylin for 10 seconds each, and rinsed with running tap water. A list of antibodies sources and dilutions used for this study are summarized in Table 3.1. To conduct semi-quantitative data analysis, the positive cells from different fields of observation were counted and normalized to the cell number per 100 total cells in each group.

Table 3.1: Antibodies Used for Immunohistochemistry (IHP)

Antibody	Source	Dilution
Anti-MMP-13	Labvision, ThermoFisher Scientific, VIC, Australia	1:50
Anti-Aggrecan (ACAN)	Merck Millipore, VIC, Australia	1:500
Anti-Collagen-X (COL10)	Abcam, Sapphire Bioscince, NSW, Australia	1:500
Anti-DIPEN	Kind gift from Prof Amanda Fosang	1.6µg/ml
Anti-NITEGE	Kind gift from Prof Amanda Fosang	5µg/ml
Anti-Malondialdehyde (MDA)	Abcam, Sapphire Bioscince, NSW, Australia	1:500
Anti-Cytochrome C (CYCS)	Cell signalling Technology, Genesearch, QLD, Australia	1:100

3.2.15 Immunofluorescence staining

Tissue slices were dewaxed and hydrated. Sections were heat-treated at 94°C for four minutes in 10mM sodium citrate solution (pH 6.0) using the decloaking chamber, then incubated with 0.1% (w/v) hyaluronidase (Sigma-aldrich, NSW, Australia) at room temperature for 30 minutes. Non-specific proteins were removed with the incubation of 2% BSA/PBS solution for one hour. Sections were then incubated with optimal dilution of primary antibody in blocking buffer overnight at 4^0C, followed by incubation with fluorescence-labelled goat anti-mouse and anti-rabbit secondary (dilution range of 1:500-1:800 in blocking buffer) antibody (Alexa Fluor® 488, Invitrogen, Austraila) for one hour. Sections were mounted using Prolong® Gold antifade reagent (Invitrogen, Australia) and visualized using a confocal microscope (Carl Zeiss Microimaging GmbH, Gottingen, Germany). To conduct semi-quantitative data analysis, the positive cells from different fields of observation were counted and normalized to the cell number per 100 total cells in each group.

3.2.16 RNA extraction and quantitative real time -PCR (qRT-PCR)

Total RNA was isolated from ACCs pellets with TRIZOL reagent (Invitogen,VIC, Australia), DNase treated and column purified using an RNeasy Mini Kit (QIAGEN Pty Ltd, VIC, Australia) as described previously in our research group (Prasadam et al., 2010). Complementary DNA was synthesized using Superscript III (Invitrogen) from 1 µg total RNA following the manufacturer's instructions. PCR primers (Table 3.2) were designed based on cDNA sequences from the NCBI Sequence database using the Primer Express® software and the primer specificity was confirmed by BLASTN searches. RT-qPCR was performed on an ABI Prism 7300 Thermal Cycler (Applied Biosystems, Scoresby, VIC, Australia) with SYBR Green detection reagent. Briefly, 2 µL of template cDNA, 20 pmol of gene-specific primers and 10 µL of 1x Master Mix were used in a 20 µL reaction volume; each sample was performed in duplicate. The thermo cycling conditions were as follows: one cycle of 10 minutes at 95°C for activation of the polymerase, 40 cycles of 10 seconds at 95°C and one minute at 60°C for amplification. Dissociation curve analysis was carried out to verify the absence of primer dimers and/or non-specific PCR products. The relative expression of the genes of interest was normalized against the α-tubulin housekeeping genes by a comparative cycle of threshold (Ct) value method (ABI user bulletin # 2). The

difference between the mean C_t values of the gene of interest and the housekeeping gene was labelled ΔC_t, and the difference between ΔC_t and the C_t value of the calibrator sample was labelled $\Delta\Delta C_t$. The $\log_2 (\Delta\Delta C_t)$ gave the relative value of gene expression.

Table 3.2: Primers Pairs Used in the qRT-PCR

Genes	Primer sequences
MMP13	FORWARD: 5'- CATTTGATGGGCCCTCTGGCCTGC-3'
	REVERSE: 5'- GTTTAGGGTTGGGGTCTTCATCTC -3'
ACAN	FORWARD: 5'- TGAGGAGGGCTGGAACAAGTACC- -3'
	REVERSE: 5'- GGAGGTGGTAATTGCAGGGAACA -3'
SREBF-2	FORWARD: 5'-GTCAGCTGCCAAGGAGAGTC -3'
	REVERSE: 5'- TCATAACCCCCACAGAGTCC -3'
NPC1L1	FORWARD: 5'- CATCTTTGCCACCAGCTACA -3'
	REVERSE: 5'- GAAAGGAACCACTTGCAGGA -3'
ABCA1	FORWARD:5'- TGGCAGTGTCCAGCATCTAA -3'
	REVERSE: 5'- AGCATGTTCCGGTGTTTCTC -3'
NR1H3	FORWARD:5'- GAGAGGCTGCAGCACACATA -3'
	REVERSE: 5'- AGACGCAGTGCAAACACTTG -3'
Beta-actin	FORWARD: 5' - CAGGTCATCACYATYGGCAATGAGC--3'
	REVERSE: 5'- CGGATGTCMACGTCACACTTCATGA -3'
RUNX2	FORWARD: 5'- ACTTTGACAATAACTGTCCT -3'
	REVERSE: 5'- GACACCTACTCTCATACTGG-3'
BAX	FORWARD: 5'- CTGAGCTGACCTTGGAGC -3'
	REVERSE: 5'- GACTCCAGCCACAAAGATG-3'
CYCS	FORWARD: 5'- GCTGGTGGTGACTTTCTCT -3'
	REVERSE: 5'- GCTTGCTTCACTGCTGCCAT -3'
SOX9	FORWARD: 5'- TGAATCTCCTGGACCCCTTC -3'
	REVERSE: 5'- TGCTGGAGCCGTTGACGCG -3'

3.2.17 Protein extraction and western blotting

Total protein was harvested by lysing the ACCs pellets in a lysis buffer containing 1 M Tris HCl (pH 8), 5 M NaCl, 20% Triton X-100, 0.5 M EDTA and a protease inhibitor cocktail (Roche, Dee Why, NSW, Australia). The cell lysate was clarified by centrifugation and the protein concentration determined by a bicinchoninic acid (BCA) protein assay (Sigma-Aldrich Pty. Ltd., NSW, Australia). 10 μg of protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 12% gel. The protein was transferred to a nitrocellulose membrane, and blocked in a Tris-Tween buffer containing 5% non-fat milk. The membranes were incubated with primary antibodies against ACAN (1:2000, rabbit anti-human/mouse; Cell Signaling Technology, Massachusetts, USA), MMP-13 (1:2000, Rabbit anti-human/mouse; Abcam-Sapphire Bioscince Pty. Ltd., NSW, Australia), phospho-ERK1/2 (1:2000) and phospho-JNK (1: 1000) and α-tubulin (1:5000, Rabbit anti-human/mouse; Abcam-Sapphire Bioscince Pty. Ltd., NSW, Australia) overnight at 4^{o}C. The membranes were washed three times in TBS-Tween buffer, and then incubated with an anti-rabbit secondary antibody at 1:2,000 dilutions for one hour. The protein bands were visualized using the ECL Plus™ Western Blotting Detection Reagents (GE Healthcare, Rydalmere, NSW, Australia) and exposed on x-ray film (Fujifilm, Stafford, QLD, Australia). Immunoblot negatives were analysed by densitometry using Image J software.

3.2.18 MTT assay

Normal chondrocytes were seeded in a 96-well tissue culture plate at the density of 5000 cells per well. After 24 hours' incubation, the culture medium was removed and replaced with DMEM supplemented with 0.2% (w/v) BSA and Chol:MβCD (30μg/ml) for 24 hours. Cells incubated with 0.2% BSA without Chol:MβCD treatment served as controls. MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was applied to test the overall metabolic activity and cell viability. 20μL of 5mg/ml MTT (Sigma-Aldrich, NSW, Australia) solution was added into each well. After incubation for four hours, the DMEM-MTT solution was carefully removed and replaced by 100μL of DMSO (dimethyl sulfoxide) to dissolve the formazan crystals. The absorbance was read at the wavelength of 595 nm by a microplate spectrophotometer (Benchmark Plus, Tacoma, Washington, USA).

3.2.19 ATP assay

Passage 0 freshly plated primary chondrocytes that retain chondrogenic phenotype were plated on T75 flasks. On the day of experiment, growth medium was removed and replaced with serum free DMEM supplemented with 30 μM Chol:MβCD for 24 h. Cells incubated with 0.2% BSA (vehicle) without Chol:MβCD served as controls. Next day, Chondrocytes with $1x10^4$ cells/ml density were cultured on a 96-well plate and the cellular ATP content of the chondrocytes was determined by a bioluminescence assay based on the luciferase requirement for ATP in producing emitted light, according to the protocol for the CellTiter-Glo^{R}Luminescent Cell Viability Assay Kit (Promega, Madison, USA). Briefly, plates with its content were equilibrated at room temperature for 30 minutes. Then, 100 μl of CellTiter-Glo reagent was added to each well. The content of each well was mixed for two minutes in an orbital shaker to induce the cell lysis. The plate was incubated at room temperature for 10 minutes to allow the luminescence signals to be stabilized. Luminescence was recorded using a Luminometer.

3.2.20 Rhodamine 123 assay (RH-123)

Passage 0 freshly plated primary chondrocytes were plated on T75 flasks and treated with 30 μM Chol:MβCD for 24 h. Cells incubated with 0.2% BSA (vehicle) without Chol:MβCD served as controls. Chondrocytes were first washed with phosphate-buffered saline (PBS) and then centrifuged (10000 g, 4 min) twice. Following measurement of viable cells using trypan blue, 90 μl of suspended chondrocytes (1 X 106 cells/ml) in a sodium citrate buffer and 10μl of Rhodamine 123 solution (500μM) (Sigma-Aldrich Pty Ltd, NSW, Australia) were incubated for 10 min at 37°C in the dark. After incubation, stained cells were centrifuged for one minute at 10 000g and the pellet was suspended in a HEPES buffer (Gibco-Invitrogen, VIC, Australia). 20μl of stained sample was read using a fluorescent plate reader at 505 nm excitation and 534 nm emission wavelengths.

3.2.21 Cellular ROS detection

Cell Cellular ROS detection assay was performed according to the protocol for the 2',7' –dichlorofluorescin diacetate (DCFDA) Cellular ROS Detection Assay Kit (Abcam, Sapphire Bioscience Pty. Ltd., NSW, Australia). Passage 0 freshly plated primary chondrocytes were seeded on the T75 flasks and left overnight to attach. On the day of experiment, growth medium was removed and replaced with serum free DMEM with 30 μM Chol:MβCD for 24h. Cells incubated with 0.2% BSA (vehicle) without Chol:MβCD served as controls. After 24 h, $1.5x10^7$cells were resuspended in DCFDA mix and incubated at 37°C for 30 minutes in the dark. Cells were washed in buffer solution by centrifugation and resepended in the supplemented buffer. $1x10^5$ stained cells/50μL were seeded per well in a 96-well dark plate. The fluorescence was read using a fluorescence plate reader to perform an endpoint read at 485 nm excitation wavelength and 535 nm emission wavelength.

3.2.22 JC-1 staining

Mitochondrial electrochemical potential gradient (ΔΨ) was assessed using fluorescent dye 5,5',6,6'-tetrachloro-1,1',3,3' tetraethylbenzimidazolylcarbocyanine iodide (JC-1). Chondrocytes were cultured at $0.2x10^6$ cells/ml and incubated overnight. The next day, media was removed and replaced with DMEM supplemented with 0.2% (w/v) BSA and Chol:MβCD (30μg/ml) for 24 hours. Cells incubated with 0.2% BSA without Chol:MβCD treatment served as controls. Cells were incubated with JC-1 dye (Sigma-Aldrich, NSW, Australia) for 20 minutes at 37°C in a humidified atmosphere containing 5% CO_2. JC-1 aggregates were visualized under fluorescence microscope (Carl Zeiss Microimaging GmbH, Gottingen, Germany) using FITC/Cy3 filters. Mitochondrial depolarization is indicated by a shift from red to green fluorescence.

3.2.23 Transmission electron microscopy (TEM)

TEM is a microscopy technique, whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. TEMs are capable of imaging at a significantly higher resolution than light microscopes and suited to study small cell organelles such as mitochondria.

Chondrocytes with $2x10^4$ cells/ml density were cultured on 6-well plate. After overnight incubation, the medium was removed and replaced with DMEM supplemented with 0.2% (w/v) BSA and Chol:MβCD (30μg/ml) for 24 hours. Cells incubated with 0.2% BSA without Chol:MβCD treatment served as controls. After treatment with cholesterol, cells were immediately fixed with glutaraldehyde. As a second fixative procedure, osmium tetroxide was used to crosslink and stabilize lipids in cells and organelle membrane. Fixation was followed by dehydration of the tissue in ethanol, followed by embedding in resin. Thin sections were cut from resin blocks and stained. Images of the stained slides were then captured using a transmission electron microscope.

3.2.24 *In situ* cell death detection (apoptosis) using TUNEL

Chondrocytes apoptosis was assessed principally at the single cell level, based on labelling of DNA strand breaks (Terminal deoxynucleotidyl transferase dUTP nick end labelling or TUNEL technology) using an *In Situ* Cell Death Detection Kit, Fluorescein (Roche, Germany) according to manufacturer's protocol. TUNEL positive chondrocytes were visualized under a fluorescence microscope (Carl Zeiss Microimaging GmbH, Gottingen, Germany).

3.2.25 Micro-CT analysis

Micro-CT analysis was performed as described previously in our group (Afara, Prasadam, Crawford, Xiao, & Oloyede, 2013). Briefly, intact joints were scanned in a 7- or 12-mm sample tube on a micro CT (Scanco 40, Switzerland) with an isotropic voxel size of 18 μm after tissues were fixed in 4% paraformaldehyde (PFA) in PBS as a scanning medium. The x-ray tube voltage was 55 kV and the current was 145μA, with a 0.5 mm aluminium filter. The exposure time was 1180 m. In the medial part of each tibia, a volume of interest was selected with an automatic contouring method. The circles were located in the middle of the load-bearing and non-load bearing sites of subchondral bone areas. Selected areas contained a subchondral plate, but did not contain a subchondral trabecular bone or growth-plate tissue. A total of 30 consecutive tomographic slices were analysed from the proximal part of the medial tibia plateau.

The meaningful subchondral bone plate measurements such as bone volume (mg HA/ccm) and BV/TV (%) were analysed using inbuilt software.

3.3 ANIMAL STUDY

3.3.1 Animal models

ApoE$^{-/-}$ mice were purchased from the Animal Resource Centre (Laboratory Animal Cenre of Central South University, China). Experiments were performed in accordance with protocols approved by the Xiangya Hospital, the Central South University of China (ethics number: 2013-053). The mice were fed either standard/control rodent chow (CD) or a high cholesterol diet (HCD) (normal diet with 1.25% cholesterol, 0.5% sodium cholate; Specialty Feeds, WA, Australia) diet two weeks prior to OA induction to raise the hypercholesterolemia phenotype in mice (9 mice per group).

In dietary induced hypercholesterolemia (DIHC) rat model, animals were purchased from the Animal Resources Centre (Perth, Western Australia) and experiments were performed in accordance with animal ethics approved by Queensland University of Technology (ethics number: 1300000283). Standard/control diet (CD) was substituted with high cholesterol diet (HCD) (10% fat, 2% cholesterol and 0.5% cholate; Specialty Feeds, WA, Australia) four weeks prior to OA induction (6 rats per group). Body mass and food consumption were measured each week and blood samples taken to assess serum lipid profiles (total cholesterol) using commercially available kits to confirm the hypercholesterolemia (HC) phenotype.

To investigate whether statin and anti-oxidant treatment can reduced OA changes in ApoE$^{-/-}$ mice and DIHC rats, atorvastatin (Merck Millipore, VIC, Australia) and Mito-tempo/MitoQ (Sigma-Aldrich Pty Ltd, NSW, Australia) drug administration started three weeks prior to the mice and rats sacrifice. Animals were given 3mg/kg/day atorvastatin orally and mito-TEMPO-MitoQ was injected to both models subcutaneously at 1.5mg/kg dose every second days.

3.3.2 Induction of OA in mice and rats

To induce OA in mice, the right knee is opened via a medial parapatellar approach and by dissecting the medial meniscus ligament to achieve destabilization of medial meniscus in the right knee joint for DMM model as described previously (Yang et al., 2010). Mice in the control group underwent a sham surgery that did not dissect the medial meniscus ligament. OA in rats was induced by medial menisectomy surgery for MSX model as described previously in our group (I. Prasadam, X. Mao, W. Shi, R. Crawford, & Y. Xiao, 2013; I. Prasadam et al., 2012; I. Prasadam, Y. Zhou, W. Shi, R. Crawford, & Y. Xiao, 2014)(Hulth A, 1970; Indira Prasadam"_ENREF_215" \o "Hulth A, 1970 #317" Hulth A, 1970; Indira Prasadam, Xinzhan Mao, Wei Shi, Ross Crawford, & Yin Xiao, 2013; Indira Prasadam et al., 2012; Indira Prasadam, Yinghong Zhou, Wei Shi, Ross Crawford, & Yin Xiao, 2014). These animals were then euthanized at five and eight weeks after surgery. Knee joint tissues were processed for histological evaluation.

3.3.3 Flourchrome injection

To assess subsequent new bone formation and turnover, fluorescence labelling dyes were injected to DIHC rats intraperitoneally. Alizarin red S (Sigma-Aldrich Pty. Ltd., NSW, Australia) (25mg/kg, three days before sacrifice) and Calcein (Sigma-Aldrich Pty. Ltd., NSW, Australia) (10mg/kg, 10 days prior to alizarin injection) were administered and the animals were euthanized. Knee samples were collected and fixed in 4% PFA followed resin embedding procedure.

3.3.4 Resin embedding

Undecalcified tissue samples were dehydrated through a graded ethanol series (70%, 80%, 90%, and 100%) for one week each and processed through three clearing in of xylene for four hours. Samples were then infiltrated with Methyl methacrylate (MMA) and subsequently incubated with a mixture of MMA and polyethylene glycol (PEG) as previously described in our research group (Jaiprakash et al., 2012). To initiate the polymerisation process, the catalyst Perkadox (0.3%) was added to a MMA/PEG (3%) solution. Samples were fully covered with the MMA/PEG/Perkadox solution and incubated at 50ºC for 10 minutes. Specimens were put to rest until fully

polymerised. Embedded samples were placed under a vacuum adhesive press and grinded to face the sample parrallel to the slide. Final mounted slides were cut into thin sections using an automated sledge microtome. The section was positioned in the grinding system and ground to a final thickness using an electronic measuring system.

3.3.5 Total cholesterol analysis

Blood samples were collected in Ethylenediaminetetraacetic acid **(**EDTA) coated tubes after four hours of food deprivation by tail incisions at eight weeks post surgeries. Blood smaples were centrifuged for 10 minutes at 600 rpm, and then plasma was separated and collected for total cholestreol analysis. For this purpose, plasma of each group was measured following cholesterol assay kit protocol (Abcam-Sappaire Bioscience pty. Ltd., NSW, Australia) and analysed for total cholesterol levels. 20µL of each smaple was added to each well of 96-well plate and adjusted to 50µl with assay buffer. Next, 50µL of total cholesterol reaction mix was added to each well and mixed well. Smaples were incubated at 37 c◦ for 60 minuets protected from light following measurement immediately on a microplate reader (Benchmark Plus, Tacoma, Washington, USA) at 570 nm wavelength.

3.3.6 Faxitron x-ray radiography

The Faxitron MX20 (Faxitron X-Ray, USA) is a commercially available x-ray machine designed for animal irradiation. After sacrificing the DIHC rats, both legs were dissected and radiographic images of both legs were taken using a Faxitron x-ray machine by placing the legs in a direction to have a frontal view of the knee.

3.4 STATISTICAL ANALYSIS

Six rats and nine mice per group were used for *in-vivo* study and all *in-vitro* experiments were performed on cells that were isolated from the tibia plateau of twenty-four different patients aged 48-79 undergoing knee replacement. In vitro, three samples were used for histology investigation while six samples were used for quantifying experiments. Three cartilage discs from three bovines were used for bovine explants culture. Data are expressed as means ± standard deviations (SDs). Analyses were carried out on the three replicate samples. Data were analyzed using

un-paired student t-test or one-way ANOVA with Dunnet's post-test when we had normal distribution between data or Kruskal-Wallis non-parametric ANOVA with a Dunnet's post hoc test, when contribution was not normal between two or more sets of data. I performed statistical analyses using GraphPad Prism version 5.01 (GraphPad software, Inc.). A probability (p) value <0.05 was considered statistically significant.

Chapter 4: Results

4.1 CHOLESTEROL DISTRIBUTION IN CARTILAGE FROM OA PATIENTS AND CHOLESTEROL REGULATION IN CHONDROCYTES *IN-VITRO.*

Data are expressed as means ± standard deviations (SDs). Analyses were carried out on the three replicate samples. Data were analyzed using un-paired student t-test using GraphPad Prism version 5.01.

Oil Red O staining of normal and OA cartilage

Oil red O staining was performed to investigate lipid distribution in OA and normal human cartilage tissues. Fresh human cartilage was obtained from patients undergoing knee replacement (n=3). Normal and OA cartilage were separated from the tibia plateau as mentioned in Section 3.2.1. Normal histological procedure on sections obtained from paraffin blocks consists of ethanol hydration and dehydration steps prior to staining which resolves the lipid content of tissue. Therefore, our intention to perform the oil red staining on fresh frozen samples was to record the lipid profile of the cartilage tissue during the histological procedure. Serial 5µm sections were cut from fresh cartilage as detailed in Section 3.2.1. Histological oil red O staining was performed as per Section 3.2.12. This histological method was used to characterize the lipid content of OA cartilage compared to normal and allowed for easy estimation of tissue lipid distribution. We compared normal and OA cartilage to characterize the lipid content of OA cartilage against normal cartilage. Oil Red O staining of normal and OA cartilage showed a different lipid profile of OA cartilage compared to normal (Figure 4.1). Using this method, we detected more lipid droplets in OA cartilage tissue than normal cartilage, which may represent excess lipid accumulation in OA cartilage compared to normal. This may suggest that excess lipid accumulation in peripheral tissue is a feature of metabolic syndromes. Higher lipid distributions in OA cartilage compared to normal tissue indicates an abnormal lipid profile of OA patients. These findings suggest that a high lipid profile may play a role in the initiation and/or development of OA.

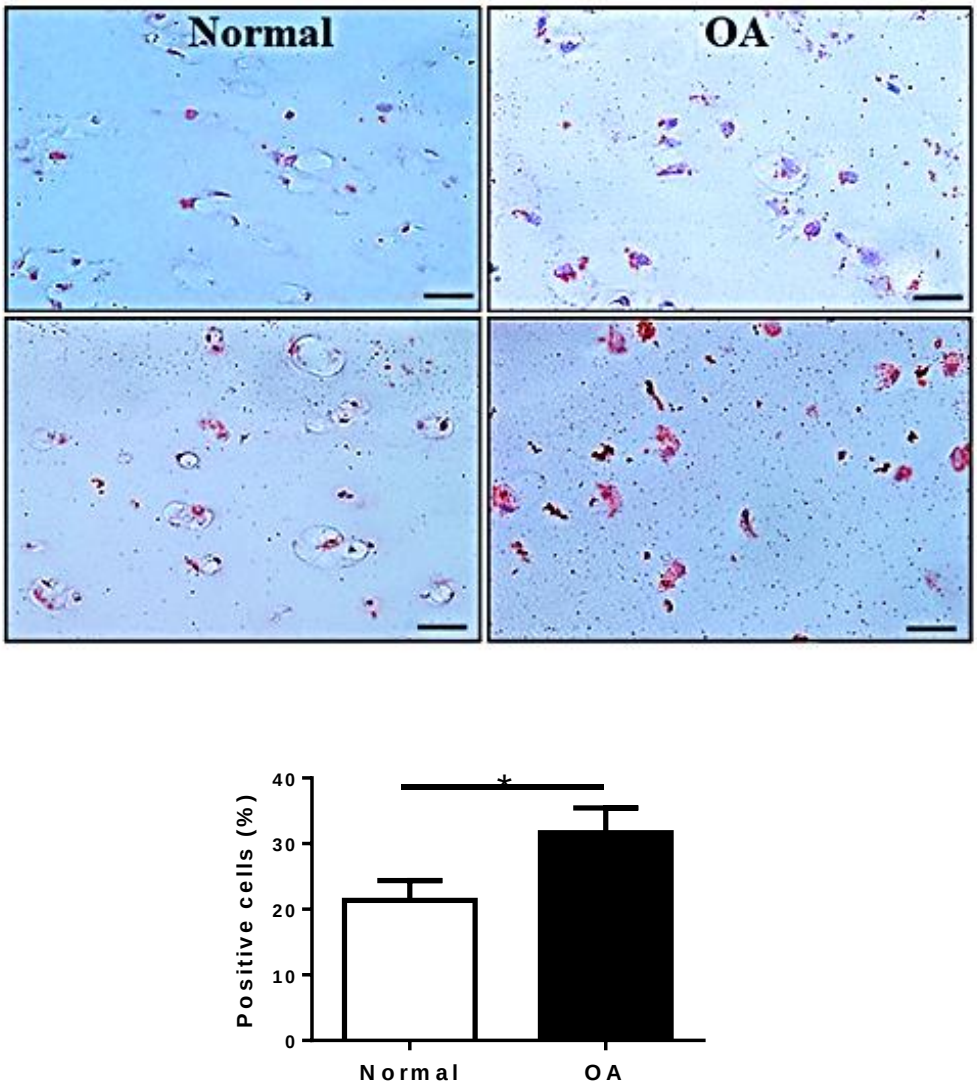

Figure 4.1: Oil Red O staining of normal (left) versus OA (right) human cartilage tissue. Fresh cartilage was obtained and classified to normal and OA (n=3). Cartilage tissues were frozen in liquid nitrogen and embedded in OCT medium. Embedded samples in OCT blocks were stored at -80 ◦c freezer for cryostat microtomy and sections were fixed by being dipped into acetone a few times. Oil Red O staining was performed to characterize lipid distribution in cartilage tissue, comparing normal and OA. Red staining represents lipid accumulation and droplets and purple counterstaining represents the nuclei. There are higher lipid extents (red droplet) in OA cartilage compared to normal. Percentages of total positive cells in OA cartilage was significantly higher compared to normal. *P<0.05 versus normal. Scale bars=50μm.

Histochemical filipin staining

Based on Oil red staining experiment, we found higher lipid distribution in OA cartilage compared to normal. Therefore, we carried out cholesterol staining technique to test specifically where cellular cholesterol is located in the cultured OA and normal ACCs. Primary normal and OA ACCs (n=3) were isolated form human cartilage as outlined in Section 3.2.2 and cells were cultured according to Section 3.2.3. Next, we tested if we could detect cholesterol location and distribution in cultured ACCs by comparing normal and OA ACCs. For this purpose, cells were cultured according to Section 3.2.3, we then performed filipin staining on cultured ACCs according to Section 3.2.13. Under normal conditions, 80-90% of total cholesterol is present at the cell membrane. Within cells, intracellular cholesterol may move to different compartments through cholesterol trafficking (efflux and efflux) pathways. Defects in these transport pathways can alter cholesterol metabolism resulting in pathological states. We used a filipin probe to visualize a cholesterol location by comparing OA and normal ACCs. Our results showed more intracellular cholesterol in OA ACCs compared to normal ACCs, whereas most cholesterol presented in cell membranes (Figure 4.2). In normal ACCs, most cholesterol presented in the cell walls, which are indicated with a yellow arrow; however, OA ACCs staining demonstrated a movement of cholesterol from the cell wall to the intracellular space where cholesterol accumulated forming a droplet shape, as indicated by red arrow. This observation indeed demonstrates that intracellular accumulation of cholesterol may highlight a dysregulated cholesterol metabolism in OA ACCs as a characteristic of OA obtained from OA patients. This result supports the contention that high lipid levels are present in OA cartilage.

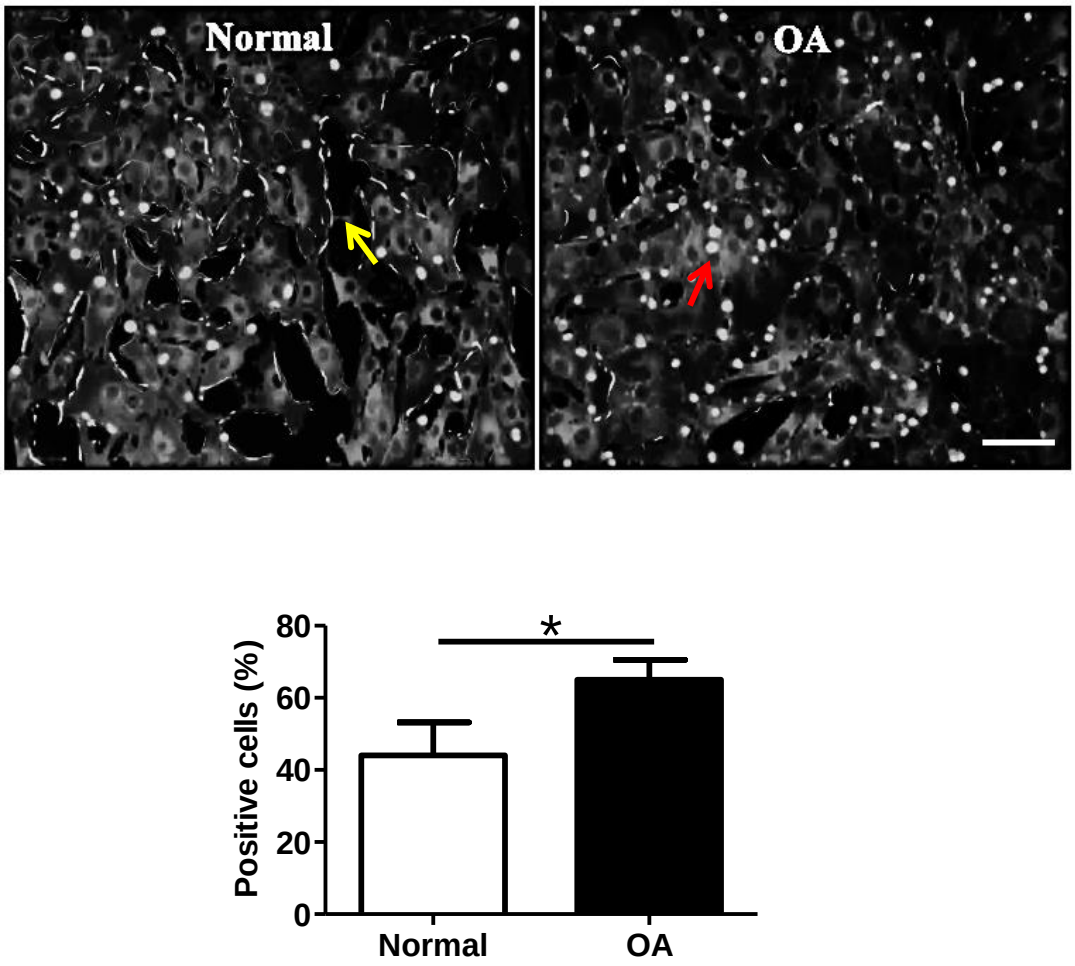

Figure 4.2: Histochemical filipin staining of normal versus OA chondrocytes. ACCs were isolated form cartilage tissue obtained from patients undergoing knee replacement (n=3). Two x 10^4 cells/well were fixed by Cell-based Assay Fixative solution and incubated with a filipin fluorescence probe in the dark. Cholesterol location was visualised using a Carl Zeiss fluorescence microscope under an excitation of 340-380 nm and emission of 385-470 nm. There is more intracellular cholesterol in OA ACCs (red arrow) compared to normal ACCs. The location of cholesterol in normal ACCs is mostly at the plasma membrane (yellow arrow). Percentages of total positive cells for pilipin staining in OA chondrocytes was significantly higher than normal cells. *P<0.05 versus normal. Scale bars=50μm.

Cholesterol Influx and efflux genes mRNA expressions level (qRT-PCR)

SREBP-2 is best known as a regulator of genes involved in cholesterol biosynthesis and uptake. NPC1L1 protein is an important efflux gene plays a critical role in the absorption of intestinal cholesterol. The liver X receptors (LXRs) act as

cholesterol sensors: when cellular oxysterols accumulate as a result of accumulative concentrations of cholesterol, LXR triggers the transcription of genes that defend cells from cholesterol overload. LXRα (NR1H3) and LXRβ (NR1H2) are two isoforms of LXR, which regulate gene expression. ABCA1 is another pivotal regulator of cholesterol efflux from cells to apolipoproteins.

Our findings demonstrated abnormal cholesterol and lipid accumulation at cellular and tissue levels in OA samples compared to normal (figure 4.2). In order to investigate molecular mechanism underlying this dysregulated cholesterol metabolism and distribution, we tested cholesterol regulatory genes expression in normal and OA ACCs pellets and cartilage. To assess the mRNA expression of cholesterol regulation genes, we extracted RNA from human cartilage tissue (n=6) and ACCs pellets (n=6) followed by qRT-PCR as outlined in Section 3.2.16.

The expression of influx genes (SREBF-2 and NPC1L1) was increased in the OA group of both cartilage and ACCs pellets compared to normal (Figure 4.3). Influx genes control cholesterol absorption into intracellular space and an increase in expression of SREBF2 and NPC1L1 genes points to excess intracellular accumulation of cholesterol (Figure 4.3). Expression of efflux genes NR1H3 was significantly decreased in both OA cartilage tissue and OA ACCs pellets, whereas expression of the other efflux gene ABCA1 was increased in the OA cartilage tissue and decreased in OA ACCs pellets, which did not reach statistical significance (Figure 4.4).

Abnormal cholesterol accumulation occurs when cholesterol influx into the tissue wall exceeds cholesterol efflux. Here, we found cholesterol influx genes such as SREBF2 and NPC1L1 mRNA levels were significantly higher in the OA group compared to normal. Moreover, cholesterol efflux genes such as NR1H3 and ABCA1 mRNA levels were lower in OA group compared to normal. The differential expression pattern of these cholesterol efflux and influx genes between the normal and OA groups may suggest a link between cholesterol related mechanisms and development and/or progression of OA. In the other word, these findings indicate that these cholesterol regulatory pathways are altered in OA patients. The results obtained from first aim support this study's hypothesis that abnormal levels of cholesterol govern signalling molecules, which may play a role in initiation and/or development of OA.

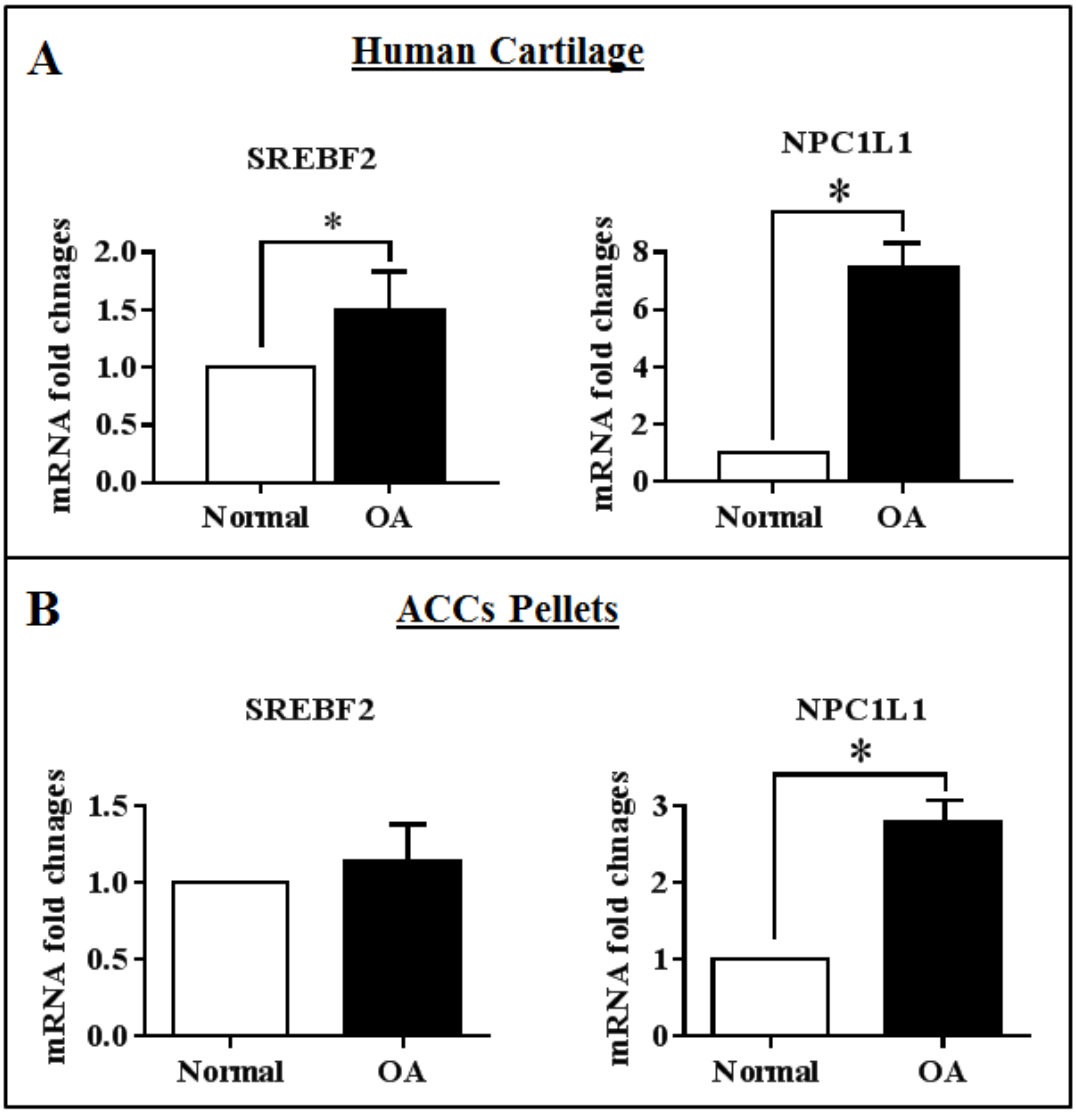

Figure 4.3: Expression of cholesterol influx genes is up-regulated in OA cartilage and ACCs pellets: mRNA was extracted from normal and OA cartilage and ACCs pellets. qRT-PCR was performed to determine the mRNA expression of cholesterol influx genes at both tissue and cellular levels **(A)** The mRNA expression of SREBF-2 and NPC1L1 was significantly increased in OA cartilage tissue compared to the normal group (n=6). Data are shown as mean ± SD. *P<0.05 versus normal. **(B)** The NPC1L1 expression was significantly up-regulated in OA ACCs pellets compared to normal (n=6). Data are mean ± SD. P0-P2 ACCs pellets were used for all the experiments. *P<0.05 versus normal. Quantitative measurements were determined using the (2−ΔΔCt); method and beta-actin expression were used as endogenous housekeeping genes. All samples were performed in triplicate. The data is normalised to normal ACCs pellets arbitrarily set to 1.

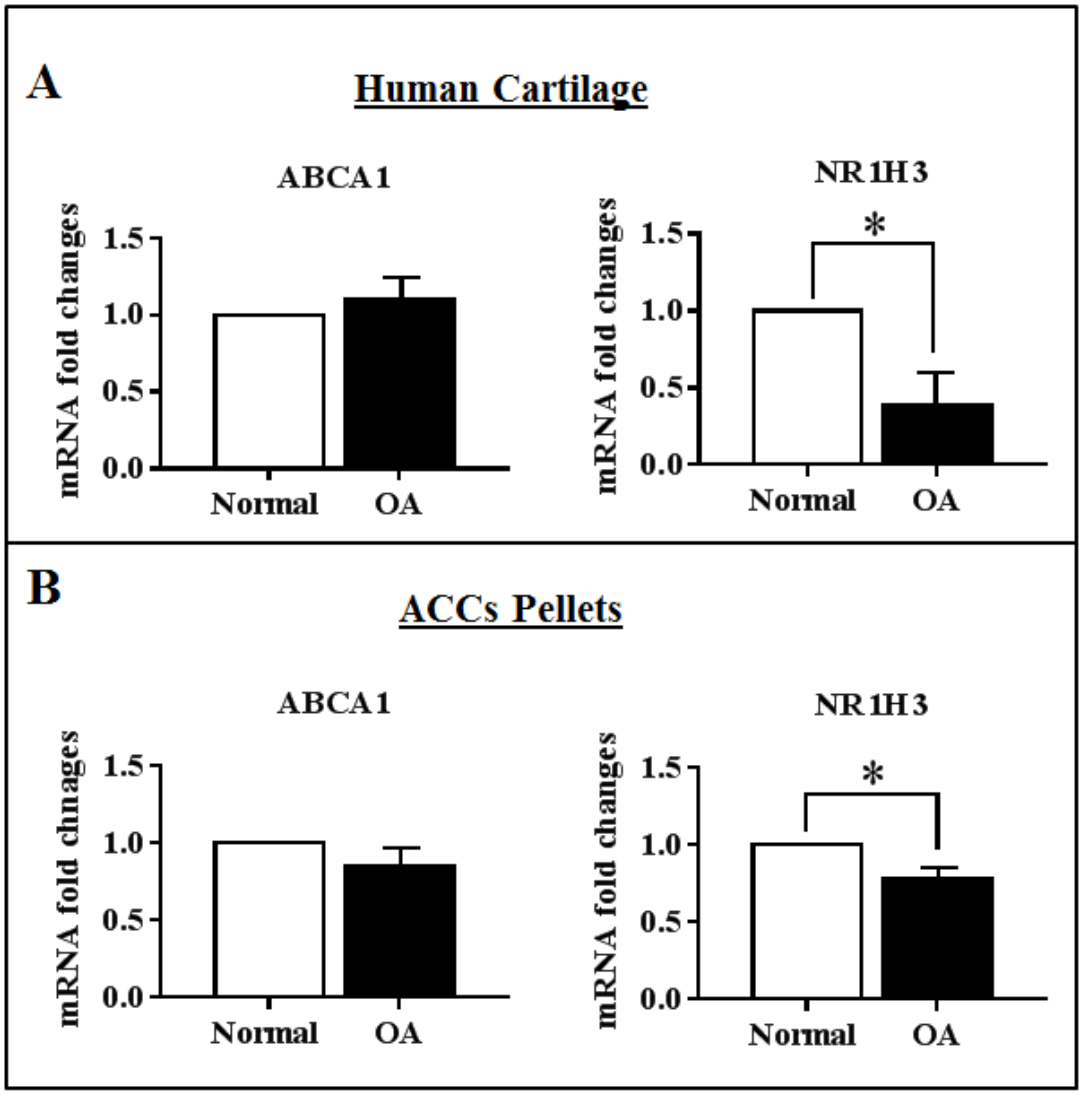

Figure 4.4: Expression of cholesterol efflux genes is down-regulated in OA cartilage and ACCs pellets: mRNA was extracted from normal and OA cartilage and ACCs pellets. qRT-PCR was performed to determined mRNA expression of cholesterol efflux genes at both tissue and cellular levels. **(A)** The mRNA expression of NR1H3 was significantly elevated in OA cartilage tissue compared to normal; however, an increase in the expression of ABCA1in OA cartilage did not reach a significant level (n=6). Data are mean ± SD. *P<0.05 versus normal. **(B)** Similar to cartilage efflux gene expression, the mRNA expression of NR1H3 was significantly increased in OA ACCs pellets compared to normal. In contrast, there was no significant difference in the expression of ABCA1 in OA and normal ACCs pellets (n=6). Data are mean ± SD. P0-P2 ACCs pellets were used for all the experiments. *P<0.05 versus normal. Quantitative measurements were determined using the

(2−ΔΔCt); method and beta-actin expression were used as endogenous housekeeping genes. All samples were performed in triplicate. The data is normalised to normal ACCs pellets arbitrarily set to 1.

4.2 ABNORMAL CHOLESTEROL CHALLENGES THE CARTILAGE PHENOTYPE AND MITOCHONDRIAL FUNCTION *IN-VITRO.*

In-vitro effect of cholesterol stimulation on ACCs pellets:

Upon completion of our first aim of this project, we already found that the differential expression of genes, which are responsible for cholesterol regulation, might be the most likely explanation for abnormal cholesterol distribution in OA samples compared to normal. Therefore, we hypothesized that those abnormal levels of cholesterol which were found in OA samples might be a contributor to initiation and/or development of OA. In orther to test our hypothesis, we performed a set of *in vitro* expriments to test the effect of abnormal cholesterol levels on chondrocytes.

To examine the effect of cholesterol stimulation *in vitro*, we performed a well-stablished ACCs 3D pellet culture model as outlined in Section 3.2.4 and ACCs pellets were treated with different doses of cholesterol, i.e. 5, 10, 30μg/ml of MβCD/chol for 14 days as detailed in Section 3.2.6. Cholesterol-stimulated pellets were stained using Safranin O staining and Alcian blue staining methods as per Section 3.2.9 and Section 3.2.10 respectively.

Safranin o and Alcian blue staining are two well-stablished methods used for histological analysis of proteoglycans. Cartilage and matrix were stained red and the background was stained green using Safranin O staining, whereas the matrix was stained blue in Alcian blue staining.

Proteoglycans are a major component of extracellular cartilage matrix and loss of proteoglycan leads to cartilage damage, which is a common incidence during OA progression. Histological observation showed a cholesterol dose dependent proteoglycan loss in both staining methods (Figure 4.5A). Proteoglycans of the matrix were remarkably lost after a high dose of cholesterol stimulation in the *in-vitro* ACCs pellet model. Moreover, Safranin O staining showed that ACCs pellets stimulation

with a high dose of cholesterol can damage the pellet's surface, representing cartilage degradation in OA development.

To quantify proteoglycans that were released from the ACCs pellets into the medium during the cholesterol stimulation, we performed a sGAG assay as per Section 3.2.7. We used condition media from ACCs 3D pellet culture to quantify the amount of sGAG. The sGAG release was determined by measuring sulphated glycosaminoglycan content compared to a chondroitin sulphate standard and expressed as μg/ml since each 1 ml of condition media contained similar amounts of cells per pellets. The amount of proteoglycans detected by the collected medium during the experiment is in line with the results obtained from the histological staining (Figure 4.5B). This experiment points to a destructive effect of a high dose of cholesterol on ACCs *in-vitro*.

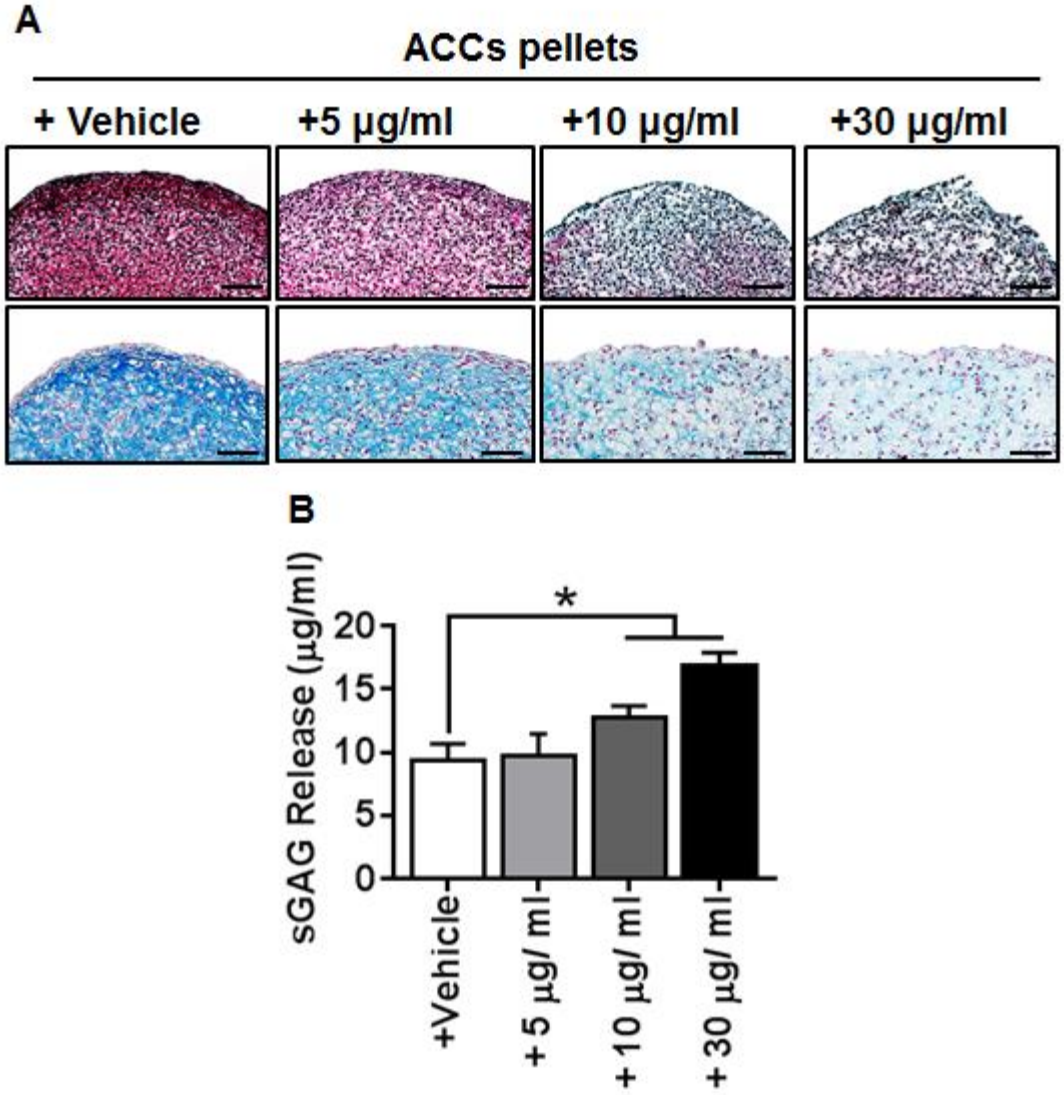

Figure 4.5: Effect of cholesterol stimulation on ACCs in 3D pellet culture. ACCs were isolated and differentiated in a chondrogenic media supplemented with 0.2% (w/v) BSA and with different doses of MβCD/chol (5, 10, 30µg/ml) for 14 days. ACCs pellets incubated with 0.2% BSA (vehicle) without Chol:MβCD served as controls. The media was collected to quantify sGAG deposition form pellets. (A) Representative Safranin O and alcian blue staining of ACCs pellets which were stimulated with different doses of cholesterol. There was a dose dependent proteoglycan loss in cholesterol–treated ACCs pellets compared to the control. At the very high doses of cholesterol (30µg/ml), pellets surface degradation was observed compared to the control. (B) sGAG content in condition medium of ACCs pellets. Deposition of sGAG increased with an acceleration in the dose of cholesterol. P0-P2 ACCs pellets were

used for all experiments (n=6). All samples were performed in triplicate. Scale bar=100µm. Data are mean± SD. *P<0.05 versus +vehicle.

Effect of cholesterol stimulation on bovine explants culture:

To test the impact of high cholesterol stimulation *in vitro*, we also performed a bovine explant culture in accordance with Section 3.2.5 as our second *in vitro* model. Bovine explant culture is widely used as an *in-vitro* model to study the collagens and proteaglycans network of the cartilage tissue (Bascoul-Colombo et al., 2015; Seol et al., 2014).

Bovine cartilage disks were stimulated with 50µg/ml cholesterol as outlined in Section 3.2.5 and the amount of sGAGs released form bovine disks into the medium were quantified using sGAG assay as per Section 3.2.7. Quantified sGAG levels showed a significant proteoglycan release from bovine cartilage; however, the \ from Saranin O staining of bovine explants did not show remarkable difference (Figure 4.6). The most possible justification for our histological observation is that the collagen degradation is typically a slower event and typically occurs subsequent to GAG release in cartilage explant models (Little et al., 2002; Shingleton, Jones, Xu, Cawston, & Rowan, 2006).

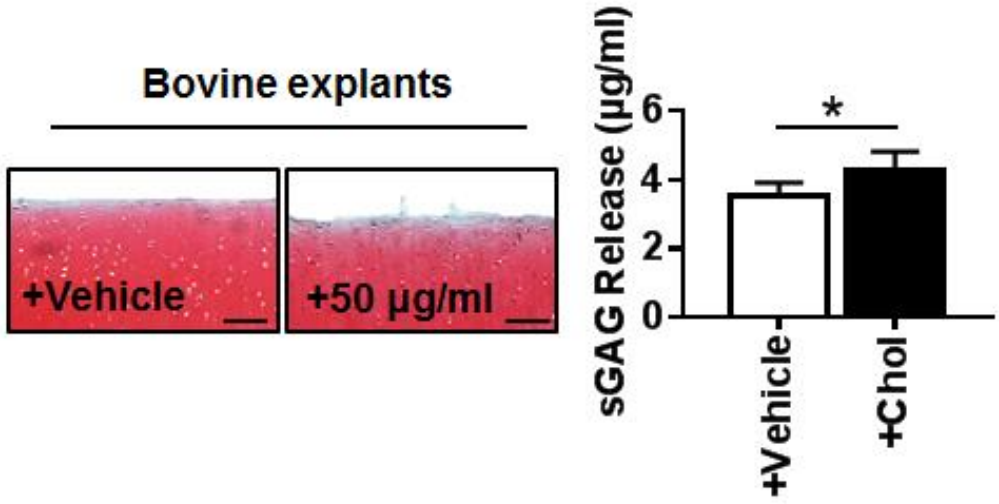

Figure 4.6: Effect of cholesterol stimulation on bovine explant culture. Bovine cartilage discs were cultured in serum free media supplemented with 0.2% (w/v) BSA and 50µg/ml Cholesterol/MβCD. Bovine discs incubated with 0.2% BSA (vehicle)

without Chol:MβCD served as controls. The media was collected to quantify sGAG deposition form bovine explants. **(A)** Safranin O staining of bovine disk stimulated with 50μg/ml of cholesterol. **(B)** sGAG content in the condition medium of the bovine explant culture (n=3). All samples were performed in triplicate. Scale bar=100μm. Data are shown as mean± SD. *P<0.05 versus +vehicle.

Immunofluorescence staining of ACCs in the 3D pellet culture

As demonstrated in figure 4.5, high level of cholesterol has a destructive effect of ACCs pellets. Here, we investigate molecular expression of genes, which are responsible for OA-like changes. To analyse the OA-like marker expression under the effect of the high cholesterol stimulation in vitro, we performed 3D ACCs pellet culture as outlined in Section 3.2.4 and stimulated the pellets with 30μg/ml cholesterol as described in Section 3.2.6. To assess the expression of COL10 (cartilage hypertrophy marker), MMP13 (cartilage degradation marker) and aggrecan (ACAN; cartilage chondrogenic marker), we performed immunofluorescence staining as per Section 3.2.15.

Similar to the *in vivo* results obtained from the immunofluorescence staining of the ACCs pellets *in vitro*, cholesterol stimulation increased COL10 and MMP13 expression in histological sections of ACCs pellets, while it decreased ACAN expression (Figure 4.7). The percentages of total positive cells stained for these markers were significantly changed after cholesterol stimulation (Figure 4.7).

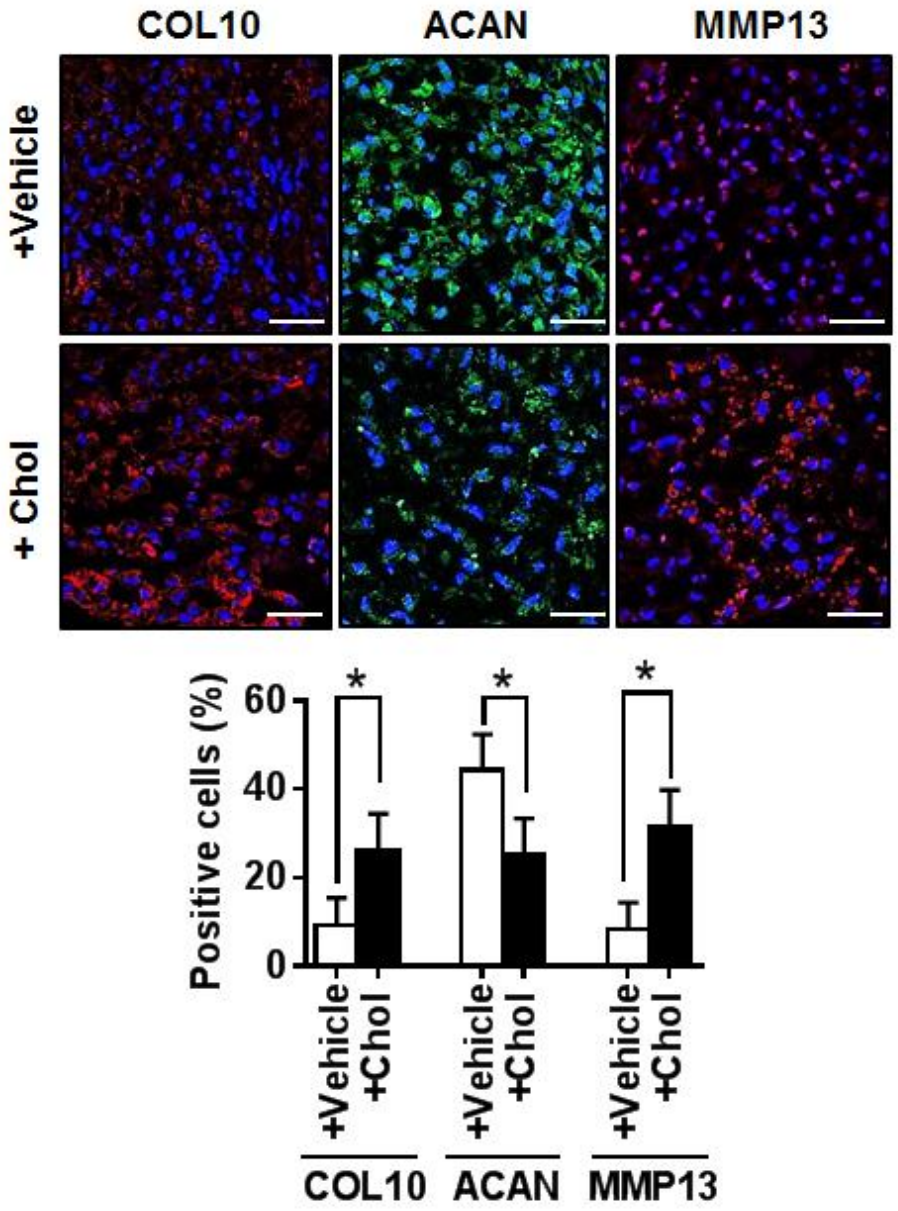

Figure 4.7: Immunofluorescence staining of chondrocytes. Immunofluorescence staining of COL10, ACAN, and MMP13 after cholesterol stimulation (30µg/ml) in 3D cultured ACCs. ACCs pellets incubated with 0.2% BSA (vehicle) without Chol:MβCD served as controls. Percentage of total positive cells was used as a standard measure to quantify. Percentages of total positive cells stained were increased for MMP13 and COL10, but significantly decreased for ACAN. P0-P2 chondrocytes pellets were used for all of the experiments (n=3). Scale bars = 75µm

ACCs 3D pellet mRNA expression after cholesterol stimulation (RT-qPCR)

Histological immunofluorescence staining of the ACCs pellets revealed increased altered expressions of COL10, ACAN, and MMP13. To further analyse whether excess cholesterol effected chondrocytes mRNA expression *in vitro*, 3D ACCs pellet culture was performed as outlined in Section 3.2.4 and ACCs pellets were treated with 30μg/ml cholesterol as described in Section 3.2.6. After completion of the cholesterol treatment, RNA was extracted from the ACCs pellet and qRT-PCR was used to assess gene expression of MMP13, ACAN, runt-related transcription factor 2 *(*RUNX2) and transcription factor SOX-9 *(*SOX9) at the mRNA level as outlined in Section 3.2.16. It has been well-demonstrated that MMP13 and RUNX2 mRNA expression levels were up-regulated, whereas ACAN and SOX9 expression were decreased in OA cartilage. MMP13 is a cartilage degradion enzyme, which breaks cartilage extracellular matrix (ECM) genes such as ACAN during OA development (Iwamoto et al., 2003; Pullig et al., 2000; Rengel, Ospelt, & Gay, 2007). RUNX 2 is a chondrocyte hypertrophy marker and is used to assess cartilage phenotypical changes as an increased expression of chondrocyte hypertrophy markers has been observed in OA cartilage. Chondrogenic transcription factor SOX9 is essential for cartilage ECM formation and is important in maintaining chondrocytes phenotypes. Therefore, we used this marker to evaluate the cartilage chondrogenic homeostasis.

At the RNA level, cholesterol treatment significantly up-regulated MMP13 and RUNX2 expression and down-regulated SOX9 expression compared to the control (Figure 4.8; $P<0.05$). There was a trend toward decreased ACAN mRNA expression in cholesterol-treated ACCs compared to the control, but this did not reach statistical significance. This may relate to the compensatory mechanism of ACCs to maintain cartilage homeostasis (Figure 4.8; $P<0.05$). Overall, our findings demonstrate the ACCs molecular changes under the effect of excess cholesterol, which are similar to those in OA progression.

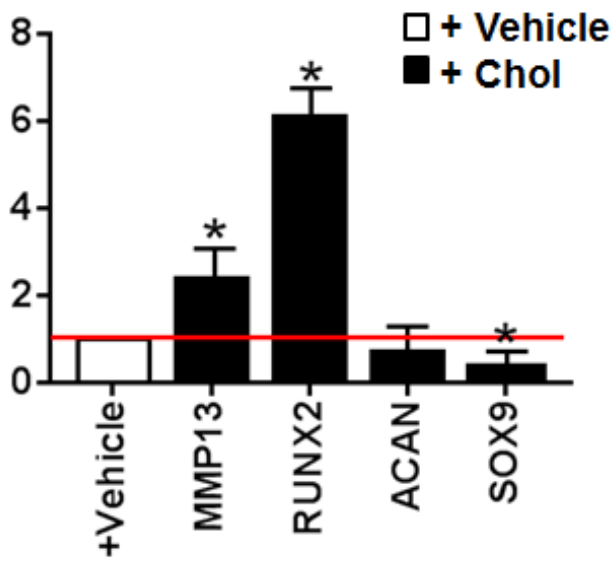

Figure 4.8: ACCs 3D pellet mRNA expression after cholesterol stimulation (RT-qPCR). The mRNA expression of MMP13, RUNX2, ACAN, and SOX9 after cholesterol stimulation (30µg/ml) in 3D cultured ACCs (n=6). ACCs pellets incubated with 0.2% BSA (vehicle) without Chol:MβCD served as controls. Data are mean ± SD. P0-P2 chondrocytes pellets were used for all the experiments. *P<0.05

versus +vehicle. Quantitative measurements were determined using the (2−ΔΔCt); method and beta-actin expression were used as endogenous housekeeping genes. All samples were performed in triplicate. The data is normalised to control ACCs pellets (+vehicle) arbitrarily set to 1.

Expression of chondrocytes haemostasis marker altered after cholesterol stimulation in ACCs 3D pellet culture (Western blot)

As qRT-PCR showed an altered mRNA expression for the OA-like marker, we also examined the effect of excess cholesterol on protein expression *in vitro*. We performed an ACCs 3D pellet culture as outlined in Section 3.2.4 and ACCs pellets were treated with 30µg/ml cholesterol as detailed in Section 3.2.6. At the end of the experiments, protein was extracted from ACCs pellets and an active MMP13 and ACAN protein expression was evaluated by Western blot analysis performed as per Section 3.2.17. As MMP13 plays a key role in cartilage matrix destruction and ACAN is the most native extracellular matrix proteoglycan, we tested the protein expression of these markers to examine ACCs homeostasis.

A single band near 50 kDa and 110 kDa corresponded to the molecular mass of active MMP13 and ACAN, which were observed in our samples respectively (Figure 4.9). The ACAN antibody we used detected 110 kDa bands; however, the ACAN predicted molecular weight is 210-250 kDa. Consistant with our qRT-PCR results, MMP13 protein expression level is higher in cholesterol-treated ACCs pellets than control. In contrast, the ACAN protein expression level was higher in the control ACCs (non-treated) than cholesterol-treated ACCs pellets. This may suggest that post-transcriptional processing of ACAN occurs in ACCs pellets treated with cholesterol, even though the mRNA expression trend is similar (Figure 4.9).

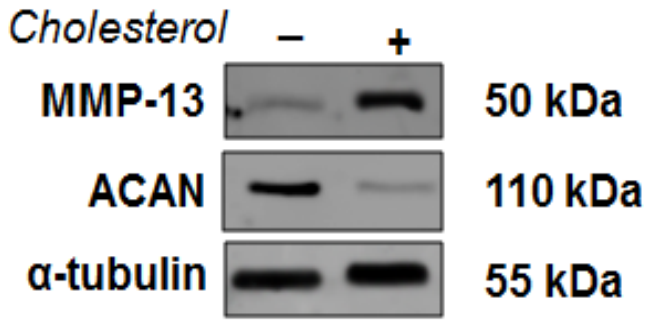

Figure 4.9: Expression of chondrocytes haemostasis marker altered after cholesterol stimulation in ACCs 3D pellet culture (Western blot). Western blot analysis of MMP13 and ACAN expression after cholesterol stimulation in 3D ACCs pellets culture (n=6). ACCs pellets incubated with 0.2% BSA without Chol:MβCD served as controls. P0-P2 ACCs pellets were used for this experiment.

Excess cholesterol altered mitochondrial function

Our above results demonstrate that cholesterol has a destructive role in cartilage homeostasis. Therefore, one potential mechanism that may play an essential role in this pathogenesis is mitochondrial dysfunction. In this experiment, we investigated the effect of high cholesterol levels on ACCs mitochondrial function.

Diverse functional assessments were performed to evaluate the mitochondrial function under excess cholesterol (30μg/ml*)* conditions. We measured four parameters: the MTT [(3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium

bromide)] assay for mitochondrial dehydrogenase activity as outlined in Section 3.2.18, RH-123 intensity for mitochondrial membrane potential as detailed in Section 3.2.20, fluorescent probe (DCFDA) assay for ROS detection as explained in Section 3.2.21, and luciferase-based assay for ATP production as per Section 3.2.19, respectively.

These four experiments are essential for evaluating mitochondrial function and morphology under excess cholesterol. The MTT assay is based on conversion of MTT to formazan crystal by living cells that determine mitochondrial activity. As for most cells populations the total mitochondrial activity is related to total number of viable cells, this assay is widely used to measure the *in vitro* cytotoxic effects of drugs on primary cells. RH-123 distributes electrophoretically into the mitochondrial matrix in response to the electric potential across the inner mitochondrial membrane and is used to measure the mitochondrial electric potential ($\Delta\psi$) (Baracca et al., 2003). Changes of $\Delta\psi$ are induced, directly or indirectly, by the proton transport occurring across the mitochondrial inner membrane during oxidative phosphorylation (ATP synthesis). Our hypothesis is that intracellular cholesterol overloadinf would alter mitochondrial membrane properties, which leads to the loss of $\Delta\psi$ and mitochondrial dysfuntion. Measuring Rh-123 levels and APT production rates in cholesterol-treated ACCs is a useful tool to test mitochondrial function. ROS is a major by-product of oxidative phosphorylation. Measuring the production rate of ROS in response to excess cholesterol is another way to further examine the mitochondrial function.

The presence of decreased mitochondrial dehydrogenase activity shown by MTT assay (Figure 4.10A; $P<0.05$), a decrease in ATP generation (Figure 4.10B; $P<0.05$), a breakdown of mitochondrial membrane potential as suggested by RH-123 assay (Figure 4.10C; $P<0.05$) and increased ROS production level in cholesterol-treated ACCs compared to non-treated ACCs, (Figure 4.10D; $P<0.05$) are all consistant with functional impairment of mitochondria after cholesterol stimulation.

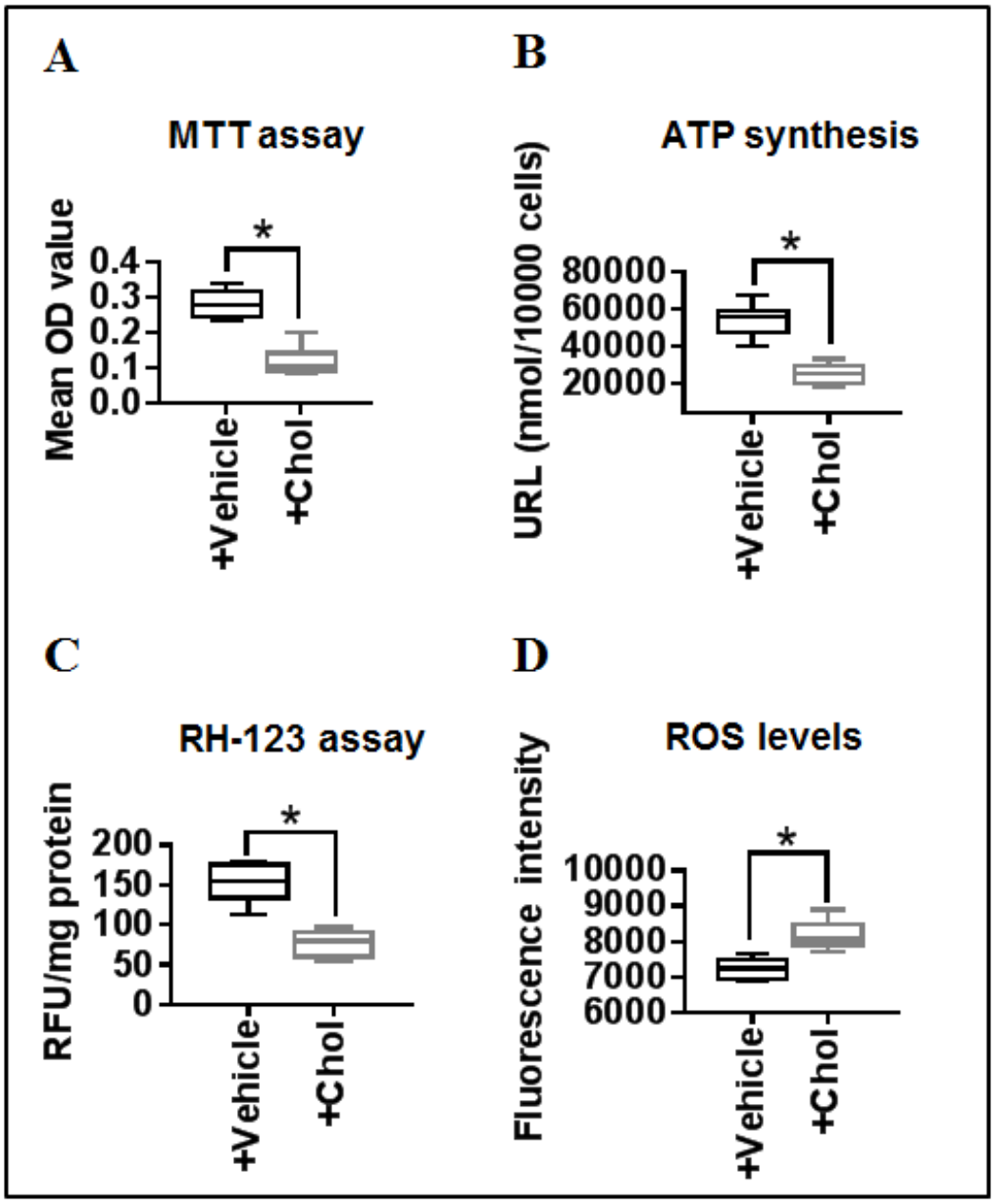

Figure 4.10: Excess cholesterol altered mitochondrial function. Four types of mitochondrial functional assays: **(A)** MTT, **(B)** ATP generation, **(C)** RH-123 intensity, and **(D)** ROS level were assessed in 30μg/ml cholesterol-treated ACCs after 24 h (n=6). ACCs incubated with 0.2% BSA (vehicle) without Chol:MβCD served as controls. P0-P2 chondrocytes were used for all the experiments. All samples were performed in triplicate. Data are mean ± SD. *P < 0.05 versus +vehicle.

Excess cholesterol changed mitochondrial morphology under TEM

Mitochondria are dynamic organelles that can change in number and morphology within a cell during development, the cell cycle, or when faced with toxic conditions. It is well-known that mitochondrial morphology is closely associated with mitochondrial function (Bereiter-Hahn & Vöth, 1994; L. B. Chen, 1988; Rizzuto et al., 1992).. In an attempt to further verify that mitochondria are involved in the pathophysiology of cholesterol-induced OA-like phenotype, we carried out morphological studies on ACCs pellets by electron microscopy as outlined in Section 3.2.23.

As demonstrated in Figure 4.11, chondrocyte from the control group showed intact, healthy mitochondrial structures with clear cristae and a lack of fragmentation. However, mitochondria in the cholesterol-treated group were characterized by fragmented double membrane and cristae disruption (yellow arrow), which are the definitive features of involvement of mitochondria in the apoptosis pathway. In addition, we found enlarged mitochondria with altered size in cholesterol-treated ACCs compared to the control group (Figure 4.11). Alteration in mitochondrial size and mass might be a mechanism by which cholesterol-treated ACCs compensate for the electron transfer deficiency resulting from mitochondrial dysfunction shown in Figure 4.11

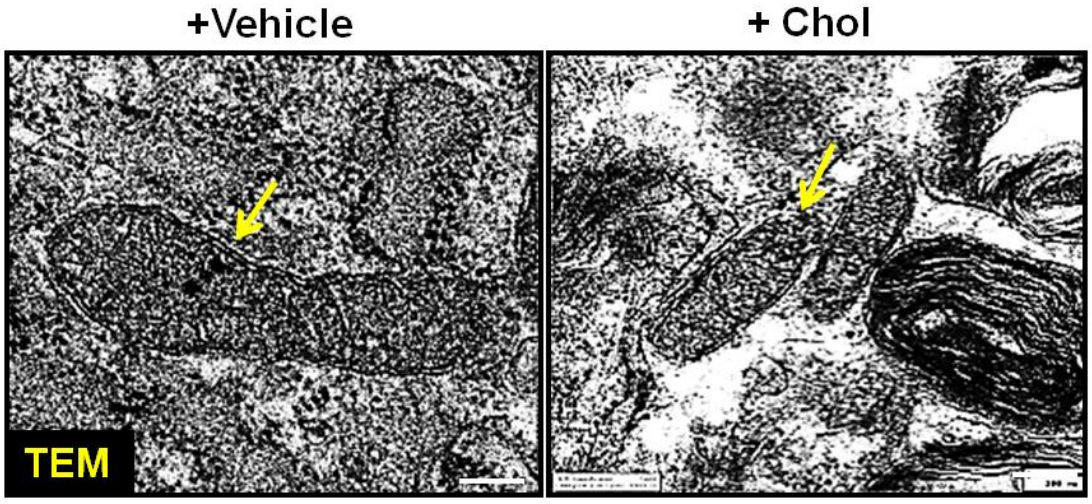

Figure 4.11: Excess cholesterol changed mitochondrial morphology. Transmission electron microscopy (TEM) of cholesterol-treated ACCs after 24 hour treatment (n=3). ACCs incubated with 0.2% BSA (vehicle) without Chol:MβCD served as controls. Scale bars= 200nm.

Excess cholesterol altered mitochondrial membrane potential (Δψ)

Mitochondrial membrane potential plays a role in both mitochondrial morphology and function. To specifically assess Δψ changes and mitochondrial function, we carried out JC-1 staining as detailed in Section 3.2.22. In normal cells, due to the electrochemical potential gradient, the dye concentrates in the mitochondrial matrix, where it forms red fluorescence. Imaging of living cells stained with JC-1 demonstrated a noticeable shift from red fluorescence (normal Δψ) to green fluorescence (altered Δψ) in cholesterol-treated ACCs compared to the control group (Figure 4.12). Excess cholesterol dissipated the accumulation of the JC-1 dye in the mitochondria, and thus, the dye was dispersed throughout the entire cell, leading to a shift from red to green fluorescence, suggesting the mitochondrial membrane was depolarised and lost in cholesterol-treated group. In contrast, there are more chondrocytes possessing normal mitochondrial polarization (red fluorescence) in the control group (Figure 4.12).

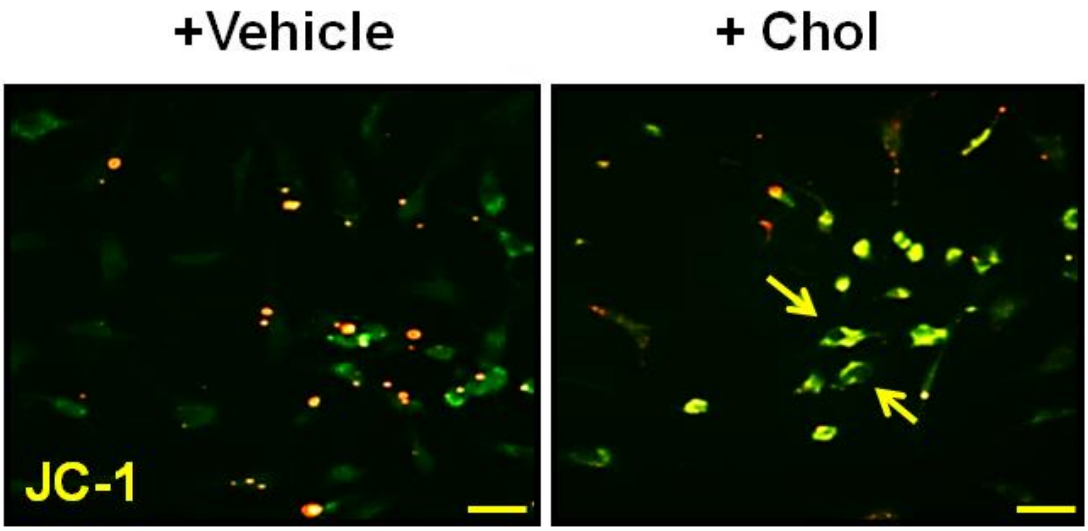

Figure 4.12: Excess cholesterol altered mitochondrial membrane potential. JC-1 staining of live ACCs after cholesterol treatment for 24 hours. ACCs incubated with 0.2% BSA (vehicle) without Chol:MβCD served as controls. Scale bars= 50μm.

Excess cholesterol increased mitochondrial apoptotic marker's expression

We demonstrated that excess cholesterol leads to the mitochondrial damage and dysfunction. Next, to test whether excess cholesterol alters mitochondria-related apoptotic markers, we measured the expression level of BAX, CYCS, and CASP9 mRNA expression. For this purpose, we performed 3D ACCs pellet culture and treated them with a high dose of cholesterol as outlined in Sections 3.2.4 and 3.2.6. We then extracted RNA from the ACCs pellets and performed qRT-PCR as detailed in Section 3.2.16 to quantify expression levels of the mitochondrial-related apoptotic markers.

BAX is widely used as the mitochondrial-related marker that initiates the apoptosis pathway by triggering cytochrome *c* release from mitochondria and down-stream activation of Caspase-9 cascades through oxidative stress-induced cell death (J. T. Liu et al., 2010). Expression of the pro-apoptotic protein, BAX was significantly increased in ACCs pellets treated with cholesterol compared to the control group (Figure 4.13; $P<0.05$). As expected, BAX translocation to the mitochondria during excess cholesterol challenge led to marked up-regulation of cytochrome *c* and Caspase-9 expression in downstream apoptotic events (Figure 4.13; $P<0.05$). This suggests that the high cholesterol levels play a role as exogenous oxidative stimuli for mitochondria that govern chondrocytes apoptosis at the molecular level.

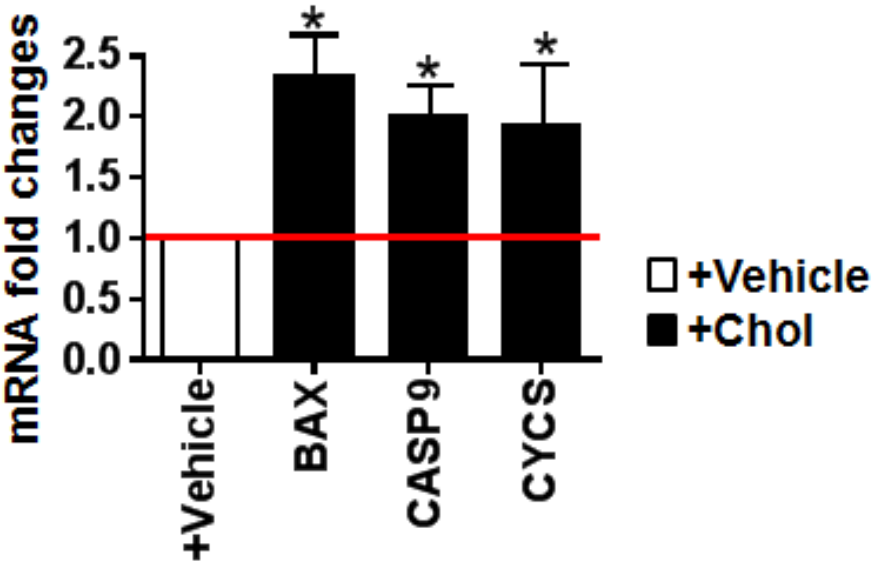

Figure 4.13: Excess cholesterol changes in mitochondrial apoptotic marker's expression. The mRNA expression of BAX, CASP9, and CYCS after cholesterol

stimulation in ACCs pellets (n=6). ACCs pellets incubated with 0.2% BSA (vehicle) without Chol:MβCD served as controls. Data are mean ± SD. P0-P2 chondrocytes pellets were used for all the experiments. *P<0.05 versus +vehicle. Quantitative measurements were determined using the ($2^{-\Delta\Delta Ct}$); method and beta-actin expression were used as endogenous housekeeping genes. All samples were performed in triplicate. The data is normalised to control ACCs pellets (+vehicle) arbitrarily set to 1.

Excess cholesterol induced mitochondrial oxidative stress-activated pathway

Our previous results showed that high cholesterol levels directly induced oxidative stress environment via ROS overproduction, which itself activates mitochondrial-related apoptosis (Fleury, C. et al., 2002). Apart from this, there are other molecular pathways that indirectly drive oxidative stress-related apoptosis independent of mitochondria, such as extracellular signal-regulated kinases (ERK) and c-Jun N-terminal kinases (JNK) pathways (Kamata, 2005). We carried out ACCs pellets and treated the pellets with a high cholesterol dose as detailed in Sections 3.2.4 and 3.2.6. Protein was extracted from the ACCs pellets and the expression of phosphorylated-JNK (p-JNK) and phosphorylated-ERK (p-ERK) were analysed using Western blot as outlined in Section 3.2.17. JNKs consist of different isoforms among which JNK1 and JNK 2 are upstream kinases following JNKs activation and found in all cells and tissues. JNK signal activation occurs through dual phosphorylation of these proteins. They are activated in response to stress stimuli such as changes in ROS level and play a role in the cellular apoptotic pathway (C. Li et al., 2012). I proposed that high levels of cholesterol may activate oxidative stress-related pathways such as ERK and JNK via overproduction of ROS and mtDNA damge.

Another signal that contributes to apoptosis in response to stress stimuli is ERKs, which belong to mitogen-activated protein kinase (MAPK) cascade. ERKs have two isoforms, ERK1 and ERK2, which are called ERK1/2 collectively. ERK1/2 is activated by oxidative stress following phosphorylation of these proteins. Though it has been reported that ERK1/2 play a pro-apoptotic role in cellular death signalling, ERK1/2 can also function as an anti-apoptotic factor after oxidative stress stimuli (Pearson et al., 2001).

Western blot analysis revealed that the expression of p-JNK1/2 signals were up-regulated in ACCs pellets after cholesterol treatment compared to non-treated ACCs.

In contrast, the expression of p-ERK1/2 pathway in ACCs was decreased after cholesterol stimuli, which may signify an anti-apoptotic function of p-ERKs cascades in response to excess amount of cholesterol (Figure 4.14).

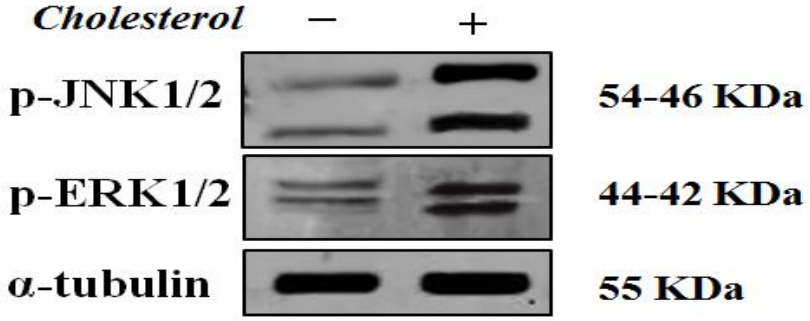

Figure 4.14: Excess cholesterol induced mitochondrial oxidative stress-activated pathway. Western blot analysis of p-JNK1/2 and p-ERK1/2 expression after cholesterol stimulation in 3D cultured ACCs (n=6). ACCs pellets incubated with 0.2% BSA (vehicle) without Chol:MβCD served as controls. P0-P2 chondrocytes pellets were used for all the experiments. Data are mean ± SD.

4.3 HIGH CHOLESTEROL LEVELS LEAD TO OA DEVELOPMENT IN APOE-KNOCKOUT (APOE-/-) MICE AND DIETARY-INDUCED HYPERCHOELSTROLEMIC (DIHC) RAT MODELS.

Characteristics of ApoE$^{-/-}$ mice model fed a control diet (CD) or high cholesterol-diet (HCD):

In order to study effect of high cholesterol levels on joint health, we developed two in vivo models: ApoE$^{-/-}$ mice and DIHC rat models. ApoE$^{-/-}$ mice, with sham or DMM surgery, are a promising small animal model, which helps to develop spontaneous hypercholesterolemia *in vivo* covering both the genetic and environmental determinants of hypercholesterolemia (Plump et al., 1992). The high-cholesterol diet that we used for this study was previously used to develop hypercholesterolemia in ApoE-/- mice model (L. Gierman et al., 2014).

To assess the effect of mechanical loading, the body mass of ApoE-/- mice, developed as per Sections 3.3.1 and 3.3.2 were measured and showed no significant changes compared to the control diet (CD) and high-cholesterol diet (HCD) groups during this study (CD ApoE-/- mice 21.61 ± 1.37 g versus HCD ApoE-/- mice 21.81 ± 1.24 g) (Figure 4.15A; P<0.05). We monitored the animals' body mass to confirm that feeding a HCD does not increase mice body mass, which would affect joint health regardless of diet.

We also performed lipoprotein analysis as per Section 3.3.5 to ensure that HCD induced high plasma cholesterol levels. The HCD group had significantly higher cholesterol levels than the CD group (Figure 4.15B; *P*<0.05). Our results show that HCD is able to increase the plasma cholesterol levels in ApoE$^{-/-}$ mice compared to CD fed animals. Normal serum cholesterol in mice varies based on strains and gender (30-100 mg/dL).

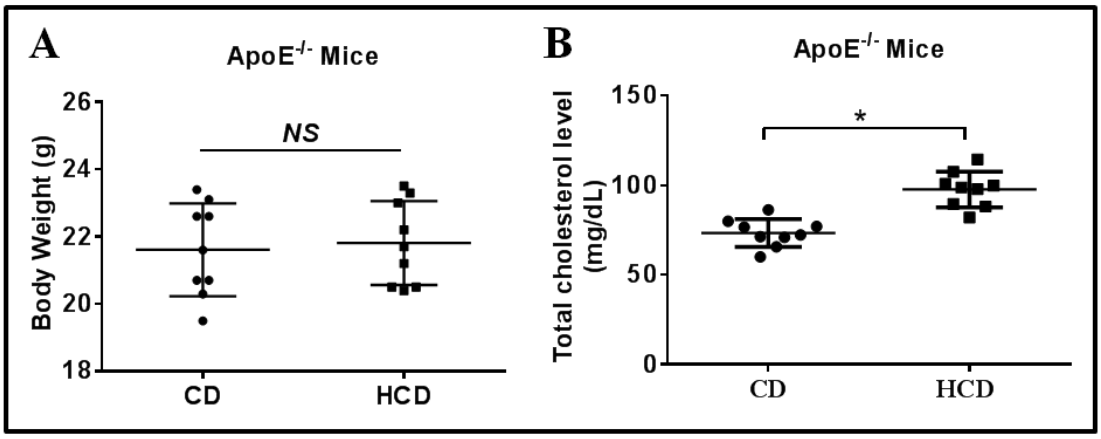

Figure 4.15: Characteristic of ApoE-/- mice on CD or HCD. (A) Body mass (g) of ApoE-/- mice (n=9 per group). Data are mean± SD. **(B)** Total serum cholesterol level in ApoE-/- mice fed a HCD and CD at the end week of the study (five weeks post-surgery). Data are mean± SD. All samples were performed in triplicate. *P<0.05.

H&E staining of knee joints from ApoE$^{-/-}$ mice model fed a CD or HCD:

The knee joint of ApoE$^{-/-}$ sham and DMM mice models developed as outlined in Sections 3.3.1and 3.3.2 respectively, were histologically assessed to examine the structural changes from HCD. We performed H&E staining, as per Section 3.2.8, on

the knee joint sections obtained from the ApoE$^{-/-}$ mice fed either a HCD or CD. Sham ApoE$^{-/-}$ mice fed a HCD showed narrowing of the joint space, which is a common phenotype in OA patients, compared to CD group. Five weeks post-surgery, when DMM-induced OA was accompanied with HCD, OA ApoE$^{-/-}$ mice demonstrated significant cartilage damage and extreme joint space narrowing, while sham HCD mice still had a normal healthy structure (Figure 4.16).

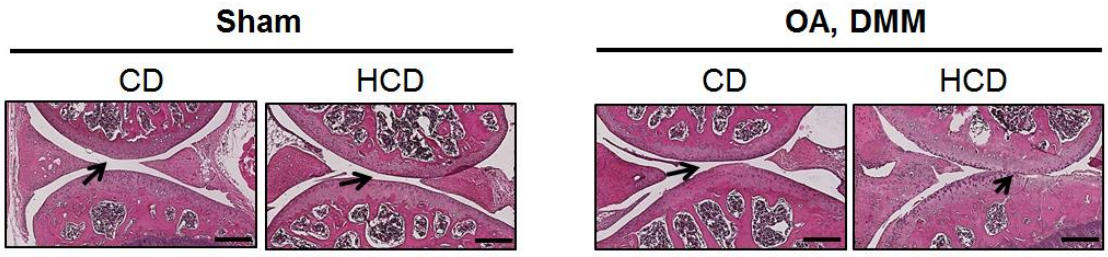

Figure 4.16: H&E staining of knee joints form ApoE-/- mice model fed a CD or HCD. HCD modified articular cartilage and joint space (black arrow) in both sham and OA ApoE$^{-/-}$ mice (n=3). Scale bar= 20µm.

Hypercholeterolemia induced OA-like changes in ApoE$^{-/-}$ mice fed a CD or HCD:

The knee joint of ApoE$^{-/-}$ sham and DMM mice models were histologically assessed to examine the effect of HCD on OA development. The knee joint sections of ApoE$^{-/-}$ mice were stained by Safranin O staining technique as per Section 3.2.9. Safranin O staining is a well-stablished method used to detect and characterize cartilage in terms of proteoglycans amount and cartilage thickness and structure. In this method, the cartilage is stained red, the nuclei are stained black and the background is stained green. Sham ApoE$^{-/-}$ mice fed a HCD showed more degradative changes compared to the CD group. Cartilage degradation was assessed based on cartilage structure and intensity of Safranin O staining representing proteoglycan content in cartilage. Five weeks post-surgery, when OA accompanied HCD, OA ApoE$^{-/-}$ mice demonstrated significantly aggravated cartilage damage, while sham HCD mice still had a smooth cartilage surface (Figure 4.17A). At this time point, the Mankin score in ApoE$^{-/-}$ mice fed a HCD was significantly higher than the CD group. Mankin scoring was performed by a blind person according to Table 2.1. This scoring system is mainly focused on the Safranin O staining intensity, chondrocytes' cellularity, and

cartilage structure integrity. Accordingly, 0 points implies normal healthy cartilage, whereas 14 points represents the most severe cartilage lesions (Figure 4.17B; $P<0.05$).

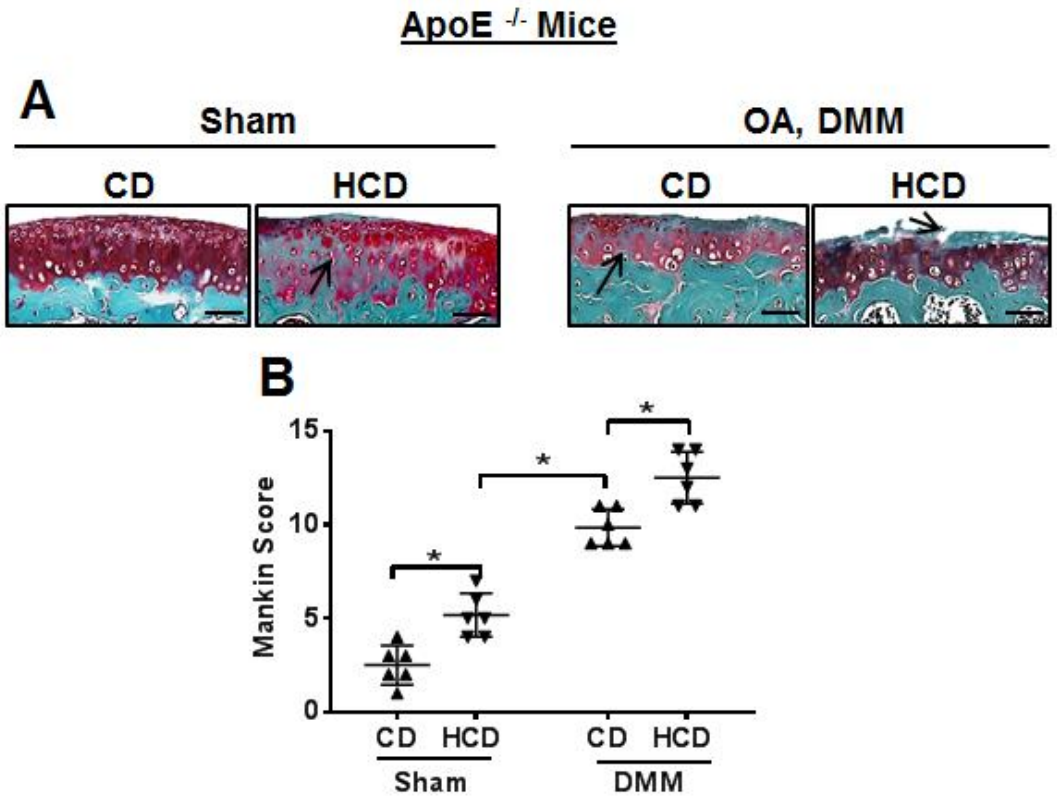

Figure 4.17: Hypercholesterolemia induced OA-like changes in APOE-knockout (ApoE-/-) mice. (A) Safranin O staining of sagittal sections of the tibia medial compartment of ApoE-/- mice fed a HCD or CD, proteoglycan (red) and background (green). Black arrows indicate loss of proteoglycan at five weeks after sham and DMM surgery. Scale bar=100μm. **(B)** Total Mankin score after OA surgery at five weeks in ApoE-/- mice. n=6 per group. Data are mean± SD. *P<0.05.

Micro-CT scanning of ApoE$^{-/-}$ mice tibia plateau

To assess potential subchondral bone changes in response to HCD, the tibia plateau of sham ApoE$^{-/-}$ mice model developed as outlined in Section 3.3.1 were scanned in a Micro-CT scanner as per Section 3.2.25 to evaluate subchondral bone changes. We used the proximal part of the medial tibia plateau to assess the ratio of bone volume over tissue volume (BV/TV). According to the Micro-CT analysis, there were significant changes in bone volume fraction (BV/TV) in the tibia plateau of the

ApoE$^{-/-}$ mice fed a HCD compared to CD. The ApoE$^{-/-}$ mice fed a HCD showed a decrease in bone volume fraction compared to the CD group (Figure 4.18; $P < 0.05$).

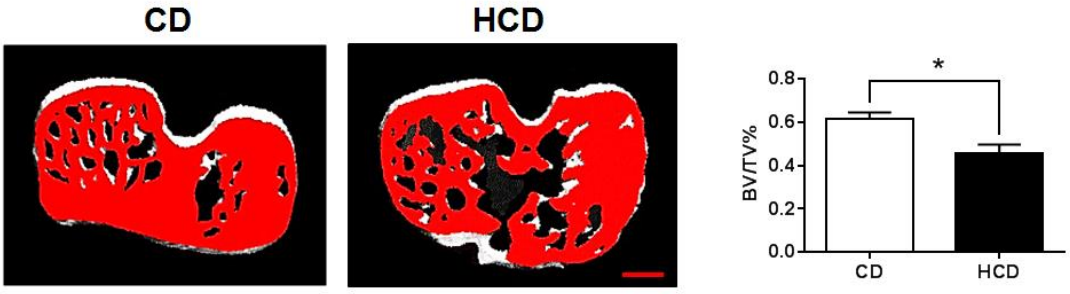

Figure 4.18: Micro-CT scanning of ApoE-/- mice tibia plateau. Micro-CT three-dimensional reconstructions of ApoE-/- mouse tibia plateau and bone volume/tissue volume ratio (BV/TV, %) measurements in the proximal part of tibia plateau (n=3). Scale bar =100μm. Data are mean ± SD. All samples were performed in triplicate. *P<0.05 versus CD.

Hypercholesterolemia altered synovial membrane thickness in ApoE$^{-/-}$ mice

To assess the effect of a HCD on the synovium, knee sections obtained from sham ApoE$^{-/-}$ mice were histologically stained as outlined in Section 3.2.9. In CD mice, histological examination showed proliferation of synovial lining cells, in places to more than five layers, associated with fibrous and disorganized intima. In the subintimal region, the reappearance of adipocytes was observed. In the ApoE$^{-/-}$ mice fed a HCD, the intima showed multi-layered lining cells, with an increase in the number of synoviocytes. Superficial synoviocytes were perpendicular rather than parallel with the joint space. Fibrosis of the normal adipose tissue was observed. Further measuring the synovial thickness, HCD mice revealed dramatic thickening of the synovial membrane. The synovium of CD ApoE$^{-/-}$ group was 23.6±5.4μm thick on average, compared to the average thickness of the HCD ApoE$^{-/-}$ mice, which was 50.28 ± 17.2μm thick (Figure 4.19).

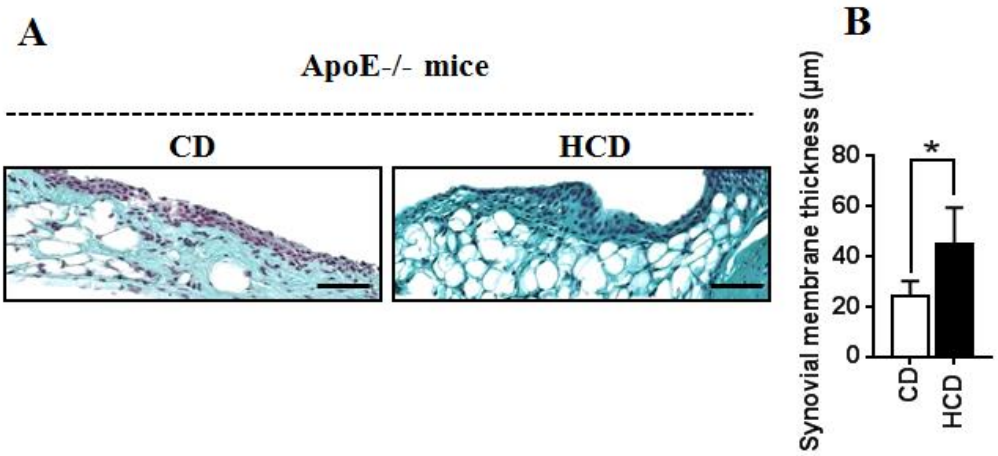

Figure 4.19: Hypercholesterolemia altered synovial membrane thickness in ApoE-/- mice. (A) Representative histological images of the synovium showing thickened lining layer and signs of synovitis in HCD ApoE-/- mice. **(B)** Overall thickness of synovial membrane (n=6). Scale bar=50µm. Data are mean ± SD. *P<0.05 versus CD.

Hypercholesterolemia induced the inflammatory infiltrate in ApoE$^{-/-}$ mice

To further test the potential inflammatory response to a HCD in the synovial membrane of ApoE$^{-/-}$ mice, we performed immunofluorescence staining for expression of F4/80 marker as described in Section 3.2.15. F4/80 is a well-characterized and extensively referenced mouse macrophage marker.

ApoE$^{-/-}$ mice fed a HCD showed more expression of macrophages general F4/80 marker compared to the CD group (Figure 4.20A). In the HCD group, there was a significant increase in positive stained cells compared to the CD mice (Figure 4.20B). Our results may signify that HCD in ApoE$^{-/-}$ mice model led to macrophage infiltration in the synovial membrane.

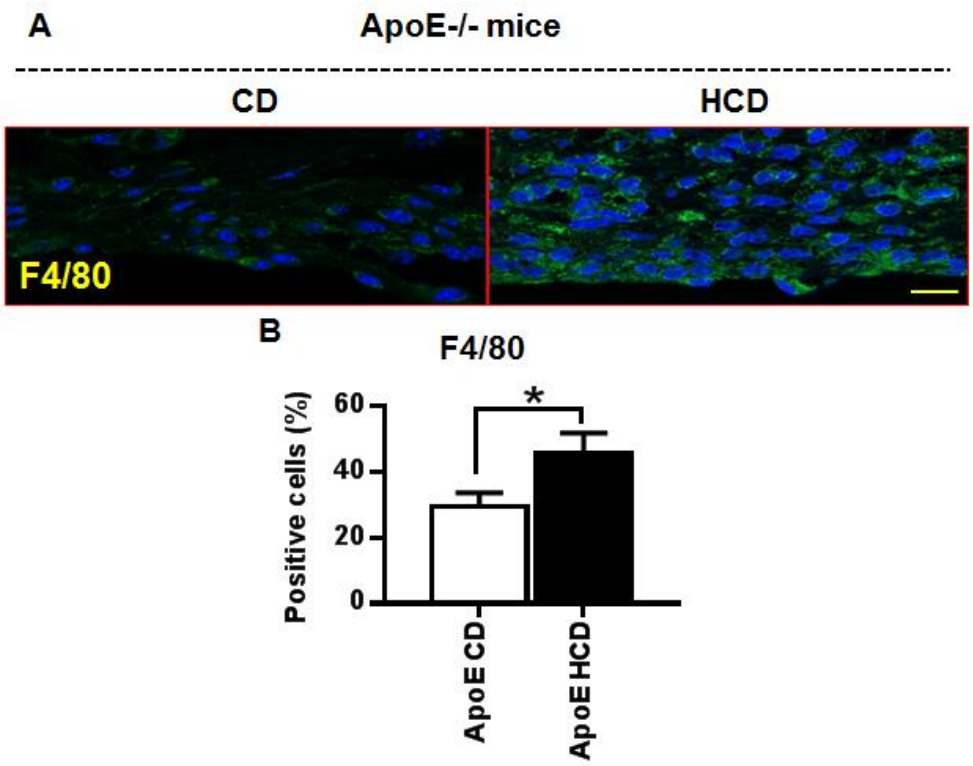

Figure 4.20: Hypercholesterolemia induced the inflammatory infiltrate in ApoE-/- mice. (A) Expression of macrophages in ApoE-/- mice (n=6). Scale bar=50μm. **(B)** Percentage of total positive macrophages stained for F4/80. Data are mean ± SD. *=P ＜0.05.

Hypercholesterolemia induced OA-like marker expression in ApoE$^{-/-}$ mice

Safranin O staining results demonstrated that HCD induce OA-like phenotype in ApoE-/- mice. In this experiment, we investigated the expression of some OA-like molecular markers in response to HCD in ApoE-/- mice. To this end, we performed immunohistochemistry staining as explained in Section 3.2.14 on sections obtained form ApoE-/- mice knee. The first marker was Collagen 10 (COL 10), which is expressed in hypertrophic chondrocytes. During OA development, COL 10 increases above the calcified zone and diffuses through the calcified matrix (Pullig O., et al., 2002). However, OA may damage superficial cartilage and induce chondrocyte hypertrophic phenotype in the superficial zone as well (G. Murphy & Nagase, 2008). DIPEN and NITEGE are two neoepitope antibodies that detect N or C terminus of Aggrecan degradation products. DIPEN distinguishes MMP- driven aggrecan loss and NITEGE distinguish aggrecanase (ADAMTS-4, 5)-driven aggrecan loss in the late-

stage disease. It has been well-demonstrated that aggrecan is degraded either with MMPs or aggercanase during OA development (Arner, 2002; Glasson et al., 2005; G. Murphy & Nagase, 2008). Our results demonstrate that the expression of COL10, NITEGE, and DIPEN in ApoE$^{-/-}$ mice fed a HCD was higher compared to the CD group, suggesting a cartilage degradative role for HCD. The total percentage of positive chondrocytes for these markers showed that only the DIPEN expression was significantly increased in the HCD group compared to control (Figure 4.21).

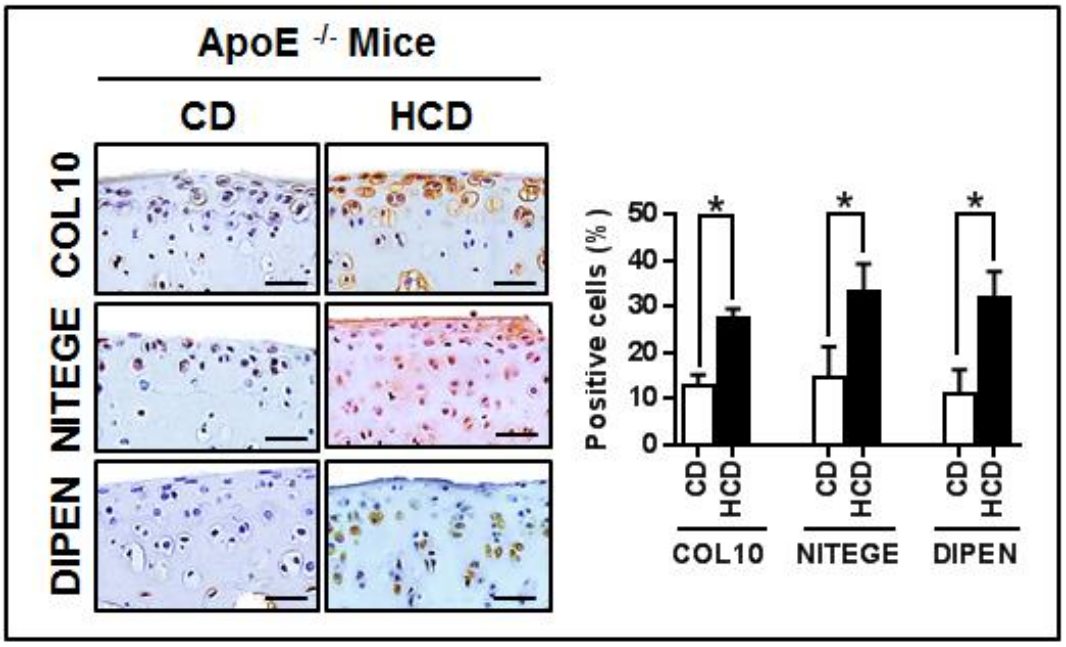

Figure 4.21: Hypercholesterolemia induced OA-like marker expression in ApoE-/- mice. Immunohistochemistry staining of COL10, NITEGE, and DIPEN five weeks after surgery in ApoE-/- mice fed a CD and HCD. Percentage of total positive cells for OA-like markers were increased after HCD feeding and achieved a significant level for DIPEN expression. Scale bars = 50μm.

Characteristics of DIHC rat model fed a CD or HCD:

We developed a dietary induced hypercholesterolemia (DIHC) rat model as described in Sections 3.3.1 and 3.3.2 to further test the effect of HCD on OA development without genetic deficiency. To characterize the DIHC rats, the body mass of the DIHC rats were measured and showed no significant changes compared to the

control diet (CD) and high-cholesterol diet (HCD) groups during this study (CD rats 304.16 ± 7.16 versus HCL rats 319.01 ± 12.81) (Figure 4.22A; $P<0.05$). Confirming that there was no significant body mass difference between the CD and HCD rats, we excluded the effect of excess mechanical loading from this study.

Food consumption of the DIHC rats was also measured. There was no significant difference in food intake of DIHC rat model (CD 16.85 ± 0.79 versus HCD 17.83 ± 0.74) (Figure 4.22B; $P<0.05$).

Plasma cholesterol levels of the DIHC rats were measured eight weeks post-surgery (mmol/l). The HCD group indicated significantly higher cholesterol levels than the CD group (Figure 4.22C; $P<0.05$). We performed this experiment to ensure that the HCD was able to increase the plasma cholesterol level significantly reaching a hypercholesterolemic condition. A normal serum cholesterol level in rats is 40-130 mg/dL.

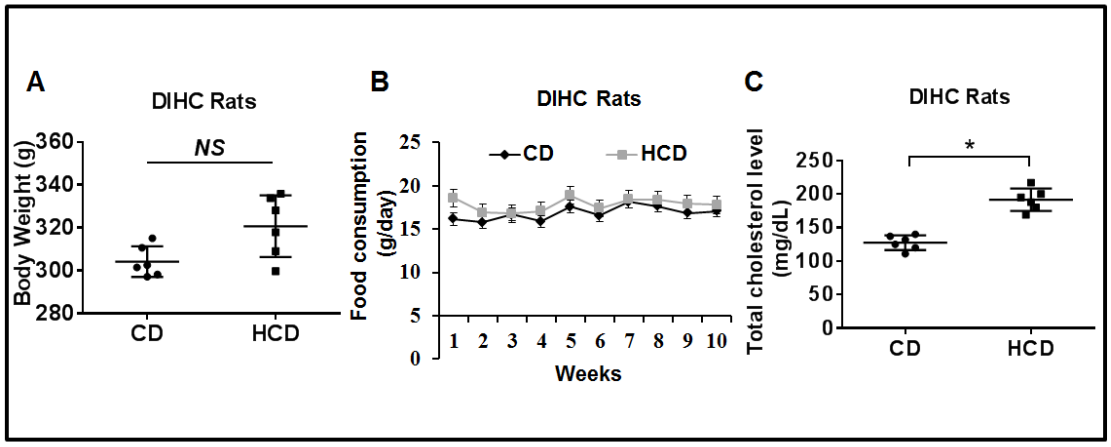

Figure 4.22: Characteristic of DIHC rat model on a CD or HCD. (A) Body weight (g) of rats (n=6 per group). Data are mean± SD. **(B)** Food consumption (g/day) of DIHC rats fed a CD or HCD. Data are mean± SD **(C)** Total serum cholesterol level in DIHC rats fed a HCD and CD at the end week of the study. Data are mean± SD. All samples were performed in triplicate. *P<0.05 versus CD.

Faxitron x-ray radiography of DIHC rats' knee joints

One of the radiographicall hallmarks in human OA patients is joint space loss. To radiographically assess the knee joint of DIHC rats, we performed x-ray radiographic as detailed in Section 3.3.6. Eight weeks after surgery, radiographs of knee joints were

obtained using the Faxitron MX20 x-ray system and semi-quantitative data obtained using Image J software and student *t-test* method used for statistcs. To adequately assess the joint space, x-ray images were taken from sham-operated rats fed either a CD or HCD. The non-uniform joint space narrowing, which is the radiographic hallmark of primary OA, was observed in rats fed a HCD compared to the CD group. Joint space in HCD fed animals was significantly decreased compared to CD. Moreover, the articular surface appears flattened in HCD group compared to CD group (Figure 4.23).

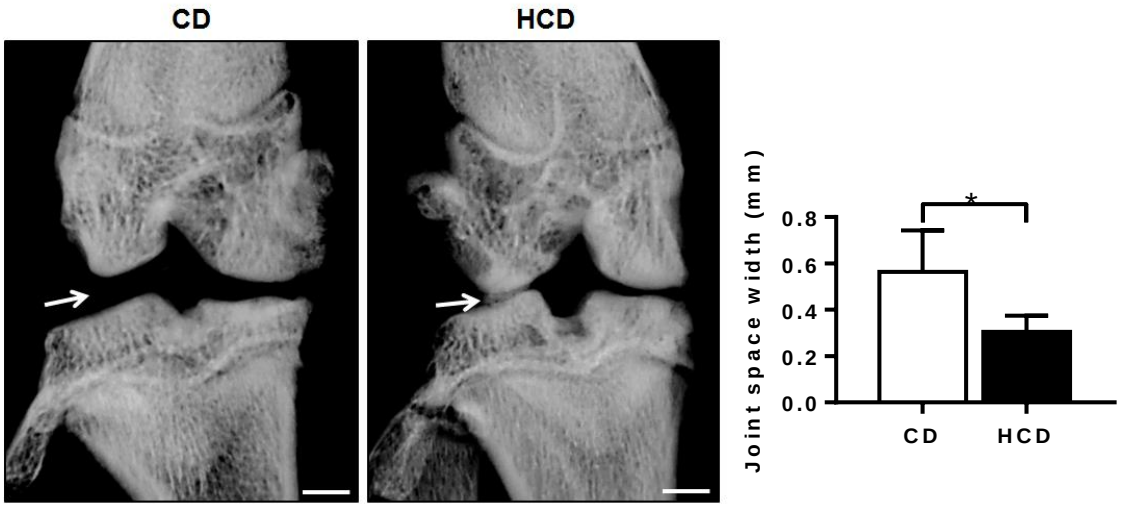

Figure 4.23: Faxitron X-Ray radiography of DIHC rat model. X-ray images of rats fed a CD or HCD at eight weeks post-surgery (n=6).

Safranin O staining and Mankin Score of tibia plateau from DIHC rat model fed a CD or HCD:

We developed a dietary induced DIHC rat model as described in Section 3.3.1 and investigated test the effect of HCD on OA development in cartilage. The knee joint of DIHC rats were histologically assessed using Safranin O staining as outlined in Section 3.2.9. The cartilage was characterized based on Safranin O staining for proteoglycans amount and cartilage thickness and lesions. Sham rats fed a HCD showed more degradative changes compared to CD group. Eight weeks post-surgery when OA was accompanied with HCD, OA rats demonstrated significant aggravated cartilage damage, while sham HCD rats still had a smooth cartilage surface (Figure

4.24A). At this time point, Mankin scoring was performed by a blind person according to Table 2.1. Mankin Score in both OA and sham DIHC rats fed a HCD was significantly higher than corresponding CD groups (Figure 4.24B; $P<0.05$).

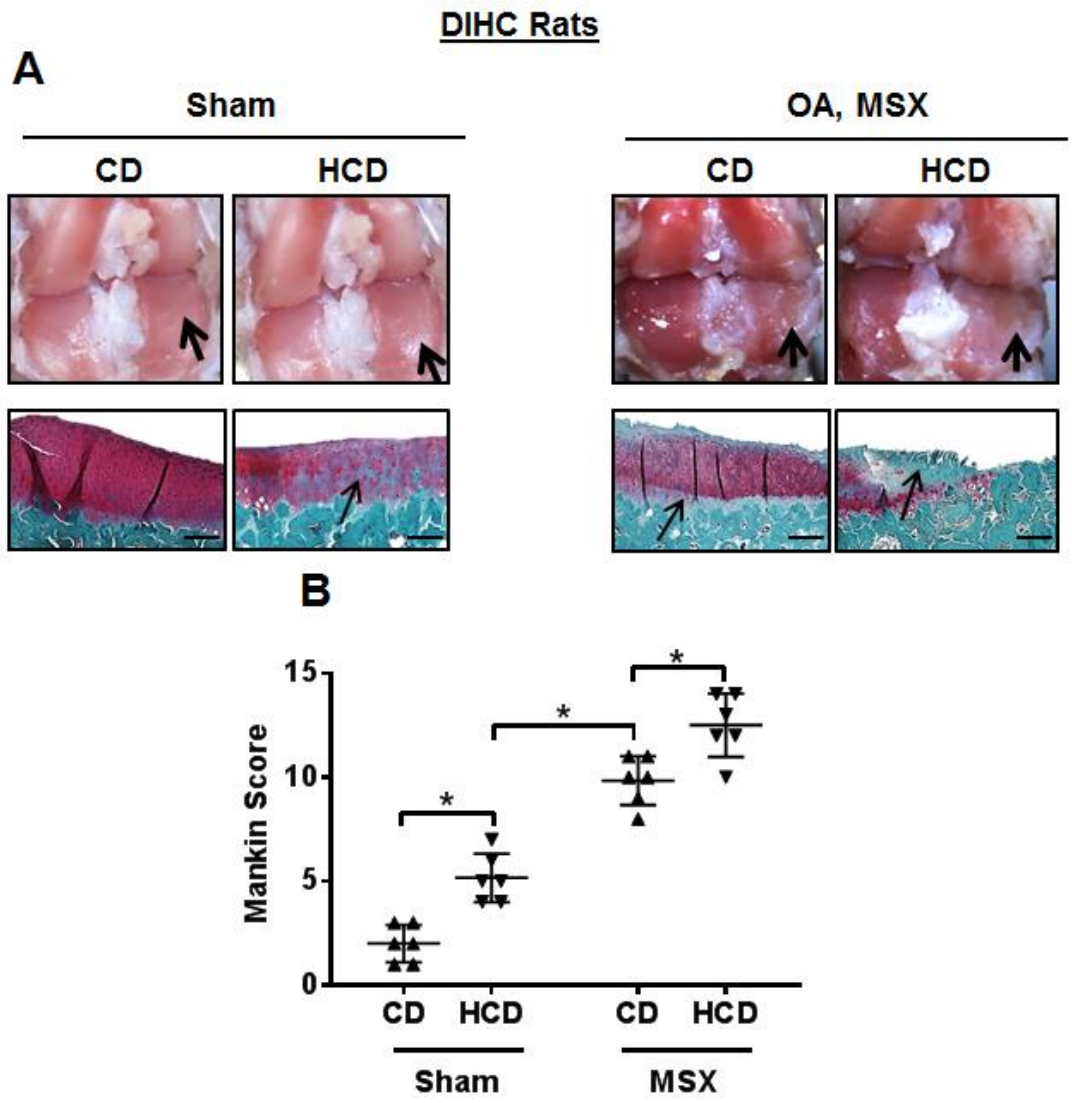

Figure 4.24: Hypercholesterolemia induced OA-like changes in DIHC rat model. **(A)** Top, gross appearance of tibia in the sham and MSX groups fed a CD or HCD at eight weeks after surgery. Black arrows indicate the surface of the cartilage in the middle region of the medial tibia. Bottom, Safranin O staining of sagittal sections of the tibia medial compartment of rats fed a HCD or CD, proteoglycan (red) and bone (blue). Black arrows indicate the loss of proteoglycan at eight weeks after sham and MSX surgery. Scale bar, 100μm. **(B)** Total Mankin score after OA surgery at eight weeks in the dietary rat model. n= 6 per group. Data are mean± SD. *P<0.05.

Micro-CT scanning of DIHC rat's tibia plateau

In this experiment, we investigated the effect of high cholesterol levels on subchondral bone. Therefore, the tibia plateau of the DIHC rat model developed as outlined in Section 3.3.1 were scanned in a Micro-CT scanner as described in Section 3.2.25 to evaluate subchondral bone changes in this animal model. We used the proximal part of the medial tibia plateau to assess the ratio of bone volume over tissue volume (BV/TV). Our Micro-CT analysis showed that there were significant changes in the bone volume fraction (BV/TV) in the tibia plateau of the DIHC rats fed a HCD compared to the CD. Subchondral bone volume was calculated using the Micro-CT scanning. Micro-CT assessment showed significant changes in BV/TV ratio in the tibia plateau of the rats on HCD. The rats fed a HCD showed a decrease in bone volume fraction compared to CD group (Figure 4.25; $P < 0.05$).

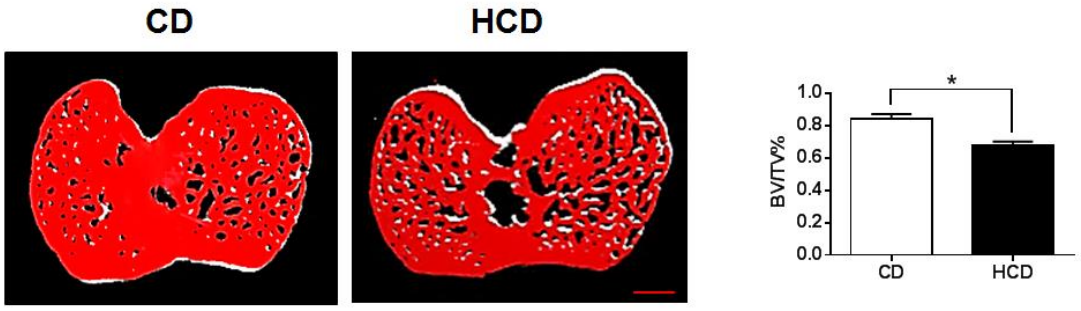

Figure 4.25: Micro-CT tibia plateau scanning of DIHC rat model. Micro-CT three-dimensional reconstructions of DIHC rats and bone volume/tissue volume ratio (BV/TV, %) measurements in the tibia plateau (n=3). Scale bar = 100μm. Data are mean± SD. All samples were performed in triplicate. *P<0.05 versus CD.

Hypercholesterolemia increased bone formation and remodelling

To further evaluate new bone formation and mineralization as well-known subchondral bone changes during the course of OA, the DIHC rat model developed as per Section 3.3.1 was subjected to fluorescence labelling as described in Section 3.3.3. Fluorochromes are fluorescent labels with calcium affinity, the most used being alizarin and calcein. When different types of fluorochromes are injected into the

animals at different moments of ossification, they bind to the available calcium that is precipitating in the mineralization areas. With the aid of filters that catch specific wavelengths for each fluorochrome, it is possible to visualize the mineralized areas in different colours for each period. Analysis of bone formation and mineral apposition are based on the distance between the two labels and percentage and/or intensity of bone labels exhibiting mineralizing activity.

Our results showed that there were more bone formation and mineralization in the subchondral bone area of the DIHC rat's model fed a HCD compared to the CD. The fluorescence band's percentage and intensity in the HCD group were higher in comparison to the CD group (Figure 4.26A). An increase in subchondral bone remodelling and mineralization in rats fed a HCD represents the subchondral bone changes occurred in OA patients.

We also found bone marrow like lesions in the subchondral bone area of DIHC rats fed a HCD compared to the CD; however, further investigation is needed to confirm this phenomenon (Figure 4.26B). Bone marrow lesions are a common symptom among OA individuals.

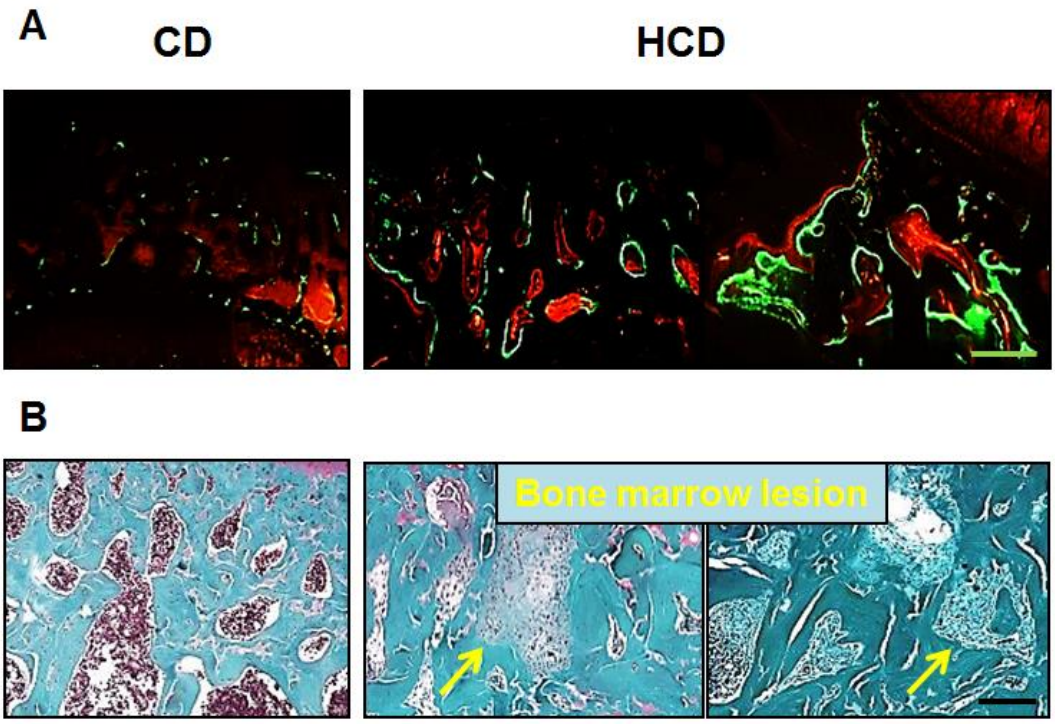

Figure 4.26: Hypercholesterolemia increased bone formation and remodelling. **(A)** Calcein and alizarin red fluorescence labelling; representative subchondral bone

remodelling in rats fed a CD or HCD at eight weeks after surgery (n=6). Scale bar = 100µm**. (B)** Bone marrow lesions in subchondral area of rats fed a HCD shown by yellow arrows. Scale bar = 50µm.

Hypercholesterolemia altered synovial membrane thickness in DIHC rats

To evaluate the effect of HCD on synovium, we performed the histological staining as described in Section 3.2.9 on the knee sections obtained from the DIHC rat's model. In rats fed a HCD, synoviocyte hypertrophy, increased number of lining cells and thickened synovial membrane was observed. The intima was fibrous and disorganized, and in the subintimal region, reappearance of adipocytes was presented in synovial membrane of HCD rats. In the CD group, the synovial membrane showed no pathological changes as indicated by the typical palisading structure of the intimal lining layer and few proliferative lining layer cells. The thickness of synovium was also decreased, compared to rats with HCD (CD = 5 ± 2.7µm thick versus HCD= 14±3.1µm thick) (Figure 4.27).

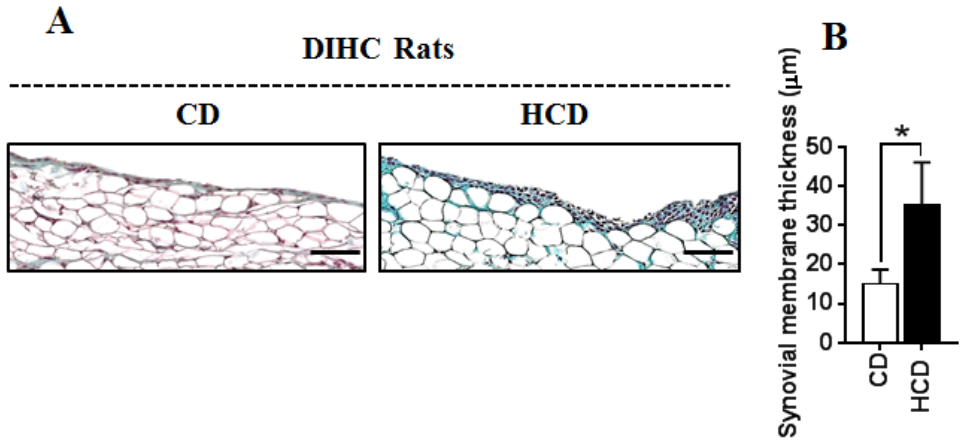

Figure 4.27: Hypercholesterolemia altered synovial membrane thickness in DIHC rats. (A) Representative histological images of the synovium showing thickened lining layer and signs of synovitis in HCD rats. **(B)** Overall thickness of synovial membrane (n=6). Scale bar=50µm. Data are mean ± SD. *P<0.05 versus CD.

Hypercholesterolemia induced the inflammatory infiltrate in DIHC rats

To further test the potential inflammatory response to a HCD in the synovial membrane of DIHC rats, we performed immunofluorescence staining for the

expression of CD68 marker as described in Section 3.2.15. CD68 is a classical inflammatory marker for rats that is expressed in monocyte/macrophages.

Immunofluorescence analysis of sections from rats showed a similar expression pattern to the mice. Rats fed a HCD had a higher CD68 fluorescence intensity compared to the CD group (Figure 4.28A). Moreover, there were significantly more CD68 positive macrophages (38.1%) in the lining cell layer and synovium of HCD rats compared to CD rats (Figure 4.28B).

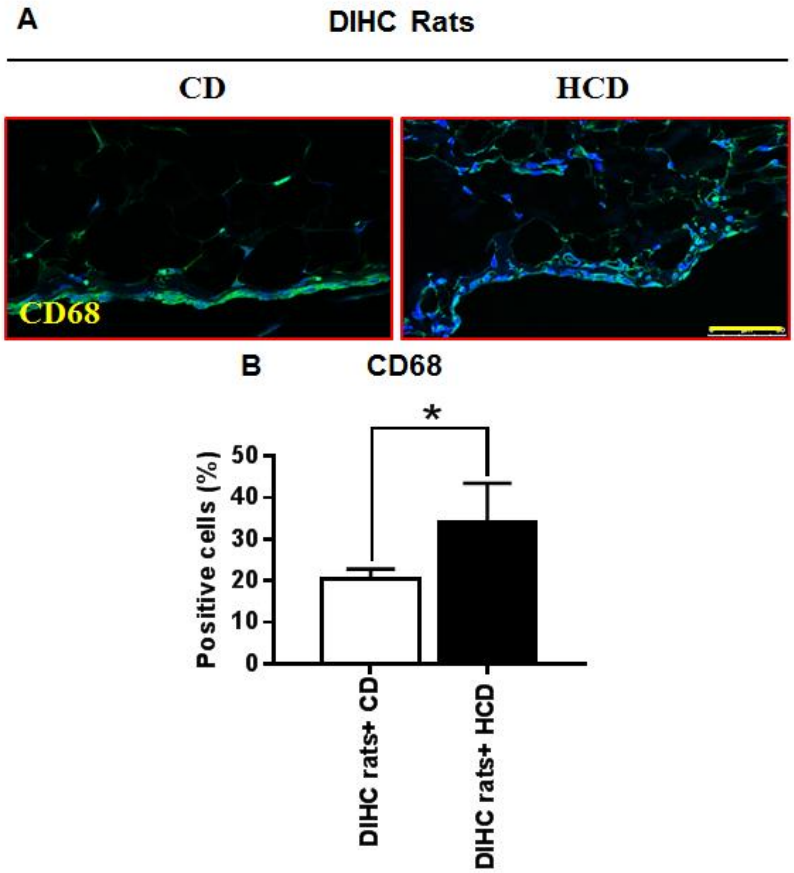

Figure 4.28: Hypercholesterolemia induced the inflammatory infiltrate in DIHC rats. (A) Expression of different macrophages in DIHC rats (n=6). Scale bar=50μm. **(B)** Percentage of total positive macrophages. Data are mean ± SD. *=P ＜0.05.

Hypercholesteremic induced OA-like marker expression in DIHC rat model

To assess the effect of a HCD on the expression of OA-like molecular markers, we performed immunohistochemistry staining as outlined in Section 3.2.14 on the knee sections obtained from the DIHC rat model. We tested COL10, DIPEN, and

NITEGE expression under the effect of HCD. Although rats fed a HCD showed increased NITEGE, COL10 and DIPEN expression compered to CD,groups (Figure 4.29).

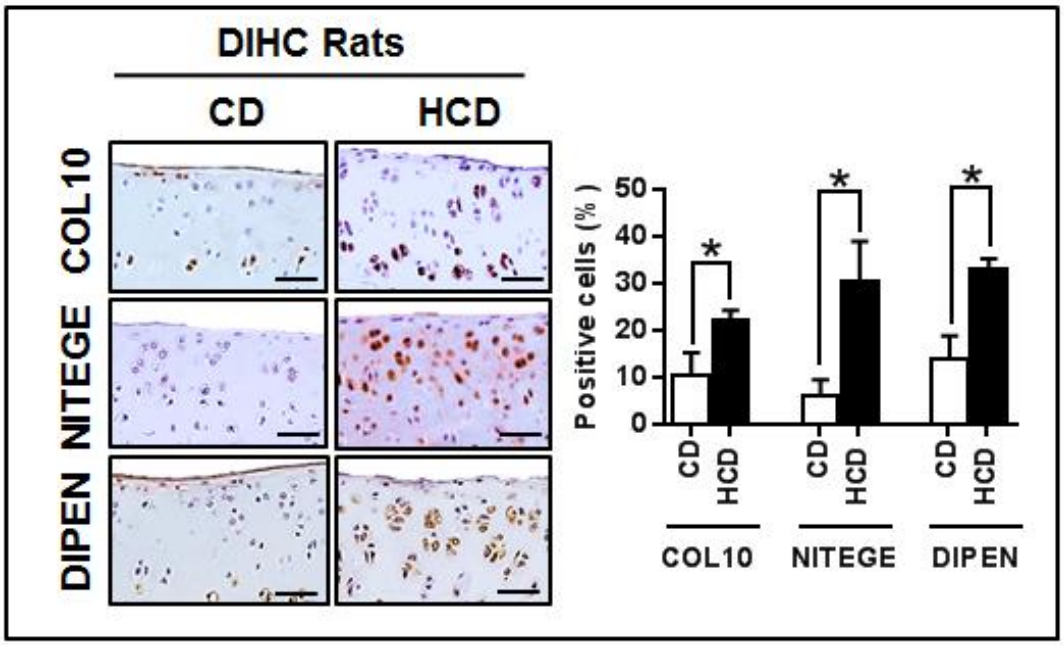

Figure 4.29: Hypercholesteremic induced OA-like marker expression in the DIHC rat model. Immunohistochemistry staining of COL10, NITEGE, and DIPEN eight weeks after surgery in rats fed a CD and HCD. Percentages of total positive cells for OA-like markers, in particular NITEGE and DIPEN, were significantly increased in HCD compared to the CD group. Scale bars = 50μm.

Expression of oxidative stress markers in ApoE$^{-/-}$ mice and rat model fed a HCD

Lipid peroxidation is an oxidative degeneration of lipids results in cell and even tissue damage. In order to demonstrate whether hypercholesterolemia induces lipid peroxidation in our animal models fed a HCD, we detected the production of Malondialdehyde (MDA), a sensitive oxidative stress marker, using immunohistochemistry as outlined in Section 3.2.14. Proteins may be modified under oxidative stress either directly by ROS or indirectly by autoxidation of lipids. Such a protein is MDA, which also termed advanced lipoxidation end products. Localization

of MDA is closely related to oxidative process and is independent evidence of local oxidative stress. In the knee cartilage of animals fed a HCD, in particular in the DIHC rat model, more positive stained MDA were found in comparison with the CD group (Figure 4.30), suggesting that hypercholesterolemia induces lipid peroxidation and oxidative stress pathway.

In order to determine whether oxidative stress induction triggers mitochondrial cytochrome C release, animals fed a HCD were immunohistochemically stained for cytochrome C expression as described in Section 3.2.14. The release of cytochrome *c*, one of the most important respiratory-chain proteins, from the mitochondria into the cytosol is the hallmark of cells passing through apoptosis. The intensity of staining in cytosol in HCD group, which represented the quantities of cytochrome *c*, was higher than those in CD group, specifically in our ApoE$^{-/-}$ mice model. The release of cytochrome *c* into the cytosol specifies the molecular basis of apoptosis, which can be induced in both ApoE$^{-/-}$ mice and DIHC rats fed a HCD compared to CD group (Figure 4.30).

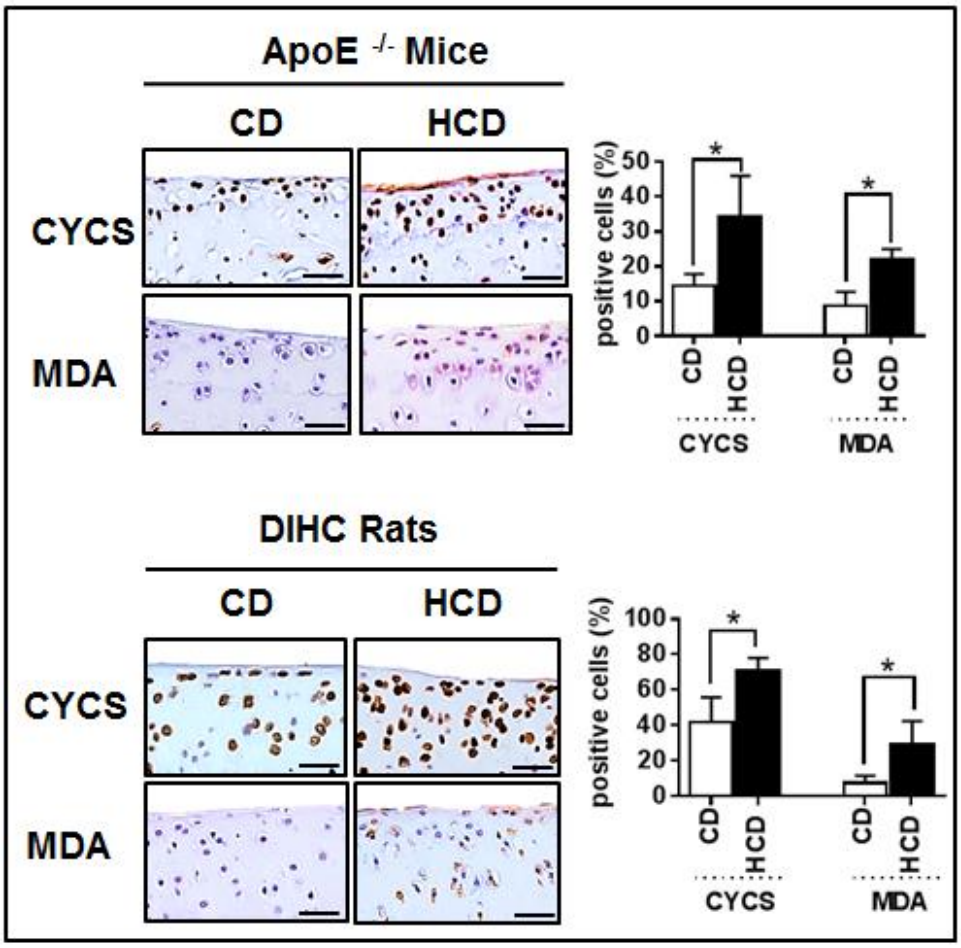

Figure 4.30: Expression of oxidative stress marker in HCD animals. Immunohistochemistry staining of CYCS and MDA, oxidative stress marker, seven and 12 weeks after HCD or CD in ApoE-/- mice and DIHC rats respectively. Scale bars= 50μm.

Hypercholesterolemia lead to apoptosis

We performed TUNEL staining assay as described in Section 3.2.24 to examine DNA fragmentation as a result of mitochondrial-induced cell death in ApoE$^{-/-}$ mice and DIHC rats fed a HCD. We used this method to further confirm a mechanism by which HCD induced cartilage damage (previously shown in Figures 4.17 and 4.24) in ApoE$^{-/-}$ mice and DIHC rat's models.

Apoptosis as a mechanism that leads to cell death can run its course very quickly, from minutes to hours. Due to this, and the focal nature of cell death, apoptosis is unusually unobtrusive in tissue sections. However, we observed more TUNEL-positive chondrocytes in the HCD groups of both animal models than the CD groups (Figure 4.31, green). Consistent with immunihistochemstry results (Figure 4.31), high levels of choelstreol targeted oxidative-induced apoptosis pathway from the most up-stream incident, CYCS release to cytosol, down to cell death and DNA damge.

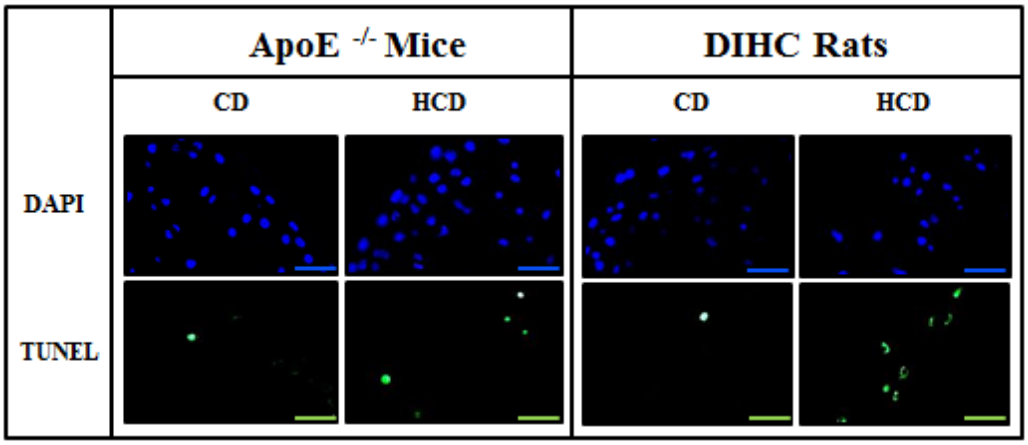

Figure 4.31: Hypercholesterolemia leads to apoptosis. Fluorescence detection (FITC) of TUNEL positive cells in ApoE-/- mice and DIHC rats seven and 12 weeks after HCD feeding, respectively. Scale bars= 50μm.

Cholesterol-lowering drug (atorvastatin) reduced plasma cholesterol levels

To study whether using cholesterol-lowering drugs could reverse cartilage degeneration, animals fed a HCD were subjected to atorvastatin oral treatment for three weeks prior to the animal's euthanasia as outlined in Section 3.3.1. Total serum cholesterol was measured in the HCD groups and HCD plus atorvastatin groups. In both $ApoE^{-/-}$ mice and DIHC rats, high cholesterol levels due to HCD were significantly reduced after atorvastatin treatment (Figure 4.32; $P<0.05$). This result confirmed that using oral atorvastatin in this study was efficient in decreasing plasma cholesterol levels. Accordingly, .this help us to rely on cholesterol-lowering effect of atorvastatin for further joint changes examination.

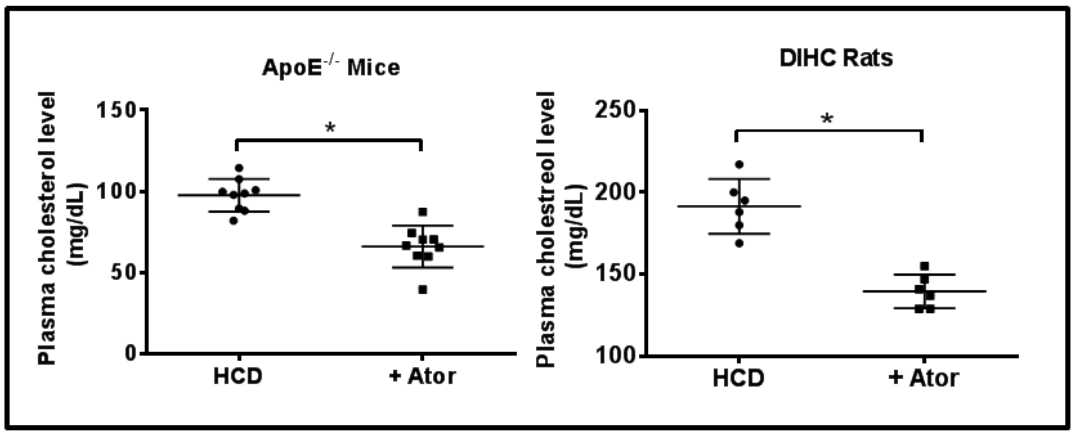

Figure 4.32: Cholesterol-lowering drug (atorvastatin) reduced plasma cholesterol levels. Total serum cholesterol level in ApoE-/- mice and DIHC rats received atorvastatin (+Ator) (n=6). Data are mean ± SD. *P<0.05 versus +HCD.

Atorvastatin as positive control attenuated cartilage damage

After confirmation of a cholesterol-lowering effect of atorvastatin administration in our animal models, the knee joint of ApoE$^{-/-}$ mice and DIHC rat models developed as per Sections 3.3.1 and 3.3.2 were histologically assessed with Safranin O staining to examine the inhibitory effect of atorvastatin on OA development as outlined in Section 3.2.9. Three weeks after atorvastatin administration, ApoE$^{-/-}$ mice showed less cartilage degenerative changes compared to the non-treated group, even with incidence of OA surgery. The Mankin score in atorvastatin-treated APOE$^{-/-}$ mice was also significantly lower than the control group (-Ator) (Figure 4.33; P<0.05). This may highlight the cartilage protective effect of atorvastatin during a course of hypercholesterolemia development.

Interestingly, consistent results were obtained from HCD rats treated with atorvastatin. We found less proteoglycan loss, better cartilage surface structure and significantly lower Mankin scores in rats treated with atorvastatin compared to the control group (Figure 4.33; P<0.05).

In general, we have demonstrated that the cartilage structure looks healthier, even in OA animals, when we diminish the impact of a HCD on joints using atorvastatin.

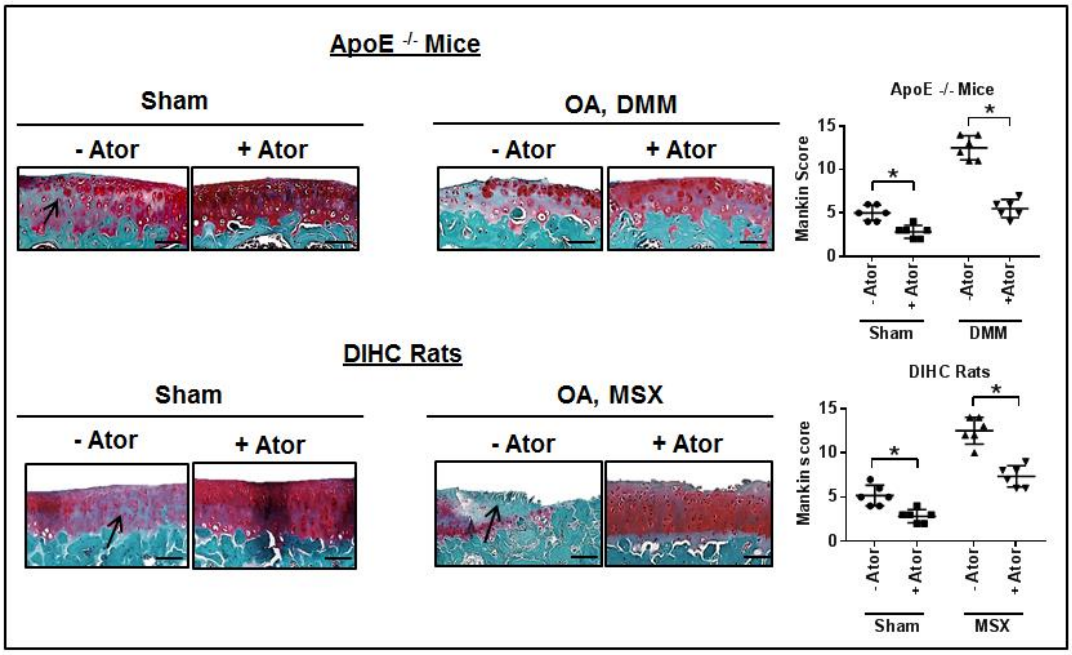

Figure 4.33: Atorvastatin attenuated cartilage damage. Safranin O staining and Mankin score of HCD ApoE-/- mice and DIHC rats who received atorvastatin (+ Ator) for three weeks. Cartilage damage caused by HCD was restored in ApoE-/- mice and DIHC rats after atorvastatin administration. This was in line with allied Mankin score graph showing a significant decrease in cartilage damage in atorvastatin-treated animals compared to the non-treated animals (-Ator). Scale bars= 100µm. Data are mean± SD. *P<0.05 versus - Ator.

Atorvastatin treatment did not change subchondral bone volume

The tibia plateau of ApoE$^{-/-}$ mice and DIHC rat models developed as outlined in Sections 3.3.1 and 3.3.2 were scanned in a Micro-CT scanner as described in Section 3.2.25 to evaluate subchondral bone changes after atorvastatin treatment in animals. We used the proximal part of the medial tibia plateau to assess the ratio of bone volume over tissue volume (BV/TV). We also have to aknowledge that due to number of animals per each group (n=3), we might have statistical analysis limitation and our Micro-CT results had low statistical power.

Micro-CT analysis showed that there were no significant changes in bone volume fraction (BV/TV) in the tibia plateau of the ApoE$^{-/-}$ mice and DIHC rats treated with atorvastatin compared to non-treated group (control). Subchondral bone volume was calculated using Micro-CT scanning. Micro-CT assessment did not show

significant changes in BV/TV ratio in the tibia plateau of the ApoE$^{-/-}$ mice and DIHC rats who received atorvastatin. As our previous Micro-CT assessment showed, in mice and rats fed a HCD, bone volume fraction was markedly decreased compared with CD group (Figures 4.18 and 4.25; P<0.05). Nevertheless, atorvastatin treatment did not significantly affect BV/TV in animals, but it tended to increase BV/TV (Figure 4.34; $P< 0.05$).

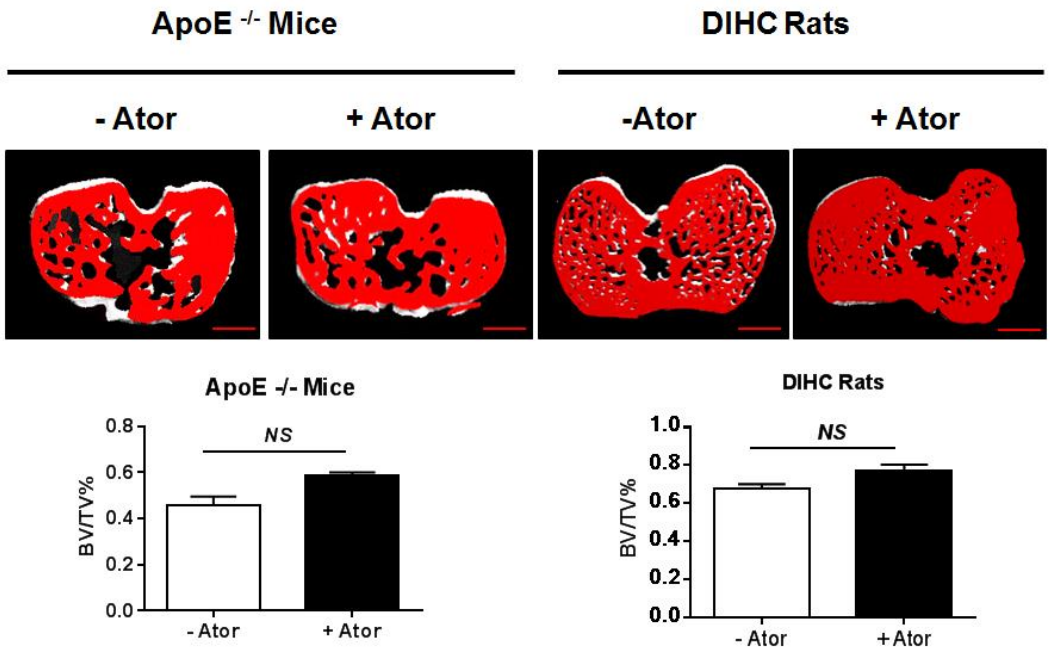

Figure 4.34: Micro-CT scanning of tibia plateau of ApoE-/- mice and DIHC rats who received atorvastatin. Micro-CT three-dimensional reconstructions of ApoE-/- mice and DIHC rats who received atorvastatin and bone volume/tissue volume ratio (BV/TV, %) measurement in the tibia plateau (n=3). Using Micro-CT analysis, no significant changes were observed after atorvastatin treatment (+Ator) in the ApoE-/- mice and DIHC rats compared to non-treated groups (-Ator). Scale bar = 100μm. Data are mean ± SD.

Atorvastatin reduced intensity and expression of OA-like markers

To assess the effect of atorvastatin on the expression of OA-like molecular markers, we performed immunohistochemistry staining as described in Section 3.2.14 on sections obtained form ApoE$^{-/-}$ mice and DIHC rat models developed as per Sections 3.3.1 and 3.3.2. We used the same OA-like marker that we used in our previous experiments as indicators of OA-like cartilage degradation. To test the effect

of atorvastatin on cartilage, we chose cartilage hypertrophic marker COL10 and cartilage degradative markers NITEGE and DIPEN.

Our results demonstrate that the intensity and/or expression amount of COL10, NITEGE, and DIPEN in ApoE$^{-/-}$ mice and DIHC rats decreased in atorvastatin-treated groups compared to non-treated ones (Figure 4.35). This finding suggests that atorvastatin treatment in both animal models is able to alter the expression of OA-like markers. Our immunohistochemistry staining results are similar to the data obtained from histological Safranin O staining (Figure 4.34) where the condro-protective effect of atorvastatin was demonstrated. Moreover, total percentages of positive cells for OA-like markers were significantly decreased in the atorvastatin-treated group compared to the non-treated animals (Figure 4.35).

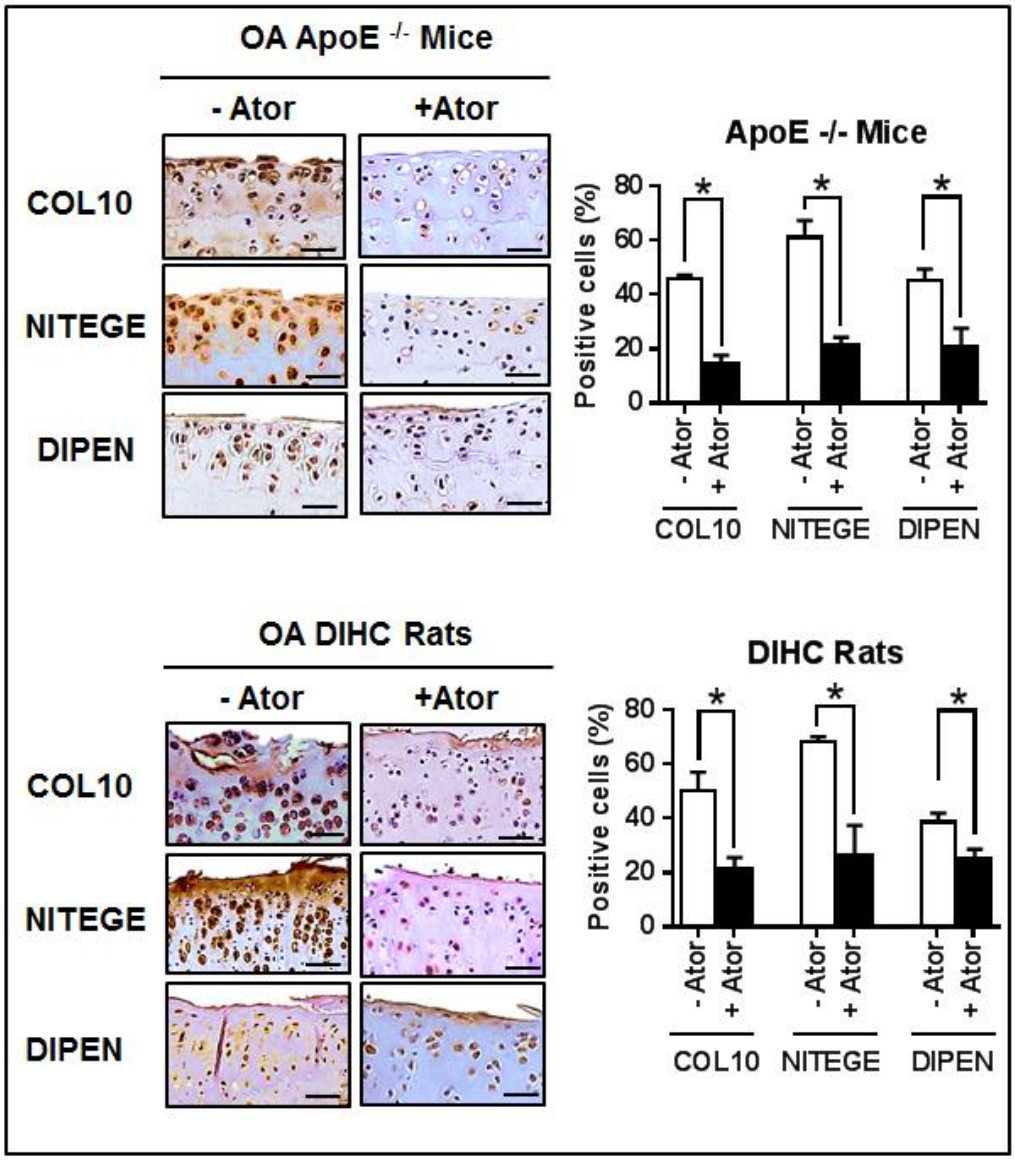

Figure 4.35: Atorvastatin reduced intensity and expression of OA markers. Immunohistochemistry staining of COL10, NITEGE, and DIPEN after atorvastatin treatment in ApoE-/- mice and DIHC rats. Expressions of OA-like markers were decreased in atorvastatin-treated (Ator) ApoE-/- mice and DIHC rats compared to non-treated (-Ator) groups (n=6). Total percentages of positive cells for all OA-like markers were significantly reduced after atorvastatin treatment in both ApoE-/- mice and DIHC rat model. Scale bars= 50μm.

Atorvastatin inhibited cell death in ApoE$^{-/-}$ mice and DIHC rats

We performed TUNEL staining assay as described in Section 3.2.24 to assess cell death as a result of apoptosis in ApoE$^{-/-}$ mice and DIHC rat models, developed as per Sections 3.3.1and 3.3.2, which received atorvastatin and compared them with the non-treated group. We used this method to further confirm that if a cholesterol level is reduced using a cholesterol lowering drug (atorvastatin), HCD-induced apoptosis (previously shown in Figure 4.28) will be decreased in ApoE$^{-/-}$ mice and DIHC rats as well.

We previously demonstrated that ApoE$^{-/-}$ mice and DIHC rats fed a HCD exhibited more apoptotic cells compared to the CD group (Figure 4.31). Here we found that the number of TUNEL positive cells were decreased after atorvastatin treatment compared to the non-treated group (Figure 4.36). This may suggest that atorvastatin inhibited the cell death program, which was induced by HCD. Although atorvastatin has multiple effects on different pathways, this result signifies the anti-apoptotic effect of this drug in ApoE$^{-/-}$ mice and DIHC rats that received it.

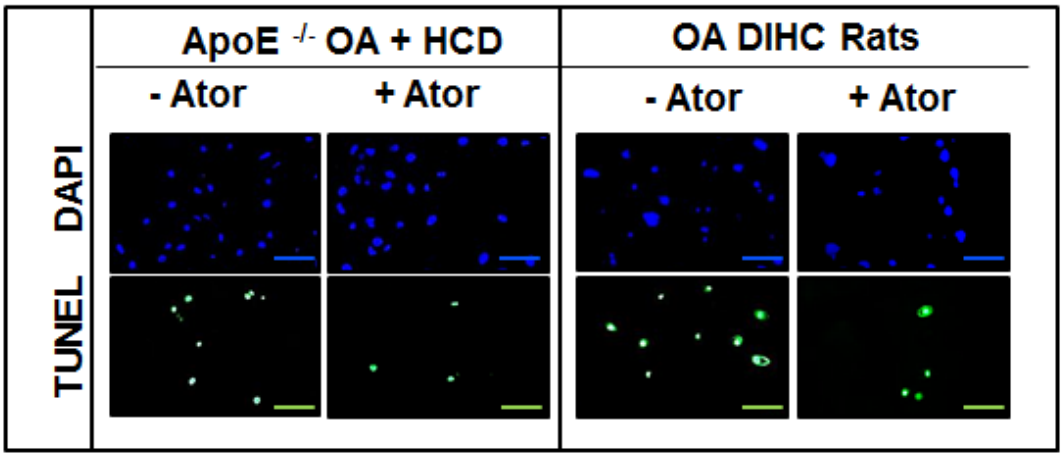

Figure 4.36: Atorvastatin inhibited cell death in ApoE-/- mice and DIHC rats. TUNEL staining of apoptotic cells in HCD ApoE-/- mice and DIHC rats after atorvastatin treatmen t(n=6). The number of TUNEL positive cells were decreased in both ApoE-/- mice and DIHC rats after atorvastatin treatment (+ Ator) compared to the non-treated group (- Ator). Scale bars= 50μm.

4.4 ADMINISTRATION OF ANTI-OXIDANT MITO-TEMPO/MITOQ COULD SLOW DOWN CHOLESTEROL-ASSOCIATED OA PROGRESSION IN OUR *IN-VIVO* AND *IN-VITRO* MODELS.

Mito-TEMPO slowed cartilage damage in-vitro

To test the effect of mito-TEMPO in our *in vitro* models, we performed ACCs pellet culture and bovine explant culture as outlined in Sections 3.2.4 and 3.2.5 respectively. We then treated the ACCs pellets and bovine explants with a combination of 30μg/ml cholesterol and 10μM mito-TEMPO as described in Section 3.2.6. ACCs pellets and bovine explants treated with cholesterol only were used as a control. Histological Safranin O staining was performed on ACCs pellets and bovine explants as per Section 3.2.9.

Our results show the protective role of mito-TEMPO in attenuating cartilage damage in-vitro. Quantifying sGAG content in the supernate of ACCs pellet culture revealed that the amount of sGAG was significantly decreased after mito-TEMPO treatment, suggesting less proteoglycan loss from ACCs pellets into the growing medium. Histological observation did not show a noticeable difference in bovine

explant culture after mito-TEMPO treatment; however, the amount of sGAG in the medium was lower in the mito-TEMPO treated group compared to the control (Figure 4.37; $P<0.05$).

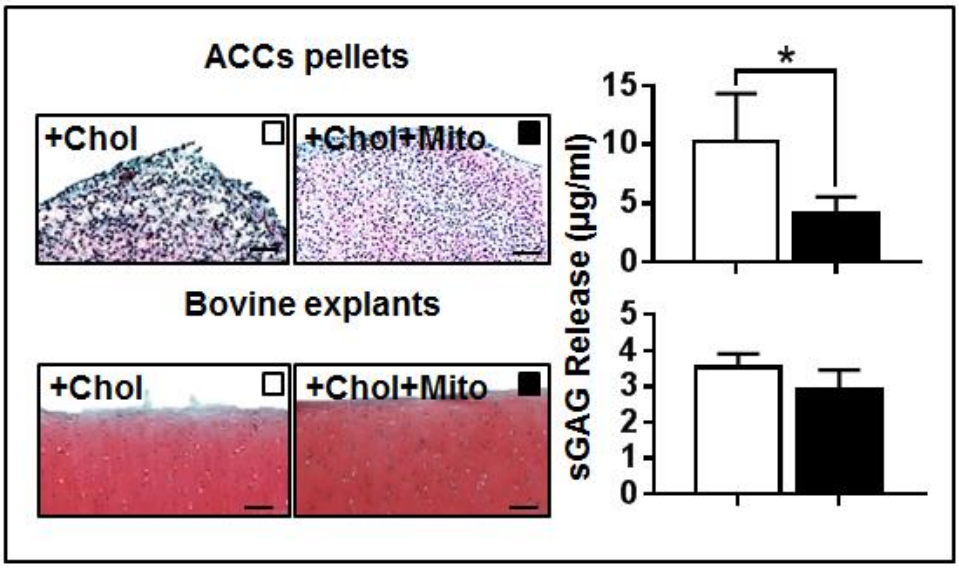

Figure 4.37: Mito-TEMPO slowed cartilage damage *in-vitro*: Safranin O staining of cholesterol stimulated ACCs in 3D culture and bovine explants and corresponding sGAG amount in growing medium after mito-TEMPO treatment (n=3). P0-P2 chondrocytes pellets were used for all of the experiments. Data are mean± SD. *P<0.05 versus cholesterol-treated (control).

Mito-TEMPO treatment changed the OA-like markers in ACCs pellets

To analyse whether an antioxidant (mito-TEMPO) is able to improve chondrocytes homeostasis *in vitro*, we performed a 3D ACCs pellet culture as described in Section 3.2.4 and stimulated the pellets with a combination of 30µg/ml cholesterol/10µM mito-TEMPO as outlined in Section 3.2.6. To assess the expression of COL10 (cartilage hypertrophic marker), MMP13 (cartilage degradative marker) and aggrecan (ACAN; cartilage chondrogenic marker), we performed immunofluorescence staining as per Section 3.2.15.

Similar results were obtained from immunofluorescence staining of ACCs pellets. We previously demonstrated that cholesterol stimulation increased COL10, and MMP13 expression in histological sections of ACCs pellets (Figure 4.7). Here we

found that the decreased expression of COL10 and MMP13 and increased expression of ACAN after mito-TEMPO treatment compared to the non-treated group (Figure 4.38).

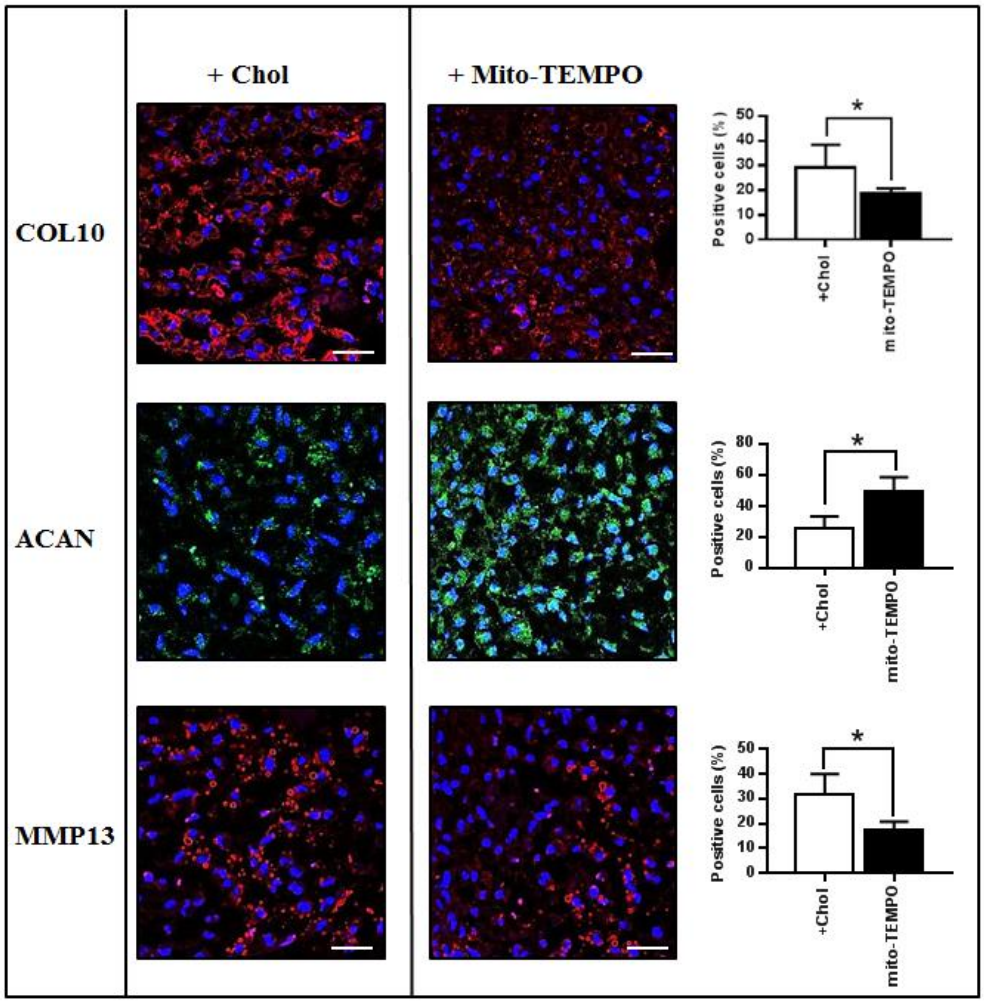

Figure 4.38: Mito-TEMPO treatment changed the OA marker in ACCs pellets. Immunofluorescence staining of COL10, ACAN, and MMP13 after mito-TEMPO treatment in 3D cultured ACCs pellets. P0-P2 chondrocytes pellets were used for all of the experiments (n=3). Scale bars = 75µm

Mito-TEMPO treatment changed the OA marker mRNA expression in ACCs pellets

The above results from the immunofluorescence staining of ACCs pellets revealed an altered expression of COL10, ACAN, and MMP13 after mito-TEMPO treatment compared to non-treated groups. To further analyse the effect of mito-

TEMPO on chondrocytes mRNA expression *in vitro*, 3D ACCs pellet culture was performed as outlined in Section 3.2.4 and ACCs pellets were treated with combination of 30µg/ml cholesterol and10µM mito-TEMPO as per Section 3.2.6. After completion of treatment, RNA was extracted from ACCs pellet and qRT-PCR was used to assess gene expression of MMP13, ACAN, RUNX2, and SOX9 at mRNA level as outlined in Section 3.2.16.

At the RNA level, mito-TEMPO treatment down-regulated the expression of the catabolic marker (MMP13) and hypertrophic marker (RUNX2); however, they could not alter the expression of ACAN to a significant level (Figure 4.39; $P<0.05$).

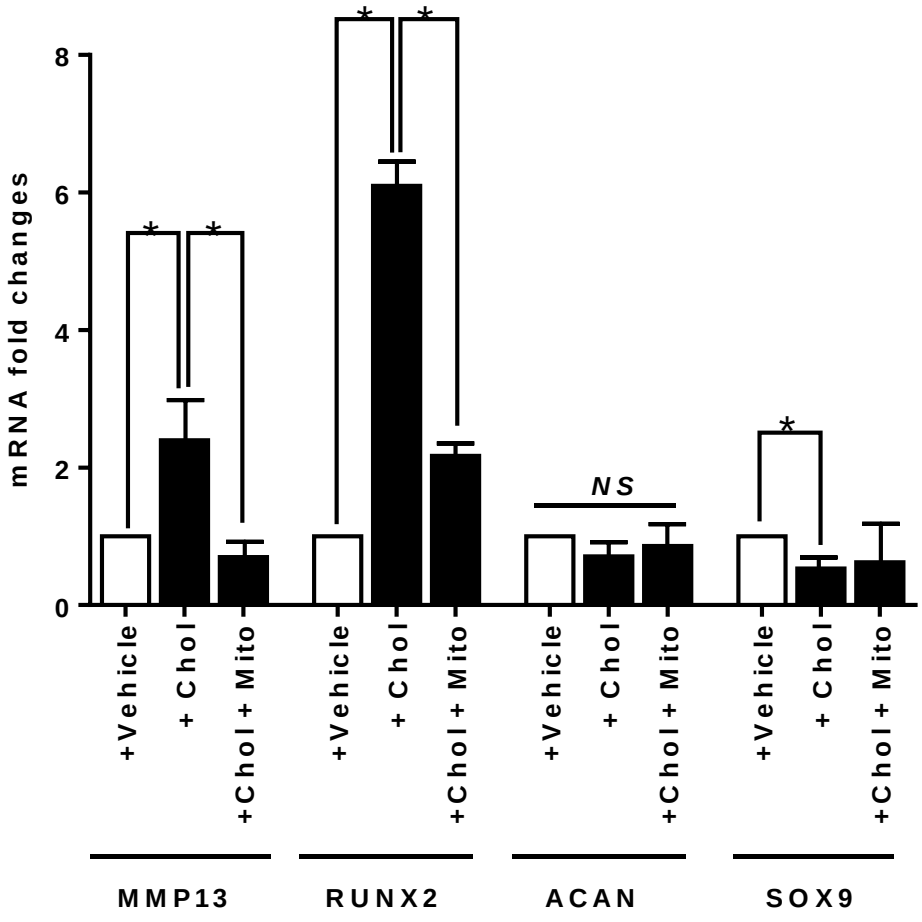

Figure 4.39: mito-TEMPO treatment changed the OA-like marker mRNA expression in ACCs pellets. The expression of MMP13, RUNX2, SOX9, and ACAN in 3D cultured ACCs after mito-TEMPO treatment (n=6). P0-P2 chondrocytes pellets were used for all of the experiments. Data are mean ± SD. Quantitative measurements

were determined using the (2−ΔΔCt); method and beta-actin expression were used as endogenous housekeeping genes. All samples were performed in triplicate. The data is normalised to control ACCs pellets (+vehicle) arbitrarily set to 1.*P<0.05 versus + Chol

Mitochondrial function is recovered using mitochondrial-targeted antioxidant Mito-tempo

Mitochondrial dysfunction was proposed as a potential mechanism playing a role in the OA-like phenotypical changes induced by excess cholesterol. We previously demonstrated that mitochondrial function is altered in ACCs after exposure to high levels of cholesterol (Figure 4.24). To test mito-TEMPO treatment effect on mitochondria, we performed the same diverse mitochondrial functional assessments. In all experiments, ACCs were treated with the combination of 30μg/ml cholesterol/10μM mito-TEMPO, and ACCs treated with 30μg/ml cholesterol was set as a control. We measured four parameters: the MTT for mitochondrial dehydrogenase activity as described in Section 3.2.18, RH-123 intensity for mitochondrial membrane potential as outlined in Section 3.2.20, DCFDA assay for ROS detection as explained in Section 3.2.21, and luciferase-based assay for ATP production as per Section 3.2.19, respectively.

Our results indicate that antioxidant mito-TEMPO is able to restore mitochondrial function by preserving mitochondrial integrity by protecting mitochondria from inner membrane potential loss (RH-123 assay). Moreover, our findings indicate that mito-TEMPO is able to specifically regulate mitochondrial ATP production in the mitochondrial inner membrane and ROS generation (Figure 4.40; $P<0.05$).

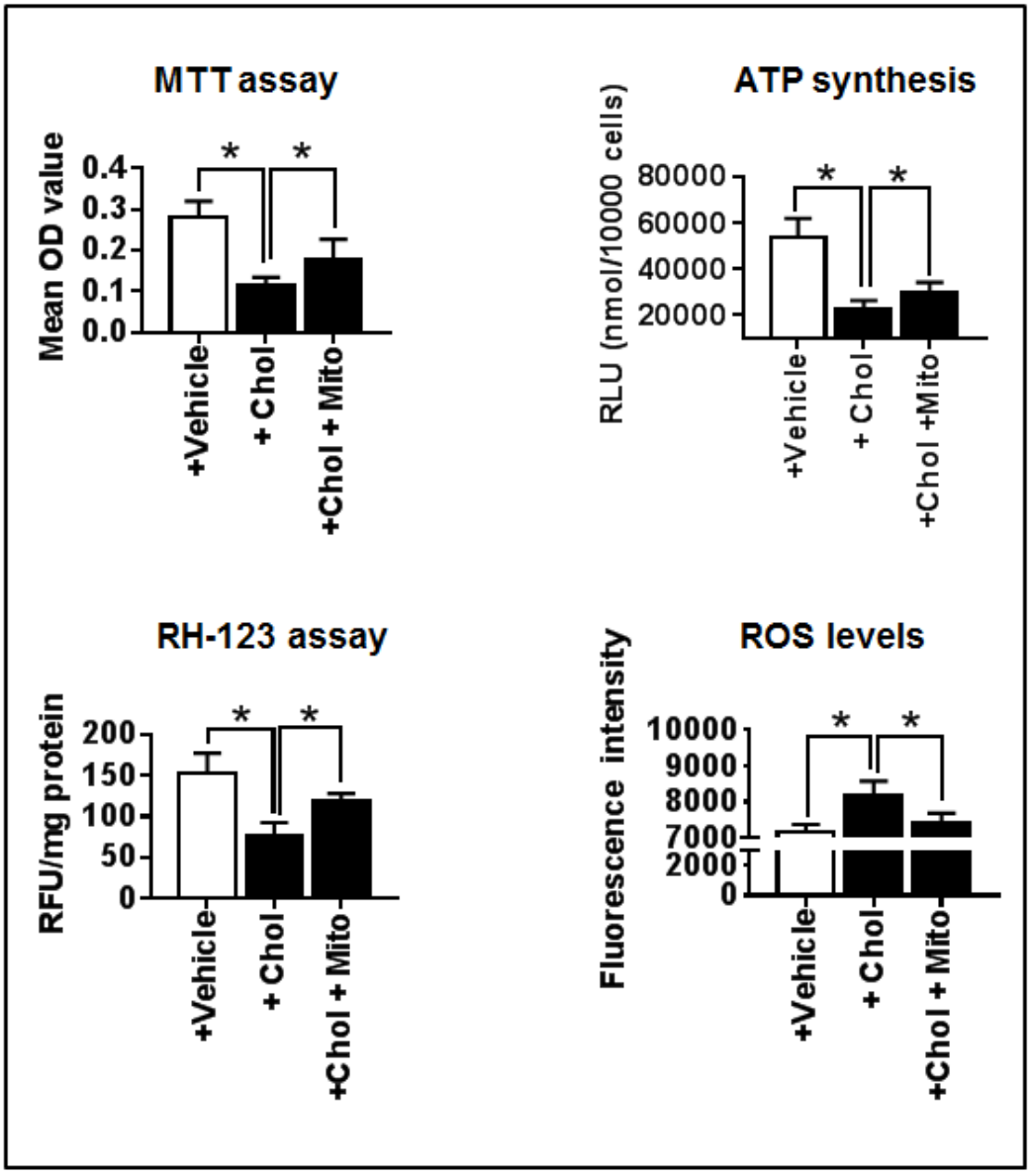

Figure 4.40: Mito-TEMPO improved mitochondrial functional. MTT assay, ATP generation, Rhodamine 123 (RH-123) intensity, and ROS level were assessed in ACCs after mito-TEMPO treatment (n=6). Data are mean ± SD. All samples were performed in triplicate. *P<0.05 versus + Chol.

Mito-TEMPO treatment altered the mitochondrial marker expression in ACCs pellets

The above results show that antioxidant mito-TEMPO is able to attenuate mitochondrial function *in vitro* cultured ACCs. To further assess the effect of mito-TEMPO on chondrocytes mRNA expression *in vitro*, a 3D ACCs pellet culture was performed as outlined in Section 3.2.4 and ACCs pellets were treated with combination

of 30μg/ml cholesterol/10μM mito-TEMPO as described in Section 3.2.6. After completion of the treatment, RNA was extracted from ACCs pellet and qRT-PCR was used to assess gene expression of BAX, CASP9, and CYCS at mRNA level as per Section 3.2.16.

It was not entirely surprising that mito-TEMPO reduced the BAX expression at the mRNA level by inhibiting the translocation of BAX to mitochondria. Therefore, the mito-TEMPO positively tapered the cytochrome *c* release into the cytoplasm led to a decrease in expression of cytochrome *c* and CASP9 as apoptotic markers (Figure 4.41; $P<0.05$).

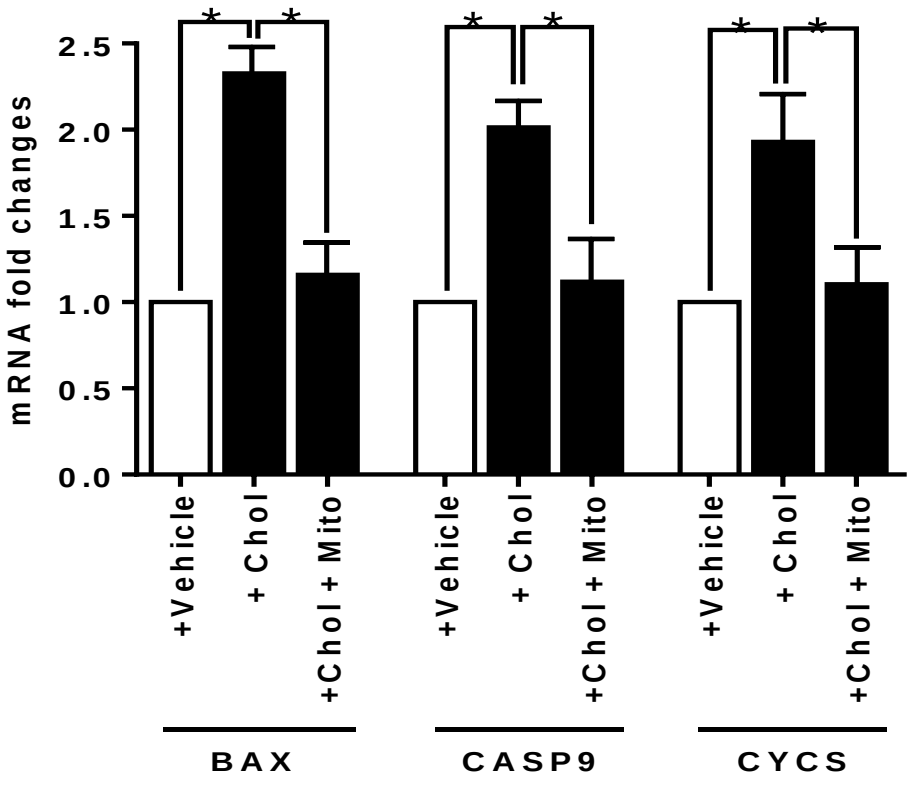

Figure 4.41: Mito-TEMPO treatment altered mitochondrial marker expression in ACCs pellets. The expression of BAX, CASP9, and CYCS in 3D cultured ACCs after mito-TEMPO treatment (n=6). P0P2 chondrocytes pellets were used for all of the experiments. Data are mean ± SD. Quantitative measurements were determined using the (2−ΔΔCt); method and beta-actin expression were used as endogenous housekeeping genes. All samples were performed in triplicate. The data is normalised to control ACCs pellets (+vehicle) arbitrarily set to 1.*P<0.05 versus + Chol.

MitoQ/mito-TEMPO antioxidant did not change the plasma cholesterol level

In the aim three of this study, we firstly demonstrated the protective effect of antioxidant against OA-like change in our *in vitro* models. To assess the potential role of oxidative stress in hypercholesterolemia-induced OA *in vivo*, we tested mitochondria-targeted antioxidants MitoQ and mito-TEMPO in our animal models. Although there are many natural antioxidants, including vitamin E, their effectiveness is limited as they do not accumulate in mitochondria, nor can they pass across lipid bilayers. Synthetic mitochondrial ROS (mtROS) scavengers such as mito-TEMPO and MitoQ have been developed to address this issue. These compounds easily pass through all biological membranes into cells and tissues affected by mtROS (M. P. Murphy & Smith, 2007). MitoQ is used to accumulate in mitochondria to protect from oxidative damage *in vivo* and mito-TEMPO protects cells from mitochondrial oxidative stress by playing a specific scavenger role against mitochondrial superoxide both *in vivo* and *in vitro*.

The ApoE$^{-/-}$ mice and DIHC rat models developed as described in Section 3.31 were subjected to MitoQ and mito-TEMPO treatment respectively as outlined in Section 3.3.1. Total serum cholesterol was measured as per Section 3.3.5 to investigate the effect of these antioxidants on plasma cholesterol levels. We did not find a significant difference in total plasma cholesterol levels in ApoE$^{-/-}$ mice and DIHC rats treated with MitoQ and mito-TEMPO respectively compared to the HCD (non-treated) group (Figure 4.42; $P<0.05$). Both MitoQ and mito-TEMPO had an antioxidant effect and no cholesterol-lowering effect in our animal models' plasma samples.

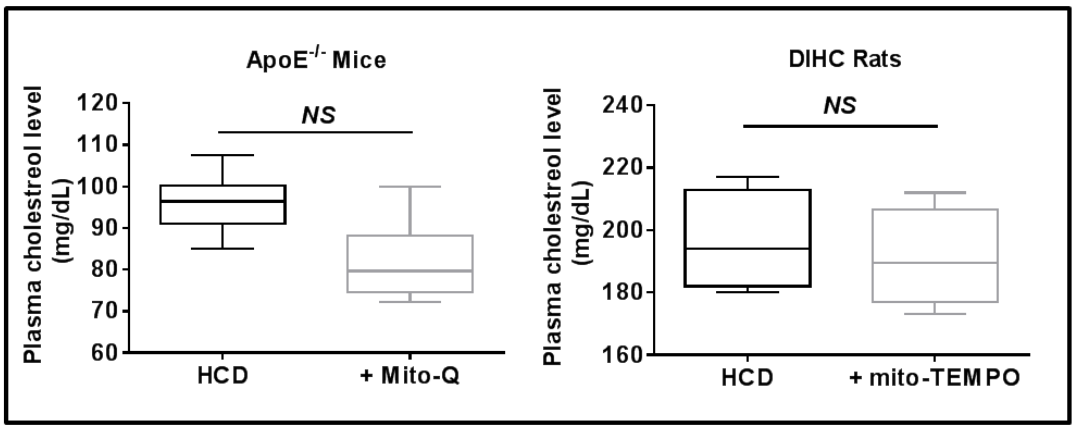

Figure 4.42: MitoQ/mito-TEMPO antioxidant did not change the plasma cholesterol level. Total serum cholesterol level in ApoE-/- mice and DIHC rats that received MitoQ and mito-TEMPO respectively (n=6). There were no significant changes in the serum cholesterol levels of DIHC rats treated with mito-TEMPO in comparison with non-treated ones. Data are mean ± SD. All samples were performed in triplicate. *P<0.05 versus HCD.

Mito-TEMPO/MitoQ antioxidants rescued cartilage integrity

The knee joint of DMM ApoE$^{-/-}$ mice and MSX DIHC rats were histologically assessed to study the scavenging effect of mito-TEMPO and MitoQ on OA development in the HCD group. Animals were developed as described in Sections 3.3.1.and 3.3.2. After three weeks of drug administration, we euthanized the animals and processed their knee joint samples for histological assessment. We performed Safranin O staining on sections obtained from the knee joints of the ApoE$^{-/-}$ mice and DIHC rats as per Section 3.2.9. Our findings demonstrated that MitoQ restored OA development in cartilage of ApoE$^{-/-}$ mice compared to animals that did not receive MitoQ. The corresponding Mankin score of the ApoE$^{-/-}$ mice that received MitoQ was significantly lower compared to the non-treated animals (-Mito) (Figure 4.43, P<0.05). Moreover, we found less proteoglycan loss, less vertical fissure, and better cartilage structure in DIHC rats treated with mito-TEMPO compared to the non-treated (-Mito) group. Mankin scoring of the cartilage in DIHC rats treated with mito-TEMPO was correlated with histological results, where cartilage integrity was saved after mito-TEMPO treatment compared to the non-treated group. Accordingly, the Mankin score

was significantly reduced after mito-TEMPO treatment in DIHC rats (Figure 4.43; $P<0.05$).

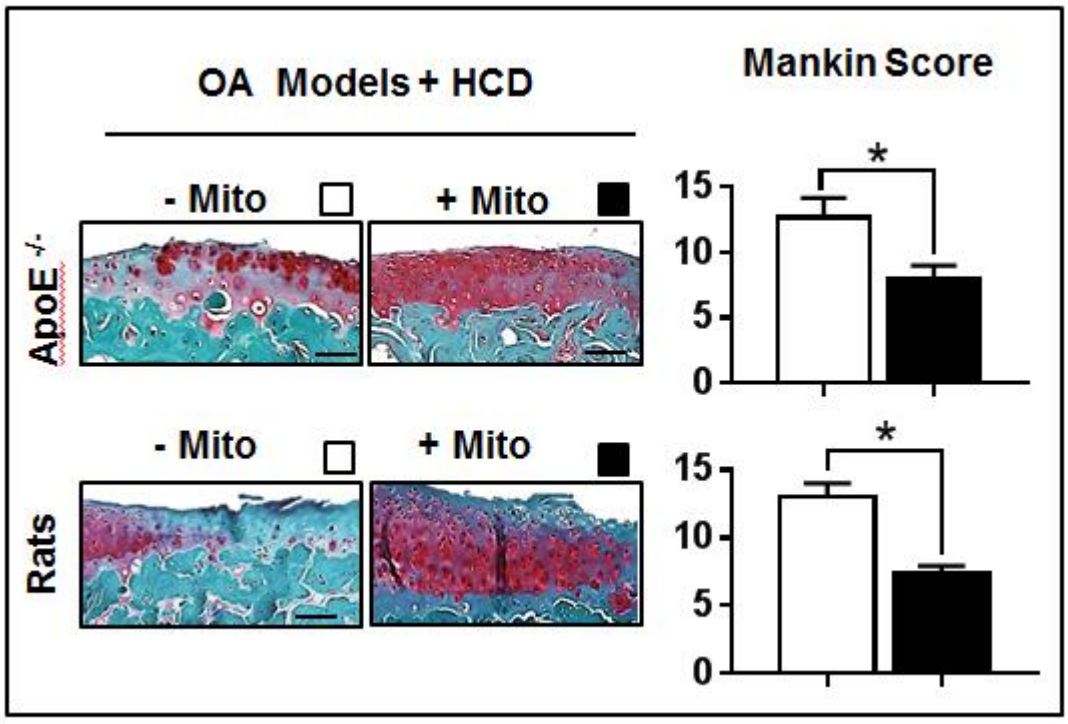

Figure 4.43: Mito-TEMPO/MitoQ antioxidants rescued cartilage integrity. Safranin O staining and Mankin score of HCD ApoE-/- mice and DIHC rats received MitoQ and mito-TEMPO respectively for three weeks (n=6). Histologically assessment of the knee joints of ApoE-/- mice and DIHC rats received antioxidants (+ Mito) revealed attenuated cartilage damage compared to non-treated (- Mito) ones. The corresponding Mankin scores were markedly decreased in both animal models treated with either MitoQ or mito-TEMPO compared to non-treated controls. Scale bars = 100μm. Data are mean ± SD. *P<0.05.

Mito-TEMPO and MitoQ antioxidants did not change subchondral bone volumes

To test the effect of antioxidants treatment on subchondral bone in ApoE$^{-/-}$ mice and DIHC rats, a Micro-Ct scanner was used. The tibia plateau of ApoE$^{-/-}$ mice and DIHC rat model developed as described in Sections 3.3.1 and 3.3.2 were scanned in a Micro-CT scanner as outlined in Section 3.2.25 to evaluate subchondral bone changes. We used a proximal part of the medial tibia plateau to assess the ratio of bone volume over tissue volume (BV/TV). According to the Micro-CT analysis, the 3D structure of

the medial tibia plateau in ApoE$^{-/-}$ mice and DIHC rats was not changed after receiving MitoQ and mito-TEMPO respectively. Micro-CT assessment exhibited that mito-TEMPO and MitoQ had a small, but not significant, additive effect on the BV/TV in tibia plateau of the ApoE$^{-/-}$ mice and rats that received treatment (+Mito) compared to the non-treated (-Mito) ones (Figure 4.44; $P<0.05$).

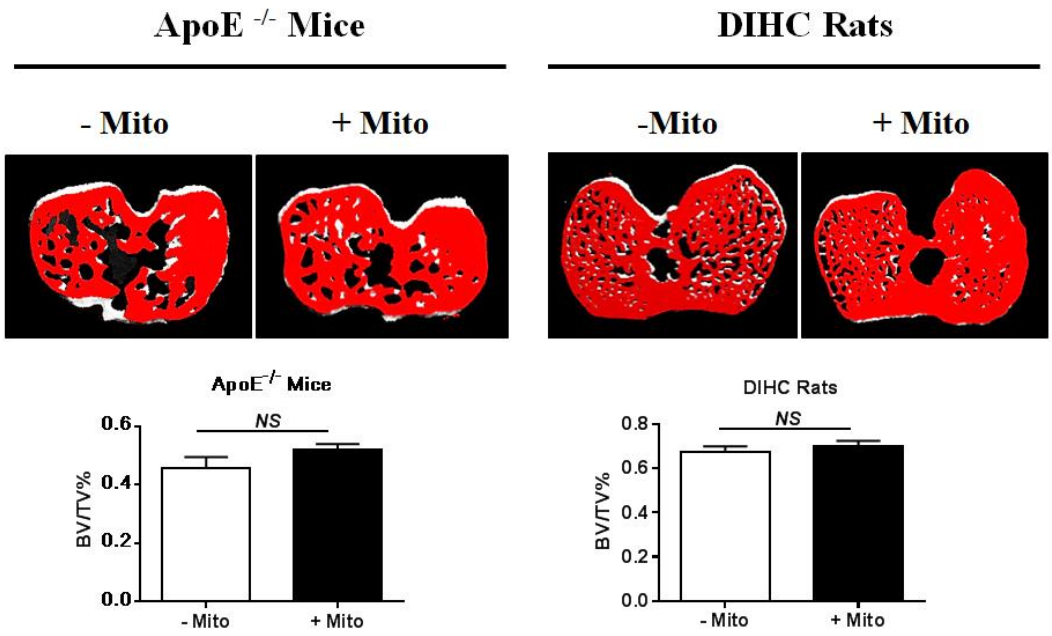

Figure 4.44: Micro-CT tibia plateau scanning of animals that received antioxidants. Micro-CT three-dimensional reconstructions of ApoE-/- mice and rats that received MitoQ and mito-TEMPO respectively, and bone volume/tissue volume ratio (BV/TV, %) measurement in the tibia plateau. Bone fraction and 3D structure of medial tibia plateau of ApoE-/- mice and DIHC rats that received antioxidants (+Mito) were not altered in comparison to non-treated groups (-Mito). Scale bar =100μm. Data are mean ± SD. All samples were performed in triplicate. *P<0.05

MitoQ and mito-TEMPO attenuated expression of OA markers

To assess the effect of antioxidants on the expression of OA-like molecular markers, we performed immunohistochemistry staining as described in Section 3.2.14 on sections obtained form ApoE$^{-/-}$ mice and DIHC rats' models developed as per Sections 3.3.1 and 3.3.2. We used the same OA-like marker as that used in our previous experiments as indicators of OA-like cartilage degradation. To test the effect of

MitoQ/mito-TEMPO on cartilage, we chose the cartilage hypertrophic marker COL10 and cartilage degeneration markers NITEGE and DIPEN.

Our results show that the expression of COL10, NITEGE, and DIPEN in ApoE$^{-/-}$ mice treated with MitoQ was decreased compared to the non-treated group (Figure 4.45). This result signifies the protective role of antioxidant MitoQ against cartilage degradation. Furthermore, we found a reduced expression of NITEGE, DIPEN, and COL10 in mito-TEMP treated DIHC rats in comparison with the non-treated group (Figure 4.46). Our immunohistochemistry staining findings are in line with our histological observation (Figure 4.45), whereas a protective effect of antioxidants treatment against OA development was revealed. In addition, the total percentage of positive cells for OA-like markers was significantly diminished in the antioxidants-treated group compared to non-treated animals (Figure 4.45).

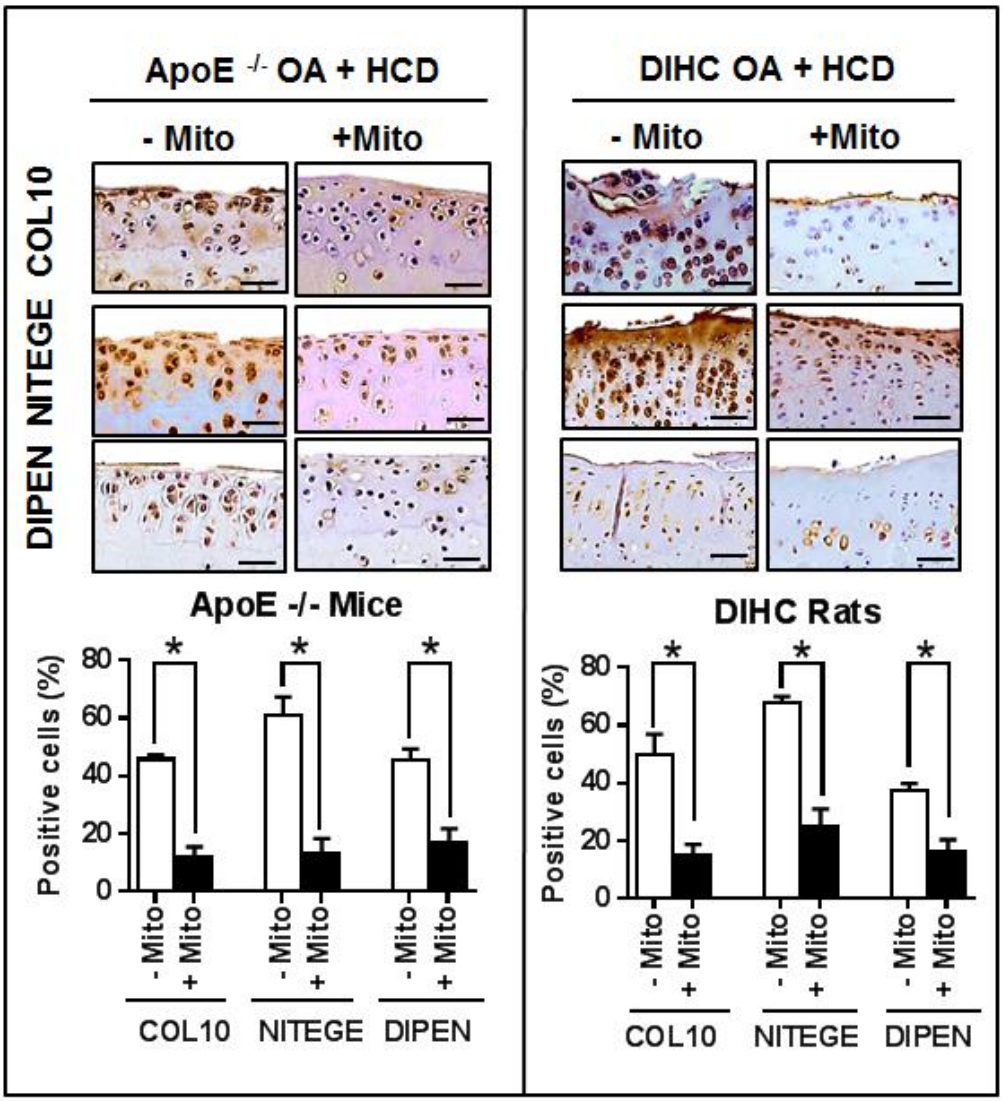

Figure 4.45: MitoQ and mito-TEMPO attenuated expression of OA markers. Immunohistochemistry staining of COL10, NITEGE, and DIPEN after MitoQ and mito-TEMPO treatment in ApoE-/- mice and DIHC rats respectively. Expressions of OA-like markers were decreased in both ApoE-/- mice and DIHC rats that received antioxidants treatment compared to the non-treated groups. Percentages of total positive cells for OA-like markers were significantly reduced after antioxidant treatment in both ApoE-/-mice and DIHC rat model. Scale bars= 50μm.

MitoQ/ mito-TEMPO antioxidants repressed mitochondrial apoptosis

We performed TUNEL staining assay as outlined in Section 3.2.24 to assess apoptotic cells in the ApoE$^{-/-}$ mice and DIHC rat models, developed as per Sections 3.3.1 and 3.3.2 that received antioxidants and compare them with the non-treated

group. We did this experiment to demonstrate that if chondrocytes' mitochondria were protected using MitoQ or mito-TEMPO, HCD-induced apoptosis (previously shown in Figures 4.28) would decrease in ApoE$^{-/-}$ mice and DIHC rats as well.

We previously demonstrated that ApoE$^{-/-}$ mice and DIHC rats fed a HCD exhibited more apoptotic cells compared to the CD group (Figure 4.31). We also found that the number of TUNEL positive cells decreased after atorvastatin treatment compared to the non-treated group (Figure 4.36). Here, we tested whether the anti-oxidant mito-TEMPO/MitoQ was able to decrease the apoptosis program by protecting mitochondria from HCD damage.

ApoE$^{-/-}$ mice and rats treated with MitoQ and mito-TEMPO respectively were evaluated for apoptosis. There were less TUNEL-positive cells in MitoQ and mito-TEMPO treated groups compared to the control (Figure 4.46). This observation may suggest that using a cholesterol-lowering drug inhibits the cell death program, which was induced by HCD.

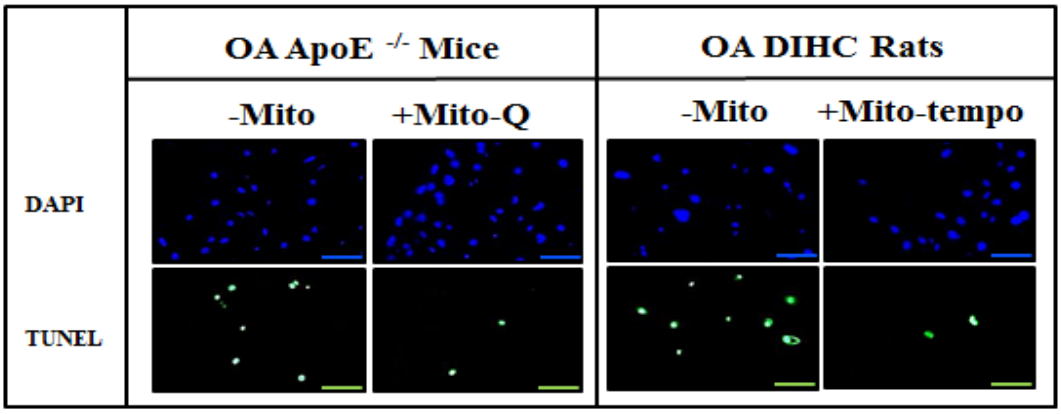

Figure 4.46: MitoQ/ mito-TEMPO antioxidants repressed mitochondrial apoptosis. TUNEL staining of apoptotic cells in HCD ApoE-/- mice and rats after MitoQ and mito-TEMPO treatment respectively. Scale bars= 50μm.

Chapter 5: Discussion

5.1 GENERAL DISCUSSION

Although still of uncertain pathology, there are many clinical factors that contribute to the risk and progression of OA, including metabolic syndromes. Metabolic syndromes are classified as obesity, dyslipidemia, high blood pressure, and diabetes (C. DAY, 2007). Metabolic OA has now been characterized as a subtype of OA, and the links between this phenotype and metabolic syndrome have been indicated in numerous studies. Related to metabolic syndrome is hypercholesterolemia that can result as an abnormal dietary cholesterol intake and/or inappropriate regulation of plasma cholesterol levels.

The role of cholesterol in the development of coronary artery disease is well documented, with numerous studies demonstrating its deleterious effects in the development of atherosclerosis (G. K. Hansson, 2005; P. Libby, Ridker, & Hansson, 2011). Its role in the etiology of OA is less understood, although epidemiological studies suggest a strong association between elevated serum cholesterol levels and OA (de Seny et al., 2015; Erb, Pace, Douglas, Banks, & Kitas, 2004; Q. Zhuo, W. Yang, J. Chen, & Y. Wang, 2012). In thelast century, the correlation between lipid abnormalities and OA was identified with the presence of increased levels of serum cholesterol in OA patients (DEBORAH J Hart et al., 1995; T. Stürmer et al., 1998). A study by Hart and colleagues showed that moderately raised serum cholesterol found in both knee and hand OA patients was independent of BMI (DEBORAH J Hart et al., 1995).

Literature data suggests that chondrocytes are able to produce cholesterol even if the mechanisms regulating this pathway remain unclear. High levels of cholesterol are naturally found in the cell membrane of chondrocytes (Ghadially, Mehta, & Kirkaldy-Willis, 1970) and proteins necessary for cholesterol biosynthesis, such as 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (HMGCS), acetyl-coenzyme A acetyltransferase 1 (ACAT1), or low density lipoprotein receptor (LDLR) are all expressed in human chondrocytes (Arkill & Winlove, 2006).

Recent evidence suggesting that cholesterol biosynthesis deregulation is presentin cartilage has given new strength to the studies focused on the cholesterol pathway in the OA. Studies have also shown aberrant regulation of cholesterol homeostasis in OA cartilage. Abnormal cholesterol accumulation occurs when cholesterol influx into the cell (through the action of apolipoproteins) exceeds cholesterol efflux. In OA chondrocytes, the gene expression of the two LXR subtypes alpha (NR1H3) and beta (NR1H2), as well as of the cholesterol transporter, APOA1 and ABCA1 are down-regulated compared to normal chondrocytes (24). We demonstrated that this differential expression pattern of cholesterol efflux genes, which are in line with recent findings (Gentili et al., 2005; A. Tsezou et al., 2010; Zerega et al., 2004), may signify a causal relationship between cholesterol homeostasis and the development and/or progression of OA. In another study, abnormal accumulation of cholesterol and fatty acids has been reported in articular cartilage, in particular, in the superficial area, during OA development. In mice, one study has shown that prolonged exposure to a high cholesterol diet can induce OA-like cartilage degenerative changes. Despite the fact that the above studies have demonstrated important and complex interactions between cholesterol and OA, the mechanisms remain unclear. More confirmatory *in vivo* evidence is required to establish the role of this risk factor in the development of OA and potential treatment options.

In our preliminary *in vitro* study, we demonstrated the abnormal lipid distribution in OA cartilage compared to the control, which might be a characteristic of OA tissues obtained from OA individuals. This hypothesis was further examined by a filipin staining where we found more intracellular cholesterol in OA ACCs compared to normal ACCs. Cholesterol is a major component of cell membrane structure. Dysregulation in cholesterol regulatory gene's expression, which is confirmed by a qRT-PCR experiment, may elucidate intracellular accumulation of cholesterol in OA samples compared to controls. As we demonstrated, cholesterol influx genes (SREBF2 and NPC1L1) were expressed more in OA tissue and ACCs pellets compared to the control, whereas cholesterol efflux genes (NR1H3 and ABCA1) were down-regulated in OA samples. Abnormal cholesterol regulatory genes expression led to intracellular accumulation of cholesterol. Our results are in line with previous evidence that highlighted the cholesterol biosynthesis deregulation role in regards to OA development cartilage (A. Tsezou et al., 2010). Moreover, we found most significant

abnormal cholesterol gene expression at the cartilage tissue level, suggesting that cholesterol abnormal uptake happens more frequently in the late stage rather than early stage, since we get most of cholesterol dysregulation in G4 cartilage obtained from late stage OA patinets; however, further investigation is required to determine the cellular mechanism behind these results. We proposed that abnormal cholesterol uptake due to genetic disorder and/or high cholesterol intake causes cellular dysfunction, which leads to cartilage damage. When Cholesterol abnormal uptake at cellular level accompany with OA pathological condition triggers vicious circle that contributes more to dysregulated cholesterol at the cellular levels. All of this may be an explanation for results we found at cartilage tissue level.

To better understand the potential association between hypercholesterolemia and OA, we designed *in vivo* and *in vitro* models. Two animal models, $ApoE^{-/-}$ mice and DIHC rats, were used to mimic human hypercholesterolemia and our results showed changes in the cartilage and subchondral bone in both models. Our confirmatory results showed that serum total cholesterol levels in animals fed a HCD significantly increased the levels considered to be hypercholesterolemia. Moreover, the data from both animal models' body mass measurement did not show any significant differences between the CD and HCD groups. This helped us to study the effect of high cholesterol levels independent of body mass; however, we have to aknowledge that potential changes in activity levels, muscular function and gait patterns alter external loading of the joint, and this contributes to loading of articular surfaces. Cartilage histopathological changes have been well-evidenced and scored during different stages of OA development. Briefly, OA cartilage changes entail cartilage degradation, loss of matrix, and surface fibrillation (Pritzker et al., 2006). Subchondral bone changes in human OA have been found to have an increase in bone mineral density, bone volume (sclerosis), and subchondral bone remodelling (Miller, Novatt, Hamerman, & Carlson, 2004). To evaluate the contribution of hypercholesterolemia to subchondral changes, we used fluorochrome bone turnover markers (Calcein and Alizarin red). We found increased bone remodelling and turnover in the subchondral bone of animals fed a HCD compared to the CD group. Increasing evidence has shown that subchondral bone turnover increases in patients with OA (Ratcliffe & Seibel, 1990). Our Micro-CT analysis presented a decreased subchondral bone volume in animals fed a HCD compared to CD. Subchondral bone volume changes during development of OA is

highly dependent on the time point post-surgery that has been chosen to analyse the subchondral bone. Early subchondral bone changes have been reported in animal models of both surgically induced and spontaneous OA (K. Brandt et al., 1997; Mason et al., 2001). Dedrick and coworkers also demonstrated subchondral bone loss in early stage OA, and subchondral bone sclerosis at the late stage of OA in a dog model of the disease (D. Dedrick, Goulet, Huston, Goldstein, & Bole, 1991; D. K. Dedrick et al., 1993). In addition, Hayami et al. also demonstrated the same trend of subchondral bone changes in their surgically induced rat model, where they found subchondral bone loss at two weeks post-surgery, but not at 10 weeks post-surgery (Hayami et al., 2004).

We also tested phenotypical changes, as well as molecular changes using a pour in vitro 3D ACCs pellets culture model. It has been known that expansion of primary ACCs using monolayer passaging results in a loss of the chondrogenic phenotype (Benya, Padilla, & Nimni, 1978; Z. Lin, Willers, Xu, & Zheng, 2006; von der Mark, Gauss, von der Mark, & Müller, 1977). To avoid this differentiation phenomenon in cartilage degradation applications and ACCs studies, primary ACCs in relatively early passages induce redifferentiation through selected medium supplements (e.g., ascorbic acid, TGFβ isoforms, insulin, transferrin, selenite, and others) (Chua, Aminuddin, Fuzina, & Ruszymah, 2005; de Haart, Marijnissen, van Osch, & Verhaar, 1999). This method is generally combined with 3D culture techniques that mimic the cartilage environment (Z. Lin et al., 2006). Moreover, the overall gene expression data showed that redifferentiation of ACCs in 2D and 3D culture systems leads to diverse chondrocyte phenotypes from which the 3D systems support the best chondrogenic differentiation, while the 2D monolayer system induces hypertrophic/mineralization markers (Caron et al., 2012). To evaluate the effect of excess cholesterol induction in our 3D ACCs pellets culture, we used the chondrocytes hypertrophic marker (COL10 and RUNX2), chondrocytes degradative marker (MMPs and ADAMTS-4,5), and chondrocytes chondrogenic (ACAN and SOX9) markers. Chondrocytes normally synthesize the chondrogenic-related genes. During OA development, it is expected that cartilage reduces the synthesis of extracellular matrix genes. However, several studies have demonstrated increased synthesis of chondrogenic components, in particular ACAN in OA (Hedbom & Häuselmann, 2002; Lippiello, Hall, & Mankin, 1977). Chondrocytes have raised anabolic activity to repair the damaged matrix during OA

development (Van der Kraan & van den Berg, 2000). Therefore, testing enzymatic degradation of matrix components might be a better hallmark to evaluate the metabolic imbalance in cartilage and 3D pellet culture, such as matrix degradative enzymes are MMPs and aggrecanase (ADAMs). Overall, we demonstrated the altered expression of ACCs anabolic and catabolic markers during cholesterol induction in our *in vitro* model.

Apart from *in vitro* phenotypical changes in response to high cholesterol induction, we also found molecular changes in our *in vivo* studies, similar to those in OA. DIPEN and NITEGE markers were used as matrix degradative markers to evaluate cartilage molecular metabolism. These two neoepitopes detect N or C terminus of aggrecan degradation products. DIPEN distinguish MMP- driven aggrecan loss and NITEGE distinguish aggrecanase (ADAMTS-4, 5)-driven aggrecan loss in the late-stage disease. It has been well-demonstrated that aggrecan is degraded, either with MMPs or aggercanase in OA patients, and the expression of both is significantly enhanced during a course of OA. Previous studies have reported that 'chondrocytes from OA cartilage have an altered phenotype, characterized by a decrease in cartilage matrix expression and increase of the terminal differentiated phenotypic changes' (Howell, 2002; O Pullig, Weseloh, Ronneberger, Käkönen, & Swoboda, 2000). Our findings showed an increased expression of COL10, DIPEN, and NITEGE in response to HCD in both models, suggesting an anabolic role for hypercholesterolemia in cartilage haemostasis.

Moreover, we found a significant increase in the synovial membrane activation induced by a HCD in both $ApoE^{-/-}$ mice and DIHC rat models. Based on histological analyses, we found that greater than 40% of animals fed a HCD exhibit synovial inflammation, with chronic inflammation appearing as diffuse infiltrates of macrophages scattered throughout the synovia of the affected joint and thickening of synovial membrane. From previous studies we know that synovial inflammation is important for OA pathology. Macrophages are a significant source of ECM degrading proteases, including MMPs, as well as of pro- and anti-inflammatory cytokines that regulate ECM remodelling, inflammatory cell recruitment and activation, and apoptosis. It has been reported that these factors, when released by inflammatory cells in the synovium, can precipitate a degenerative phenotype change of the cartilage (Andujar, Serrano, & Gomez-Zurita, 2012), a finding similar to those found in the

present study and that implicates high-cholesterol in macrophage mediated inflammation of the synovia. Interestingly, LDL receptor deficient mice ($LDLr^{-/-}$) have an accumulation of oxidized LDL in macrophages lining the synovium, which leads to activation of TGF-β and osteophyte formation in experimental OA (Hendrich, Pons, Ribera, & Balke, 2010). These studies, together with our own, support the hypothesis that cholesterol induced OA shares many similarities with atherosclerosis, especially the initial stages of macrophage in-filtration of the arteries. They also support the notion that hypercholesterolemia induces OA-like symptoms of the whole-joint, which include cartilage and subchondral bone changes, osteophyte formation and synovial inflammation. Our findings may also point to a potentially pro-inflammatory effect of hypercholesterolemia on joint health.

The knee joint synovial circulation is physically connected to the systemic circulation via the synovial capillaries and lymphatic system (Levick & McDonald, 1995). The most obvious difference between the synovial and systemic circulations is that the former operates at low pressure and the latter at high pressure. It is still an open question regarding whether or not metabolic factors, such as high cholesterol, affect the synovial circulation in a similar way to that of the systemic vasculature. The changes seen in the cartilage have been attributed to both direct and indirect effects of high cholesterol. A significant level of cholesterol has been found in the synovial fluid of OA patients and correlates with serum cholesterol levels (F. Oliviero et al., 2012). Under such circumstances, chondrocytes are exposed to elevated cholesterol levels in the synovial fluid, which then may diffuse into the cartilage (Ananth, Prete, & Kashyap, 1993; F. Oliviero et al., 2012; F. Oliviero et al., 2009). This could alter the fluidity of the cell membrane and activate the membrane lipid, signalling pathways causing damage to chondrocytes. Alternatively, it is known that high cholesterol levels in the $ApoE^{-/-}$ mice and rat models can trigger the inflammatory cytokine expression such as TNF-α, IL-1β and IL-6 (Rajamaki et al., 2010). Several studies have previously reported that some of these factors released by the synovium inflammatory cells can lead to alterations in the degenerative phenotype of the cartilage using co-culture studies (Chowdhury, Akanji, Salter, Bader, & Lee, 2008; S. R. Goldring & Goldring, 2004).

Increased body weight did not appear to have an effect on the development of OA, as no correlation was found between body mass and cartilage changes in either

animal model. This is, therefore, a strong indication that the effects of excess cholesterol have a greater role in OA development, particularly in conjunction with the mechanical instability caused by surgically induced OA (DMM or MSX) in these models. The changes observed in this study can, therefore, be directly and indirectly attributed to the effects of high cholesterol, independent of variables such as body mass. Althought, subchondral bone loss has been suggested to be, in part, related to disuse or limb and joint unloading, by muscular atrophy (Abusara et al., 2016; Egloff et al., 2014) and hind limb suspension (Anderson et al., 2016; Christiansen et al., 2012) which suggest there are multiple biomechanical and biochemical factors at work in the OA pathways. To better answer the question whether high cholesterol affect subchodral bone loss using same mechanisms, further investigation needed. Corroborating our findings is a recent study that showed dietary cholesterol played a role in the development of OA in an APOE*3Leiden mouse model. In that study, the drug ezetimibe was used to lower plasma cholesterol by inhibiting intestinal cholesterol absorption. Treatment with ezetimibe did not reduce the severity of OA in animals fed a cholesterol-rich diet, which suggests that factors other than cholesterol exposure contributed to the development of OA (L. M. Gierman et al., 2013). Another study has shown that overal HDL-C plasma levels may be less critical compared to impaired HDL functionality as a measure of predisposition to OA (Laakmann, Auel, & Kochzius, 2012).

In the present study, the results of the *in vitro* experiments mirrored the *in vivo* results, in as much as chondrocytes treated with physiological concentrations of free cholesterol had impaired cartilaginous gene expression and displayed degenerative changes. The first sign of obvious histological characteristics of OA is the onset of fibrillation at the surface of articular cartilage as matrix molecules, including that type II collagen and aggrecan are damaged (A. Robin Poole et al., 1996). This is associated with increased cleavage of the collagen by collagenases and aggrecan loss (Mwale, Tchetina, Wu, & Poole, 2002). ‘After the initial cleavage of type II collagen by collagenases it is denatured and lost. Chondrocytes subsequently undergo further phenotypic change becoming hypertrophic and expressing and secreting COL10’ (A. R. Poole, Kobayashi, M., Yasuda, T., Laverty, S., Mwale, F., Kojima, T., ... & Tchetina, E, 2002). We demonstrated decreased aggrecan and increased MMP-13 expressions in cholesterol stimulated chondrocytes; however, aggrecan expression at

the molecular level did not demonstrate a lessening trend after cholesterol treatment, which might be due to the compensatory mechanism of cells to maintain cartilage matrix homeostasis. Furthermore, increased COL10 expression in cholesterol-treated chondrocytes supported chondrocytes' hypertrophic phenotype as a late marker of OA. The final phase of OA seems to reflect a failure of the reparative process, resulting in degradation of the matrix, cell death, and total loss of cartilage integrity (Blanco, Guitian, Vázquez-Martul, de Toro, & Galdo, 1998). These findings suggest that cholesterol directly affects the function of the chondrocytes, possibly through changes to the fluidity and function of the cell membrane, resulting in abnormal cell behaviour and cholesterol accumulation (N. Wang et al., 2000).

We also demonstrated, for the first time, an increased incidence of bone marrow lesions in the subchondral bone area of cholesterol fed animals, an observation that corresponds to a recent epidemiology study that found a positive correlation between the level of serum cholesterol and triglycerides and an increased incidence of bone marrow lesions, a common symptom in OA patients (M. L. Davies-Tuck et al., 2009). In addition to cartilage changes, we also found significant changes to the subchondral bone, as well as osteophyte formation—both symptomatic of OA development—in the $ApoE^{-/-}$ and DIHC animals fed a high cholesterol diet.

The detrimental effects of cholesterol on chondrocytes and resulting OA changes can be attributed to a number of mechanisms. Mitochondria are vital organelles that produce energy for cells, particularly in the cartilage. Mitochondria are involved, not only in energy production, but also in other cellular activity including apoptosis. Mitochondrial dysfunction is known to be induced by hypercholesterolemia, hyperglycemia, hypertriglyceridemia, as well as by the process of aging (Lee & Wei, 2012; Puddu et al., 2005). A number of studies have shown an association between increased cholesterol oxidation products and mitochondrion-derived oxidative stress, which typically leads to increased production of mitochondrial mt-ROS (Lordan et al., 2009). Mitochondrial oxidative pathways produce ROS to synthesise ATP to maintain normal cartilage homeostasis when subject to physiological mechanical strains, with the signal cascade stimulated by mechanical distortion of the mitochondria (Brouillette et al., 2014; Montagne et al., 2014; Wolff et al., 2013). Excess ROS is typically produced by mitochondria and, under optimal conditions, is neutralized by the mitochondrial cellular defence mechanisms. Excessive ROS can overwhelm the

mitochondrial oxidative stress defences, inducing irreparable harm in susceptible cells, thereby causing permanent damage to the associated tissues. Increased production of ROS, in particular, superoxide and radicals derived from superoxide, has been associated with cartilage dysfunction in human disease (Ostalowska et al., 2006) and animal models (Johnson et al., 2004), and evidence shows a link between oxidative stress and cartilage degeneration in humans (Afonso et al., 2007; Regan et al., 2005). On the other hand, chondrocyte matrix synthesis and mineralization are modulated by the balance between ATP generation and consumption; therefore, it is essential to regulate mitochondrial function as a source of energy production to maintain cartilage homeostasis. Recent ex vivo studies have found mitochondrial dysfunction in human OA chondrocytes, and analyses of mitochondrial electron transport chain activity in these cells shows decreased activity of Complexes I, II and III compared to normal chondrocytes. In Maneiro et al.'s study, mitochondrial mass was increased in OA, as revealed by a significant increase in enzymatic activity (Maneiro et al., 2001). Consequently, a rise in mitochondrial mass might be a compensatory mechanism of OA chondrocytes against electron transfer deficiency via complexes II and III leading to low ATP production per mitochondrion (Hsin-Chen, Pen-Hui, Ching-You, Chin-Wen, & Yau-Huei, 2000). In addition, OA cells demonstrated a loss of the mitochondrial membrane potential ($\Delta\psi m$) as shown by means of the fluorescent probe JC-1. It has been revealed that OA chondrocytes have a lower red/green fluorescence ratio than normal chondrocytes as demonstrated by flow cytometry, indicating the mitochondria depolarization (Blanco, López-Armada, & Maneiro, 2004). Previous studies also reported that loss of $\Delta\psi m$ is related to the outer mitochondrial membrane disruption, mitochondrial swelling, and the discharge of pro-apoptotic molecules, including apoptosis-inducing factor, cytochrome *c*, and pro-caspases from the intermembrane space (Crompton, 1999). This evidence highly supports chondrocyte mitochondrial impairment as a mediator of the initiation and/or development of OA. However, it remains unknown whether excess cholesterol levels can affect chondrocyte mitochondrial function due to the impaired mitochondria potential and ROS production. Numerous studies have shown that a major source of nutrients for cartilage is synovial fluid, and because synovial fluid is an ultrafiltrate of plasma, chondrocytes receive their nutrients via a double diffusion system. Accordingly, it is highly possible that a high level of plasma cholesterol directly influences chondrocytes function through synovial fluid high levels of cholesterol in plasma.

In the present study, we found that cholesterol induced OA-like changes were triggered by means of mitochondrial pathways. Strong evidence for this hypothesis was the observation that cells subjected to a cholesterol challenge suffered a loss of mitochondrial membrane integrity that activated the intrinsic apoptotic pathway resulting in the release of a number of cell-death promoting molecules, including cytochrome *c*. The other major clue of a mitochondrial involvement was the observation that cholesterol treated cells produced excess ROS commensurate with cartilage degeneration. Mitochondrial dysfunction affects other pathways implicated in cartilage degradation, including 'oxidative stress, defective chondrocyte biosynthesis, increased cytokine-induced chondrocyte inflammation and matrix catabolism, cartilage matrix calcification, and increased chondrocyte apoptosis' (L. Wang et al., 2013).

To support this finding, we reported that cholesterol-treated cells trigger the intrinsic apoptosis pathway by causing the loss of mitochondrial membrane integrity, leading to the release of multiple death-promoting molecules such as cytochrome *c*. Moreover, mitochondria is a major source of ROS in the cells and we found that cholesterol treated cells produce excess ROS that can lead to cartilage degradation. Mitochondrial dysfunction can impact on some pathways that have been associated with cartilage degradation, including 'oxidative stress, increased cytokine-induced chondrocyte inflammation and matrix catabolism, defective chondrocyte cartilage matrix calcification, biosynthesis and growth responses, and increased chondrocyte apoptosis' (Blanco, Rego, & Ruiz-Romero, 2011). This is in line with our findings, suggesting that cholesterol stimulation of cells can alter mitochondrial morphology and structure led to mitochondrial functional impairment activating mitochondrial apoptosis pathway. Here we tested oxidative stress-induced apoptosis as growing evidence supporting the strong link between MetS and oxidative stress condition (Furukawa et al., 2004; Matsuda & Shimomura, 2013; Roberts et al., 2006). We demonstrated for the first time that cholesterol challenge influences cartilage haemostasis resulting in chondrocytes apoptosis. We stressed that oxidative stress pathways such as P-ERK and P-JNK are involved in the chondrocytes apoptosis, which play a role in down-stream events of oxidative stress-induced apoptosis under excess cholesterol environment.

Atorvastatin is an HMG-CoA reductase inhibitor that has been prescribed as a cholesterol lowering drug for the past two decades. We tested the efficacy of atorvastatin to attenuate cholesterol induced OA symptoms in both *in vivo* and *in vitro* experiments. The *in vivo* results showed a statistically significant protective effect from atorvastatin conferred on articular cartilage and subchondral bone, against the progression of OA-like symptoms. Similar effects were also observed *in vitro*, in the form of a significant reduction of catabolic and hypertrophic marker expressions. Apart from their cholesterol lowering properties, statins also exert pleiotropic effects that include immunomodulation, anti-inflammation, anti-angiogenesis, and anti-apoptosis (Bhandari et al., 2015; Khurana, Gupta, Bhalla, Nandwani, & Gupta, 2015; Nezic et al., 2009; Tremoulet, 2015; Zhao et al., 2015). Statins can also affect several target proteins that are related to mitochondrial ROS production (Lim & Barter, 2014), proteins whose expression was increased in $ApoE^{-/-}$ mice and DIHC rats fed a high cholesterol diet. The *in vitro* experiments revealed that atorvastatin treatment had anti-oxidative effects, decreased expression of matrix metalloproteinases and interleukins, and increased the expression of aggrecan and type II collagen in chondrocytes. In synovial cells, atorvastatin reduced the production of matrix metalloproteinases, interleukins, chemokines, and induced apoptosis of synovial fibroblasts. It is possible that statins may affect the progression of osteoarthritis by inhibiting osteoclastogenesis and vascular pathology, which in turn stimulates bone formation and counteracts the underlying mechanisms of OA. Therefore, at this stage we cannot draw definitive conclusions as to which of atorvastatin's effects is the most important in terms of reducing cartilage degeneration; be that the reduction of plasma cholesterol in the $ApoE^{-/-}$ mice and DIHC rats or the drug's potent anti-inflammatory effects.

The *in vitro* results pointed to mitochondrial dysfunction as a likely culprit for the adverse effects of high cholesterol on chondrocytes. This prompted us to evaluate a mitochondrial specific anti-oxidant that targets the most upstream mitochondrial event to prevent OA development induced by high cholesterol levels. Mito-TEMPO was chosen over more common antioxidants such as ascorbic acid or tocopherols due to its demonstrated specificity against mitochondrial ROS and superoxide production (Dikalova et al., 2010). Mito-TEMPO is a physicochemical compound as one of SOD mimics. It has an ability to pass through lipid bilayers easily and accumulate selectively in mitochondria (Dakilova et al., 2010). Superoxide and alkyl radical

scavenging properties A mito-TEMPO as a mitochondria-targeted antioxidant have been confirmed both Bin vitro and in vivo studies (Liang et al. , 2010, Dakilova et al., 2010, Liu, M., Liu, H., & Dudley, S. C., 2010). It has been reported that mito-TEMPO preserves physiological ROS signalling in non-malignant skin fibroblasts and selectively targets tumour cells with abnormal mitochondrial activities (Nazarewicz et al., 2013). Mito-TEMPO decreases mitochondrial superoxide levels by mimicking the action of super oxide dismutase. Therefore, I hypothesised that mito-TEMPO prevents the formation of O_2 derived ROS, including cytosolic H_2O_2 and self-propagating lipid peroxides and allows the cells to clear out excess ROS. All of this toghether would prevent cartilage tissue from ROS damage.

In particular, we report that mito-TEMPO prevents high cholesterol-induced OA changes in both animal models and *in vitro* cell culture studies. Mito-TEMPO specifically targeted inner membrane potential of mitochondria and prevented chondrocytes from overwhelming production of ROS, which influenced triggers oxidative stress pathways. These findings offer a rationale to test mitochondria-targeted anti-oxidants as a genuine treatment option against hypercholesterolemia induced OA.

5.2 FUTURE PERSPECTIVES AND CLINICAL IMPLICATIONS

A new concept of OA is emerging, indicating that OA is not a unique disease, but is a heterogeneous syndrome with different clinical phenotypes, eventually leading to common clinical manifestations. Although all phenotypes of OA demonstrate the same clinical symptoms, the mechanisms involved in such a manifestation are not the same.

OA is a heterogeneous condition affecting whole joint heath and whole animal/human behaviour, e.g. physical activity and movement, which will also affect biomechanical pathways for disease. Moreover, pathological changes of OA happen at cartilage, bone, synovium and menisci and muscles covered joint capsule. Therefore, it is important to study the effect of cholesterol on different tissue involved in OA pathogenesis and also tight relationship between altered biomechanics, biochemistry and tissue structure and morphology needs to explored and understood. One future direction of this study is to investigate whether the destructive effect of the

cholesterols starts from the subchondral bone or cartilage and better understand which tissue will be secondarily affected. Using animal models of hypercholesterolemia will be an effective tool to OA pathogenesis at different time points to clarify the etiology of cholesterol-induced OA.

Another interesting future perspective that should be tested is whether cholesterol plays a role in the angiogenesis process. As we demonstrated that an excess amount of cholesterol induced alterations in the dynamical interactions of the biomechanical, biochemical with mitochondria structural changes result in a system that is seen as mitochondria dysfunction, hypercholesterolemia may also play a role in angiogenesis. Both *in vivo* and *in vitro* testing of effect of high cholesterol level on angiogenesis markers would be interesting to determine other possible pathways from which excess cholesterol begins to affect cartilage and synovial inflammation. Studying the potential role of hypercholesterolemia in angiogenesis is another angle that might be approached to address the pathogenesis of cholesterol-induced OA.

Moreover, further *in vitro* and *in vivo* experimental models are required to study the role of synovial macrophages in promoting inflammatory and destructive responses in OA of hypercholesterolemia associated metabolic syndrome phenotypes and to better understand the potential synergic mechanism underlying this process. This will assist clinicians to modify strategies for managing OA patients. Successful completion of these studies will thus provide a platform from which to develop novel treatments based on careful manipulation of the immune system that can be applied to OA patients.

In addition, targeting mitochondrial dysfunction as a mechanism involving in cholesterol-induced OA requires further *in-vivo* and *in-vitro* models. Using commercially available mitochondrial trackers for both *in vivo* and *in vitro* models would be a complementary method to observe mitochondrial structure and function during the course of cholesterol-induced OA.

This PhD study has demonstrated a strong association between OA and hypercholesterolemia independent of obesity or any other known risk factors for OA development. This conception has great importance for the potential development of ‘disease-modifying anti-OA drugs (DMOADs. A future real world implication of this project would be testing the clinical trial of antioxidant mito-TEMPO to provide a new therapeutical drug targeting OA development.

5.3 CONCLUDING REMARKS

In this study we demonstrated in two animal models that elevated plasma cholesterol levels could trigger oxidative stress in the mitochondria and chondrocytes apoptosis that led to OA development. We further showed that the cholesterol lowering drug atorvastatin and the mitochondria specific antioxidant Mito-tempo/Q could have protective effects that attenuate OA symptoms caused by high cholesterol.

Current knowledge on the association between hypercholesterolemia and OA mostly rely on epidemiological studies. These studies investigated the link between these two disorders in OA individuals as a study group, however; their outcomes were controversial. There is only one experimental study in this field, which evidenced a direct link between hypercholesterolemia and OA. Although the author claimed a direct association between OA and hypercholesterolemia, his conclusion was drawn based on basic histological changes of cartilage only. Moreover, the molecular mechanism behind this association was not answered. The current understanding of OA is that alterations are not only limited to cartilage, but the underlying subchondral bone tissues and synovium also undergo pathological changes, described mostly by subchondral sclerosis and elevated subchondral bone remodelling and synovial inflammation (Lajeunesse, Hilal, Pelletier, & Martel-Pelletier, 1999). Therefore, there is an increasing evidences that recognize OA as a whole-of-joint whole-of-person pathological condition (Andriacchi et al., 2004). An ideal investigation for effect of high levels of cholesterol on OA-like changes and disease treatment should target the structural alteration to all of these tissues. Hence, it is essential that the specific factors responsible for these structural alteration, to be identified and targeted in order to accomplish such outcomes. In this project we have investigated role of hypercholesterolemia in OA initiation and/or progression.

In order to investigate our aims, we characterized cholesterol distribution and regulation in OA samples comparing to normal. Differential cholesterol regulation pathways between OA and normal samples, which were concluded from our first aim, led us to test direct effect of high levels of cholesterol on ACCs phenotype and function. Upon completion of the second aim of this project, we have demonstrated strong destructive effect of excess cholesterol on chondrocytes phenotype. For the first

time, we have indicated that chondrocytes phenotypical changes in response to high levels of cholesterol may be due to mitochondrial dysfunction. In addition, we also showed that mitochondrial morphology and function changes trigger oxidative stress pathway, which is one of the key molecular drivers in OA development. As a part of aim two of this study, we investigated molecular mechanism underlying excess cholesterol and ACCs changes which has been further tested in our *in vivo* models

Next, we have used two animal models to further manipulate our in vitro results in the in vivo condition. ApoE-/- mice and dietary induced hypercholesterolemia rats were used in order to test the impact of cholesterol regulatory gene deficiency and high cholesterol food intake on joint health. These two risk factors are the most important contributing elements in human hypercholesterolemia. In this part of this project we have shown a direct association between hypercholesterolemia and OA-like changes in the whole-joint tissues including cartilage, subchondral bone and synovium. Moreover, we found mitochondrial-related changes in our animals models fed HCD compared to CD group supporting our in vitro results.

In the last part of our study, we have used specific antioxidant mito-TEMPO in both *in vivo* and *in vitro* models. These antioxidants specifically target mitochondrial inner-membrane potential and function. Our in vitro findings suggest that use of antioxidant in combination with cholesterol can restore mitochondrial function and ACCs phenotype consequently. For the first time, we have reported that OA-like changes in response to the condition mimicked hypercholesterolemia were attenuated by the use of antioxidant in our animal models.

It is important to understand how metabolic risk factors such as hypercholesterolemia contribute biologically to the development of OA because most of the risk factors can be modified with simple life style changes without the need of surgical interventions or expensive drug and medical treatments. This study has advanced our understanding of how high cholesterol levels influence the joint biology. In summary, our results indicate that hypercholesterolemia is a critical mediator of increased mitochondrial oxidative stress which leads to the progression of OA. Inhibition of mitochondrial oxidative stress and dysfunction by the use of mitochondria-targeted antioxidants offer therapeutic potential for the prevention and treatments tailored specifically for this risk group.

References

Abusara, Z., Von Kossel, M., Herzog, W., 2016. In Vivo Dynamic Deformation of Articular Cartilage in Intact Joints Loaded by Controlled Muscular Contractions. PloS one 11, e0147547.

Acehan, D., Jiang, X., Morgan, D. G., Heuser, J. E., Wang, X., & Akey, C. W. (2002). Three-Dimensional Structure of the Apoptosome: Implications for Assembly, Procaspase-9 Binding, and Activation. *Molecular Cell, 9*(2), 423-432. doi: http://dx.doi.org/10.1016/S1097-2765(02)00442-2

Adams, J. M. (2003). Ways of dying: multiple pathways to apoptosis. *Genes & development, 17*(20), 2481-2495.

Adorni, M. P., Zimetti, F., Puntoni, M., Bigazzi, F., Sbrana, F., Minichilli, F., . . . & Sampietro, T. (2012). Cellular cholesterol efflux and cholesterol loading capacity of serum: effects of LDL-apheresis. *Journal of Lipid Research, 53*(5), 984-989. Doi: jlr.P024810 [pii] 10.1194/jlr.P024810

Adouni, M., Shirazi-Adl, A., 2014. Evaluation of knee joint muscle forces and tissue stressesstrains during gait in severe OA versus normal subjects. Journal of orthopaedic research : official publication of the Orthopaedic Research Society 32, 69-78.Afara, I. O., Prasadam, I., Crawford, R., Xiao, Y., & Oloyede, A. (2013). Near infrared (NIR) absorption spectra correlates with subchondral bone micro-CT parameters in osteoarthritic rat models. Bone, 53(2), 350-357. doi: http://dx.doi.org/10.1016/j.bone.2012.12.042

Afonso, V., Champy, R., Mitrovic, D., Collin, P., & Lomri, A. (2007). Reactive oxygen species and superoxide dismutases: role in joint diseases. *Joint Bone Spine, 74*(4), 324-329. doi: S1297-319X(07)00159-5 [pii]10.1016/j.jbspin.2007.02.002

Ai, M., Tanaka, A., & Schaefer, E. (2014). Apolipoprotein B-48: a unique marker of chylomicron metabolism. *Advances in clinical chemistry, 64*, 117.

Aiello, R. J., Brees, D., & Francone, O. L. (2003). ABCA1-Deficient Mice Insights Into the Role of Monocyte Lipid Efflux in HDL Formation and Inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology, 23*(6), 972-980.

Aigner, T., & Schmitz, N. (2011). Pathogenesis and pathology of osteoarthritis. *Rheumatology*, 1741-1759.

Akasaki, Y., Matsuda, S., & Iwamoto, Y. (2009). [Progress of research in osteoarthritis. The anti-inflammatory effects of intra-articular injected statin on experimental osteoarthritis]. *Clinical Calcium, 19*(11), 1653-1662. Doi: 091116531662 [pii] CliCa091116531662

Akasaki, Y., Matsuda, S., Nakayama, K., Fukagawa, S., Miura, H., & Iwamoto, Y. (2009). Mevastatin reduces cartilage degradation in rabbit experimental osteoarthritis through inhibition of synovial inflammation. *Osteoarthritis Cartilage, 17*(2), 235-243. doi: S1063-4584(08)00210-0 [pii] 10.1016/j.joca.2008.06.012

Al-Arfaj, A. S. (2003). Radiographic osteoarthritis and serum cholesterol. Saudi Medical Journal, 24(7), 745-747.

Alshehri, A. M. (2010). Metabolic syndrome and cardiovascular risk. Journal pf Family and Community Medicine, 17(2), 73-78. doi: 10.4103/1319-1683.71987

Altman, R., Asch, E., Bloch, D., Bole, G., Borenstein, D., Brandt, K., . . . & Hochberg, M. (1986). Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. *Arthritis & Rheumatism, 29*(8), 1039-1049.

Amin, S., Baker, K., Niu, J., Clancy, M., Goggins, J., Guermazi, A., . . . & Felson, D. T. (2009). Quadriceps strength and the risk of cartilage loss and symptom progression in knee osteoarthritis. *Arthritis & Rheumatism, 60*(1), 189-198.

Ananth, L., Prete, P. E., & Kashyap, M. L. (1993). Apolipoproteins A-I and B and cholesterol in synovial fluid of patients with rheumatoid arthritis. *Metabolism, 42*(7), 803-806.

Anderson, M.J., Diko, S., Baehr, L.M., Baar, K., Bodine, S.C., Christiansen, B.A., 2016.Contribution of mechanical unloading to trabecular bone loss following non-invasive kneeinjury in mice. Journal of Orthopaedic Research (in Press).Andriacchi, T. P., Mündermann, A., Smith, R. L., Alexander, E. J., Dyrby, C. O., & Koo, S. (2004). A framework for the in vivo pathomechanics of osteoarthritis at the knee. *Annals of biomedical engineering, 32*(3), 447-457.

Andujar, C., Serrano, J., & Gomez-Zurita, J. (2012). Winding up the molecular clock in the genus Carabus (Coleoptera: Carabidae): assessment of methodological decisions on rate and node age estimation. *BMC Evolutionary Biology, 12*, 40. doi: 10.1186/1471-2148-12-40

Arkill, K. P., & Winlove, C. P. (2006). Fatty acid transport in articular cartilage. *Archives of Biochemistry and Biophysics, 456*(1), 71-78. doi: S0003-9861(06)00345-6 [pii]10.1016/j.abb.2006.09.014

Arner, E. C. (2002). Aggrecanase-mediated cartilage degradation. *Current opinion in pharmacology, 2*(3), 322-329. Arthritis research foundation. *Australia, www.arthritisfoundation.com.au*

Australian Institute of health and Welfare website: http://www.aihw.gov.au/arthritis-and-musculoskeletal-conditions-publications/

Bagge, E., Bjelle, A., Eden, S., & Svanborg, A. (1991). Factors associated with radiographic osteoarthritis: results from the population study 70-year-old people in Goteborg. *Journal of Rheumatology, 18*(8), 1218-1222.

Baigent, C., Landray, M. J., Reith, C., Emberson, J., Wheeler, D. C., Tomson, C., . . . & Craig, J. (2011). The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. *The Lancet, 377*(9784), 2181-2192.

Balaban, R. S., Nemoto, S., & Finkel, T. (2005). Mitochondria, Oxidants, and Aging. *Cell, 120*(4), 483-495. doi: http://dx.doi.org/10.1016/j.cell.2005.02.001

Baliga, B., & Kumar, S. (2003). Apaf-1//cytochrome c apoptosome: an essential initiatorof caspase activation or just a sideshow? *Cell Death & Differentiation, 10*(1), 16-18.

Bao, Q., & Shi, Y. (2007). Apoptosome: a platform for the activation of initiator caspases. *Cell Death & Differentiation, 14*(1), 56-65.

Baracca, A., Sgarbi, G., Solaini, G., & Lenaz, G. (2003). Rhodamine 123 as a probe of mitochondrial membrane potential: evaluation of proton flux through F 0 during ATP synthesis. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1606(1), 137-146.

Barbier, O., Torra, I. P., Duguay, Y., Blanquart, C., Fruchart, J. C., Glineur, C., & Staels, B. (2002). Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology, 22*(5), 717-726.

Bascoul-Colombo, C., Garaiova, I., Plummer, S. F., Harwood, J. L., Caterson, B., & Hughes, C. E. (2015). Glucosamine Hydrochloride but Not Chondroitin Sulfate Prevents Cartilage Degradation and Inflammation Induced by Interleukin-1α in Bovine Cartilage Explants. *Cartilage*, 1947603515603762.

Bastiaansen-Jenniskens, Y. M., Clockaerts, S., Feijt, C., Zuurmond, A. M., Stojanovic-Susulic, V., Bridts, C., . . . & van Osch, G. J. (2012). Infrapatellar fat pad of patients with end-stage osteoarthritis inhibits catabolic mediators in cartilage. *Annals of the rheumatic diseases, 71*(2), 288-294. doi: 10.1136/ard.2011.153858

Bendele, A. (2001). Animal models of osteoarthritis. *Journal of Musculoskeletal Neuronal Interactions, 1*(4), 363-376.

Bendele, A. M., & Hulman, J. F. (1991). Effects of body weight restriction on the development and progression of spontaneous osteoarthritis in guinea pigs. *Arthritis & Rheumatism, 34*(9), 1180-1184.

Benito, M. J., Veale, D. J., FitzGerald, O., van den Berg, W. B., & Bresnihan, B. (2005). Synovial tissue inflammation in early and late osteoarthritis. *Annals of the rheumatic diseases, 64*(9), 1263-1267. doi: 10.1136/ard.2004.025270

Benya, P. D., Padilla, S. R., & Nimni, M. E. (1978). Independent regulation of collagen types by chondrocytes during the loss of differentiated function in culture. *Cell, 15*(4), 1313-1321.

Bereiter-Hahn, J., & Vöth, M. (1994). Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. *Microscopy research and technique, 27*(3), 198-219.

Berenbaum, F. (2012). Diabetes-induced osteoarthritis: from a new paradigm to a new phenotype. *Postgraduate medical journal, 88*(1038), 240-242.

Bernardi, P. (1996). The permeability transition pore. Control points of a cyclosporin A-sensitive mitochondrial channel involved in cell death. *Biochimica et*

Biophysica Acta (BBA) - Bioenergetics, 1275(1–2), 5-9. doi: http://dx.doi.org/10.1016/0005-2728(96)00041-2

Bernardi, P., Krauskopf, A., Basso, E., Petronilli, V., Blalchy-Dyson, E., Di Lisa, F., & Forte, M. A. (2006). The mitochondrial permeability transition from in vitro artifact to disease target. *FEBS Journal, 273*(10), 2077-2099. doi: 10.1111/j.1742-4658.2006.05213.x

Bhandari, S., Gupta, P., Quinn, P., Sandhu, J., Hakimi, A., Jones, D., & Ng, L. (2015). Pleiotropic effects of statins in hypercholesterolaemia: a prospective observational study using a lipoproteomic based approach. *Lancet, 385 Suppl 1*, S21. doi: 10.1016/s0140-6736(15)60336-1

Bierma-Zeinstra, S. M., & Koes, B. W. (2007). Risk factors and prognostic factors of hip and knee osteoarthritis. *Nature Clinical Practice Rheumatology, 3*(2), 78-85. Doi: ncprheum0423 [pii] 10.1038/ncprheum0423

Blagojevic, M., Jinks, C., Jeffery, A., & Jordan, K. (2010). Risk factors for onset of osteoarthritis of the knee in older adults: a systematic review and meta-analysis. *Osteoarthritis and Cartilage, 18*(1), 24-33.

Blanchette-Mackie, E. J. (2000). Intracellular cholesterol trafficking: role of the NPC1 protein. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1486*(1), 171-183.

Blanco, F. J., Guitian, R., Vázquez-Martul, E., de Toro, F. J., & Galdo, F. (1998). Osteoarthritis chondrocytes die by apoptosis: A possible pathway for osteoarthritis pathology. *Arthritis & Rheumatism, 41*(2), 284-289. doi: 10.1002/1529-0131(199802)41:2<284::AID-ART12>3.0.CO;2-T

Blanco, F. J., López-Armada, M. J., & Maneiro, E. (2004). Mitochondrial dysfunction in osteoarthritis. *Mitochondrion, 4*(5–6), 715-728. doi: http://dx.doi.org/10.1016/j.mito.2004.07.022

Blanco, F. J., Rego, I., & Ruiz-Romero, C. (2011). The role of mitochondria in osteoarthritis. *Nature Reviews Rheumatology, 7*(3), 161-169.

Bondeson, J. (2010). Activated synovial macrophages as targets for osteoarthritis drug therapy. *Current Drug Targets, 11*(5), 576-585.

Bondeson, J., Wainwright, S. D., Lauder, S., Amos, N., & Hughes, C. E. (2006). The role of synovial macrophages and macrophage-produced cytokines in driving aggrecanases, matrix metalloproteinases, and other destructive and inflammatory responses in osteoarthritis. *Arthritis Research & Therapy, 8*(6), R187.

Bonora, E., Kiechl, S., Willeit, J., Oberhollenzer, F., Egger, G., Bonadonna, R., & Muggeo, M. (2003). Metabolic syndrome: epidemiology and more extensive phenotypic description. Cross-sectional data from the Bruneck Study. *International journal of obesity, 27*(10), 1283-1289.

Bosch, M., Marí, M., Herms, A., Fernández, A., Fajardo, A., Kassan, A., . . . & Pol, A. (2011). Caveolin-1 Deficiency Causes Cholesterol-Dependent Mitochondrial

Dysfunction and Apoptotic Susceptibility. *Current Biology, 21*(8), 681-686. doi: http://dx.doi.org/10.1016/j.cub.2011.03.030

Bossy-Wetzel, E., Newmeyer, D. D., & Green, D. R. (1998). Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarization. *The EMBO Journal, 17*(1), 37-49.

Brandt, K., Schauwecker, D., Dansereau, S., Meyer, J., O'Connor, B., & Myers, S. (1997). Bone scintigraphy in the canine cruciate deficiency model of osteoarthritis. Comparison of the unstable and contralateral knee. *The Journal of Rheumatology, 24*(1), 140-145.

Brandt, K. D., Dieppe, P., & Radin, E. L. (2008). Etiopathogenesis of osteoarthritis. *Rheumatic Disease Clinics of North America, 34*(3), 531-559.

Brouillette, M.J., Ramakrishnan, P.S., Wagner, V.M., Sauter, E.E., Journot, B.J., McKinley, T.O.,Martin, J.A., 2014. Strain-Dependent Oxidant Release in Articular Cartilage Originates from Mitochondria. *Biomechanics and modeling in mechanobiology 13*, 565-572.

Brown, M. S., & Goldstein, J. L. (1986). A receptor-mediated pathway for cholesterol homeostasis. *Science, 232*(4746), 34-47.

Brown, M. S., & Goldstein, J. L. (1999). A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proceedings of the National Academy of Sciences, 96*(20), 11041-11048.

Brown, M. S., Kovanen, P. T., & Goldstein, J. L. (1981). Regulation of plasma cholesterol by lipoprotein receptors. *Science, 212*(4495), 628-635.

Buckland-Wright, C. (2004). Subchondral bone changes in hand and knee osteoarthritis detected by radiography. *Osteoarthritis and Cartilage, 12, Supplement*, 10-19. doi: http://dx.doi.org/10.1016/j.joca.2003.09.007

Buckland-Wright, J., Lynch, J., & Dave, B. (2000). Early radiographic features in patients with anterior cruciate ligament rupture. *Annals of the rheumatic diseases, 59*(8), 641-646.

Buckland-Wright, J. C., Macfarlane, D. G., & Lynch, J. A. (1992). Relationship between joint space width and subchondral sclerosis in the osteoarthritic hand: a quantitative microfocal radiographic study. *The Journal of Rheumatology, 19*(5), 788-795.

Buckwalter, J. A., & Mankin, H. J. (1997). Instructional Course Lectures, The American Academy of Orthopaedic Surgeons-Articular Cartilage. Part I: Tissue Design and Chondrocyte-Matrix Interactions*†. *Journal Bone Joint Surgery, 79*(4), 600-11.

Bultel, S., Helin, L., Clavey, V., Chinetti-Gbaguidi, G., Rigamonti, E., Colin, M., . . . & Lestavel, S. (2008). Liver X receptor activation induces the uptake of cholesteryl esters from high density lipoproteins in primary human macrophages. *Arteriosclerosis, thrombosis, and vascular biology, 28*(12), 2288-2295. doi: ATVBAHA.108.175042 [pii]10.1161/ATVBAHA.108.175042

Burr, D. B. (1998). The importance of subchondral bone in osteoarthrosis. *Current Opinion in Rheumatology, 10*(3), 256-262.

Burr, D. B. (2004). Anatomy and physiology of the mineralized tissues: role in the pathogenesis of osteoarthrosis. *Osteoarthritis and Cartilage, 12*, 20-30.

Burr, D. B., & Gallant, M. A. (2012). Bone remodelling in osteoarthritis. *Nature Reviews Rheumatology, 8*(11), 665-673.

Burr, D. B., & Radin, E. L. (2003). Microfractures and microcracks in subchondral bone: are they relevant to osteoarthrosis? *Rheumatic Disease Clinics North America, 29*(4), 675-685.

Cadenas, E., & Davies, K. J. A. (2000). Mitochondrial free radical generation, oxidative stress, and aging1. *Free Radical Biology and Medicine, 29*(3–4), 222-230. doi: http://dx.doi.org/10.1016/S0891-5849(00)00317-8

Carman, W. J., Sowers, M., Hawthorne, V. M., & Weissfeld, L. A. (1994). Obesity as a risk factor for osteoarthritis of the hand and wrist: a prospective study. *American Journal of Epidemiology, 139*(2), 119-129.

Caron, M. M. J., Emans, P. J., Coolsen, M. M. E., Voss, L., Surtel, D. A. M., Cremers, A., . . . & Welting, T. J. M. (2012). Redifferentiation of dedifferentiated human articular chondrocytes: comparison of 2D and 3D cultures. *Osteoarthritis and Cartilage, 20*(10), 1170-1178. doi: http://dx.doi.org/10.1016/j.joca.2012.06.016

Castañeda, S., Roman-Blas, J. A., Largo, R., & Herrero-Beaumont, G. (2013). Osteoarthritis: a progressive disease with changing phenotypes. *Rheumatology*, ket247.

Cawston, T. E., & Wilson, A. J. (2006). Understanding the role of tissue degrading enzymes and their inhibitors in development and disease. *Best Practice & Research Clinical Rheumatology, 20*(5), 983-1002.

Chan, D. C. (2006). Mitochondrial fusion and fission in mammals. *Annu. Rev. Cell Dev. Biol., 22*, 79-99.

Chang, T. Y., Chang, C. C., & Cheng, D. (1997). Acyl-coenzyme A: cholesterol acyltransferase. *Annual review of biochemistry, 66*, 613.

Chawla, A., Repa, J. J., Evans, R. M., & Mangelsdorf, D. J. (2001). Nuclear receptors and lipid physiology: opening the X-files. *Science, 294*(5548), 1866-1870.

Chen, F., Frenkel, S., & Di Cesare, P. (1999). Repair of articular cartilage defects: part I. Basic Science of cartilage healing. *American journal of orthopedics (Belle Mead, NJ), 28*(1), 31-33.

Chen, H., Detmer, S. A., Ewald, A. J., Griffin, E. E., Fraser, S. E., & Chan, D. C. (2003). Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *The Journal of Cell Biology, 160*(2), 189-200.

Chen, L. B. (1988). Mitochondrial membrane potential in living cells. *Annual review of cell biology, 4*(1), 155-181.

Cheung, H. S. (2005). Role of calcium-containing crystals in osteoarthritis. *Front Biosci, 10*, 1336-1340.

Choi, Y. H., & Toyoda, Y. (1998). Cyclodextrin removes cholesterol from mouse sperm and induces capacitation in a protein-free medium. *Biology of reproduction, 59*(6), 1328-1333.

Chowdhury, T., Akanji, O., Salter, D., Bader, D., & Lee, D. (2008). Dynamic compression influences interleukin-1β-induced nitric oxide and prostaglandin E2 release by articular chondrocytes via alterations in iNOS and COX-2 expression. *Biorheology, 45*(3-4), 257-274.

Christian, A., Haynes, M., Phillips, M., & Rothblat, G. (1997). Use of cyclodextrins for manipulating cellular cholesterol content. *Journal of lipid research, 38*(11), 2264-2272.

Christiansen, B.A., Anderson, M.J., Lee, C.A., Williams, J.C., Yik, J.H., Haudenschild, D.R., 2012. Musculoskeletal changes following non-invasive knee injury using a novel mouse model of post-traumatic osteoarthritis. Osteoarthritis and Cartilage 20, 773-782.

Christie, W. W., & Han, X. (2010). Lipid analysis: isolation, separation, identification and lipidomic analysis. *Oily Press, an imprint of PJ Barnes & Associates, 24*, xviii, 428.

Chua, K., Aminuddin, B., Fuzina, N., & Ruszymah, B. (2005). Insulin-transferrin-selenium prevent human chondrocyte dedifferentiation and promote the formation of high quality tissue engineered human hyaline cartilage. *Eur Cell Mater, 9*(9), 58-67.

Clayton, P. T. (1998). Disorders of cholesterol biosynthesis. *Archives of disease in childhood, 78*, 185–189.

Clockaerts, S., Van Osch, G. J., Bastiaansen-Jenniskens, Y. M., Verhaar, J. A., Van Glabbeek, F., Van Meurs, J. B., . . . & Bierma-Zeinstra, S. M. (2012). Statin use is associated with reduced incidence and progression of knee osteoarthritis in the Rotterdam study. *Annals of the rheumatic diseases, 71*(5), 642-647. Doi: annrheumdis-2011-200092 [pii] 10.1136/annrheumdis-2011-200092

Cohen-Solal, M., Funck-Brentano, T., & Hay, E. (2013). Animal models of osteoarthritis for the understanding of the bone contribution. *BoneKEy Rep, 2*. doi: 10.1038/bonekey.2013.156

Colell, A., García-Ruiz, C., Lluis, J. M., Coll, O., Mari, M., & Fernández-Checa, J. C. (2003). Cholesterol impairs the adenine nucleotide translocator-mediated mitochondrial permeability transition through altered membrane fluidity. *Journal of Biological Chemistry, 278*(36), 33928-33935.

Collins, S., Martin, T. L., Surwit, R. S., & Robidoux, J. (2004). Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. *Physiology & behavior, 81*(2), 243-248.

Collins, T. J., & Bootman, M. D. (2003). Mitochondria are morphologically heterogeneous within cells. *Journal of experimental biology, 206*(12), 1993-2000.

Conaghan, P. G., Vanharanta, H., & Dieppe, P. A. (2005). Is progressive osteoarthritis an atheromatous vascular disease? *Annals of the rheumatic diseases, 64*(11), 1539-1541. doi: ard.2005.039263 [pii] 10.1136/ard.2005.039263

Cooper, C., Inskip, H., Croft, P., Campbell, L., Smith, G., McLearn, M., & Coggon, D. (1998). Individual risk factors for hip osteoarthritis: obesity, hip injury and physical activity. *American Journal of Epidemiology, 147*(6), 516-522.

Cooper, C., Snow, S., McAlindon, T. E., Kellingray, S., Stuart, B., Coggon, D., & Dieppe, P. A. (2000). Risk factors for the incidence and progression of radiographic knee osteoarthritis. *Arthritis & Rheumatism, 43*(5), 995.

Cooper, G. M. (2000). The Cell: A Molecular Approach. 2nd edition. *Sunderland (MA): Sinauer Associates*.

Costet, P., Lalanne, F., Gerbod-Giannone, M. C., Molina, J. R., Fu, X., Lund, E. G., . . . & Tall, A. R. (2003). Retinoic acid receptor-mediated induction of ABCA1 in macrophages. *Molecular and cellular biology, 23*(21), 7756-7766.

Crain, R. C., Clark, R. W., & Harvey, B. E. (1983). Role of lipid transfer proteins in the abnormal lipid content of Morris hepatoma mitochondria and microsomes. *Cancer research, 43*(7), 3197-3202.

Crompton, M. (1999). The mitochondrial permeability transition pore and its role in cell death. *Biochemical journal, 341*(2), 233-249.

Dahaghin, S., Bierma-Zeinstra, S. M., Koes, B. W., Hazes, J. M., & Pols, H. A. (2007). Do metabolic factors add to the effect of overweight on hand osteoarthritis? The Rotterdam Study. *Annals of the rheumatic diseases, 66*(7), 916-920. doi: ard.2005.045724 [pii] 0.1136/ard.2005.045724

Daniels, S. R., & Greer, F. R. (2008). Lipid screening and cardiovascular health in childhood. *Pediatrics, 122*(1), 198-208.

Davies-Tuck, M. L., Hanna, F., Davis, S. R., Bell, R. J., Davison, S. L., Wluka, A. E., . . . & Cicuttini, F. M. (2009). Total cholesterol and triglycerides are associated with the development of new bone marrow lesions in asymptomatic middle-aged women—a prospective cohort study. *Arthritis Research and Therapy, 11*(6), 181-187.

Davies-Tuck, M. L., Hanna, F., Davis, S. R., Bell, R. J., Davison, S. L., Wluka, A. E., . . . & Cicuttini, F. M. (2009). Total cholesterol and triglycerides are associated with the development of new bone marrow lesions in asymptomatic middle-aged women - a prospective cohort study. *Arthritis Research Therapy, 11*(6), R181. doi: ar2873 [pii] 10.1186/ar2873

Davis, H. R., Zhu, L. J., Hoos, L. M., Tetzloff, G., Maguire, M., Liu, J., . . . & Lund, E. G. (2004). "Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol

and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis." *Journal of Biological Chemistry, 279*(32), 33586-33592.

Davis, M. A., Ettinger, W. H., & Neuhaus, J. M. (1988). The role of metabolic factors and blood pressure in the association of obesity with osteoarthritis of the knee. *Journal of Rheumatol, 15*(12), 1827-1832.

Davis, M. A., Neuhaus, J. M., Ettinger, W. H., & Mueller, W. H. (1990). Body fat distribution and osteoarthritis. *American Journal of Epidemiology, 132*(4), 701-707.

Day, C. (2007). Metabolic syndrome, or What you will: definitions and epidemiology. *Diabetes and Vascular Disease Research, 4*, 32-38. doi: 10.3132/dvdr.2007.003

Day, J., Ding, M., Van der Linden, J., Hvid, I., Sumner, D., & Weinans, H. (2001). A decreased subchondral trabecular bone tissue elastic modulus is associated with pre-arthritic cartilage damage. *Journal of Orthopaedic Research, 19*(5), 914-918.

de Boer, T. N., van Spil, W. E., Huisman, A. M., Polak, A. A., Bijlsma, J. W. J., Lafeber, F. P. J. G., & Mastbergen, S. C. (2012). Serum adipokines in osteoarthritis; comparison with controls and relationship with local parameters of synovial inflammation and cartilage damage. *Osteoarthritis and Cartilage, 20*(8), 846-853. doi: http://dx.doi.org/10.1016/j.joca.2012.05.002

de Haart, M., Marijnissen, W. J., van Osch, G. J., & Verhaar, J. A. (1999). Optimization of chondrocyte expansion in culture: Effect of TGFβ-2, bFGF and L-ascorbic acid on bovine articular chondrocytes. *Acta Orthopaedica Scandinavica, 70*(1), 55-61.

de Munter, W., Blom, A. B., Helsen, M. M., Walgreen, B., van der Kraan, P. M., Joosten, L. A., . . . & van Lent, P. L. (2013). Cholesterol accumulation caused by low density lipoprotein receptor deficiency or a cholesterol-rich diet results in ectopic bone formation during experimental osteoarthritis. *Arthritis Research & Therapy, 15*(6), R178.

de Seny, D., Cobraiville, G., Charlier, E., Neuville, S., Lutteri, L., Le Goff, C., . . . & Malaise, M. G. (2015). Apolipoprotein-A1 as a damage-associated molecular patterns protein in osteoarthritis: ex vivo and in vitro pro-inflammatory properties. *PloS one, 10*(4), e0122904. doi: 10.1371/journal.pone.0122904

Dedrick, D., Goulet, R., Huston, L., Goldstein, S., & Bole, G. (1991). Early bone changes in experimental osteoarthritis using microscopic computed tomography. *The Journal of rheumatology. Supplement, 27*, 44-45.

Dedrick, D. K., Goldstein, S. A., Brandt, K., O'Connor, B., Goulet, R. W., & Albrecht, M. (1993). A longitudinal study of subchondral plate and trabecular bone in cruciate-deficient dogs with osteoarthritis followed up for 54 months. *Arthritis & Rheumatism, 36*(10), 1460-1467.

DeGrella, R., & Simoni, R. (1982). Intracellular transport of cholesterol to the plasma membrane. *Journal of Biological Chemistry, 257*(23), 14256-14262.

DeGroot, J. (2004). The AGE of the matrix: chemistry, consequence and cure. *Current opinion in pharmacology, 4*(3), 301-305.

Desroches, S., & Lamarche, B. (2007). The evolving definitions and increasing prevalence of the metabolic syndrome. *Applied Physiology, Nutrition, and Metabolism, 32*(1), 23-32.

Di, D., Wang, Z., Liu, Y., Luo, G., Shi, Y., Berggren-Soderlund, M., . . . & Xu, N. (2012). ABCA1 upregulating apolipoproein M expression mediates via the RXR/LXR pathway in HepG2 cells. *Biochemical and biophysical research communications, 421*(1), 152-156. doi: S0006-291X(12)00658-4 [pii]10.1016/j.bbrc.2012.04.022

Dietschy, J. M., & Turley, S. D. (2001). Cholesterol metabolism in the brain. *Current Opinion in Lipidology, 12*(2), 105-112.

Dikalova, A. E., Bikineyeva, A. T., Budzyn, K., Nazarewicz, R. R., McCann, L., Lewis, W., . . . & Dikalov, S. I. (2010). Therapeutic targeting of mitochondrial superoxide in hypertension. *Circulation research, 107*(1), 106-116. doi: 10.1161/CIRCRESAHA.109.214601

Donovan, M., & Cotter, T. G. (2004). Control of mitochondrial integrity by Bcl-2 family members and caspase-independent cell death. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1644*(2), 133-147.

Doulabi, A. H., Mequanint, K., & Mohammadi, H. (2014). Blends and nanocomposite biomaterials for articular cartilage tissue engineering. *Materials, 7*(7), 5327-5355.

Driban, J. B., Sitler, M. R., Barbe, M. F., & Balasubramanian, E. (2010). Is osteoarthritis a heterogeneous disease that can be stratified into subsets? *Clinical rheumatology, 29*(2), 123-131.

Du, C., Fang, M., Li, Y., Li, L., & Wang, X. (2000). Smac, a mitochondrial protein that promotes cytochrome c–dependent caspase activation by eliminating IAP inhibition. *Cell, 102*(1), 33-42.

Ducy, P., Amling, M., Takeda, S., Priemel, M., Schilling, A. F., Beil, F. T., . . . & Karsenty, G. (2000). Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell, 100*(2), 197-207.

Dumond, H., Presle, N., Terlain, B., Mainard, D., Loeuille, D., Netter, P., & Pottie, P. (2003). Evidence for a key role of leptin in osteoarthritis. *Arthritis & Rheumatism, 48*(11), 3118-3129.

Echegoyen, S., Oliva, E., Sepulveda, J., Diaz-Zagoya, J., Espinosa-Garcia, M., Pardo, J., & Martinez, F. (1993). Cholesterol increase in mitochondria: its effect on inner-membrane functions, submitochondrial localization and ultrastructural morphology. *Biochemical Journal, 289*, 703-708.

Egloff, C., Sawatsky, A., Leonard, T., Hart, D. A., Valderrabano, V., & Herzog, W. (2014). Effect of muscle weakness and joint inflammation on the onset and progression of osteoarthritis in the rabbit knee. *Osteoarthritis and Cartilage*, 22(11), 1886-1893.

Erb, N., Pace, A. V., Douglas, K. M., Banks, M. J., & Kitas, G. D. (2004). Risk assessment for coronary heart disease in rheumatoid arthritis and osteoarthritis. *Scandinavian journal of rheumatology, 33*(5), 293-299. doi: 10.1080/03009740410006899

Eva Hurt-Camejo, U. O., Olov Wiklund, Göran Bondjers, Germán Camejo, & (1997). Cellular Consequences of the Association of ApoB Lipoproteins With Proteoglycans. *Arteriosclerosis, Thrombosis, and Vascular Biology, 17*, 1011-1017. doi: 10.1161/01.ATV.17.6.1011

Faggiotto, A., & Ross, R. (1984). Studies of hypercholesterolemia in the nonhuman primate. II. Fatty streak conversion to fibrous plaque. *Arteriosclerosis, 4*(4), 341-356.

Faggiotto, A., Ross, R., & Harker, L. (1984). Studies of hypercholesterolemia in the nonhuman primate. I. Changes that lead to fatty streak formation. *Arteriosclerosis, 4*(4), 323-340.

Felson, D. T. (1996). Weight and osteoarthritis. *The American journal of clinical nutrition, 63*(3), 430S-432S.

Felson, D. T. (2010). Identifying different osteoarthritis phenotypes through epidemiology. *Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society, 18*(5), 601.

Felson, D. T., & Chaisson, C. E. (1997). 2 Understanding the relationship between body weight and osteoarthritis. *Bailliere's clinical rheumatology, 11*(4), 671-681.

Felson, D. T., Chaisson, C. E., Hill, C. L., Totterman, S. M., Gale, M. E., Skinner, K. M., ... & Gale, D. R. (2001). The association of bone marrow lesions with pain in knee osteoarthritis. *Annals of internal medicine, 134*(7), 541-549.

Felson, D. T., Zhang, Y., Hannan, M. T., Naimark, A., Weissman, B., Aliabadi, P., & Levy, D. (1997). Risk factors for incident radiographic knee osteoarthritis in the elderly. The Framingham Study. *Arthritis & Rheumatism, 40*(4), 728-733.

Feng, B., Yao, P. M., Li, Y., Devlin, C. M., Zhang, D., Harding, H. P., . . . & Fisher, E. A. (2003). The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. *Nature cell biology, 5*(9), 781-792.

Feo, F., Canuto, R., Garcea, R., & Gabriel, L. (1975). Effect of cholesterol content on some physical and functional properties of mitochondria isolated from adult rat liver, fetal liver, cholesterol-enriched liver and hepatomas AH-130, 3924A and 5123. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 413*(1), 116-134.

Field, F. J., Born, E., Murthy, S., & Mathur, S. N. (1998). Transport of cholesterol from the endoplasmic reticulum to the plasma membrane is constitutive in CaCo-2 cells and differs from the transport of plasma membrane cholesterol to the endoplasmic reticulum. *Journal of lipid research, 39*(2), 333-343.

Fielding, C. J., & Fielding, P. E. (2001). Cellular cholesterol efflux. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1533*(3), 175-189.

Findlay, D. M. (2007). Vascular pathology and osteoarthritis. *Rheumatology (Oxford), 46*(12), 1763-1768. doi: kem191 [pii] 10.1093/rheumatology/kem191

Firestein, G. S., Budd, R., O'Dell, J. R., Gabriel, S. E., & McInnes, I. B. (2012). *Kelley's textbook of rheumatology*: Saunders.

Fosslien, E. (2001). Mitochondrial medicine--molecular pathology of defective oxidative phosphorylation. *Annals of Clinical & Laboratory Science, 31*(1), 25-67.

Freeman, M. A. R. (1973). *Adult articular cartilage*. London: Pitman.

Frey, T. G., & Mannella, C. A. (2000). The internal structure of mitochondria. *Trends in Biochemical Sciences, 25*(7), 319-324. doi: http://dx.doi.org/10.1016/S0968-0004(00)01609-1

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., . . . & Shimomura, I. (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of Clinical Investigation, 114*(12), 1752-1761.

Galluzzi, L., Larochette, N., Zamzami, N., & Kroemer, G. (2006). Mitochondria as therapeutic targets for cancer chemotherapy. *Oncogene, 25*(34), 4812-4830.

Garcia-Calvo, M., Lisnock, J., Bull, H. G., Hawes, B. E., Burnett, D. A., Braun, M. P., . . . & Detmers, P. A. (2005). The target of ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). *Proceedings of the National Academy of Sciences of the United States of America, 102*(23), 8132-8137.

Gavriilidis, C., Miwa, S., von Zglinicki, T., Taylor, R. W., & Young, D. A. (2013). Mitochondrial dysfunction in osteoarthritis is associated with down-regulation of superoxide dismutase 2. *Arthritis & Rheumatism, 65*(2), 378-387. doi: 10.1002/art.37782

Gegout, P. P., Francin, P.-J., Mainard, D., & Presle, N. (2008). Adipokines in osteoarthritis: friends or foes of cartilage homeostasis? *Joint Bone Spine, 75*(6), 669-671. doi: http://dx.doi.org/10.1016/j.jbspin.2008.07.008

Gelber, A. C., Hochberg, M. C., Mead, L. A., Wang, N. Y., Wigley, F. M., & Klag, M. J. (1999). Body mass index in young men and the risk of subsequent knee and hip osteoarthritis. *The American Journal of Medicine, 107*(6), 542-548.

Gentili, C., Tutolo, G., Pianezzi, A., Cancedda, R., & Descalzi Cancedda, F. (2005). Cholesterol secretion and homeostasis in chondrocytes: a liver X receptor and retinoid X receptor heterodimer mediates apolipoprotein A1 expression. *Matrix Biol, 24*(1), 35-44. doi: S0945-053X(04)00157-X [pii] 10.1016/j.matbio.2004.12.003

Gevers, G., Dequeker, J., Martens, M., Van Audekercke, R., Nyssen-Behets, C., & Dhem, A. (1989). Biomechanical characteristics of iliac crest bone in elderly women according to osteoarthritis grade at the hand joints. *The Journal of rheumatology, 16*(5), 660-663.

Ghadially, F. N., Mehta, P. N., & Kirkaldy-Willis, W. H. (1970). Ultrastructure of articular cartilage in experimentally produced lipoarthrosis. *Journal of Bone & Joint Surgery America, 52*(6), 1147-1158.

Gibson, B. W. (2005). The human mitochondrial proteome: oxidative stress, protein modifications and oxidative phosphorylation. *The International Journal of Biochemistry & Cell Biology, 37*(5), 927-934. doi: http://dx.doi.org/10.1016/j.biocel.2004.11.013

Gierman, L., Kühnast, S., Koudijs, A., Pieterman, E., Kloppenburg, M., van Osch, G., . . . & Zuurmond, A. M. (2014). Osteoarthritis development is induced by increased dietary cholesterol and can be inhibited by atorvastatin in APOE* 3Leiden. CETP mice—a translational model for atherosclerosis. *Annals of the rheumatic diseases, 73*(5), 921-927.

Gierman, L. M., Kuhnast, S., Koudijs, A., Pieterman, E. J., Kloppenburg, M., van Osch, G. J., . . . & Zuurmond, A. M. (2013). Osteoarthritis development is induced by increased dietary cholesterol and can be inhibited by atorvastatin in APOE*3Leiden.CETP mice--a translational model for atherosclerosis. *Annals of the rheumatic diseases*. doi: 10.1136/annrheumdis-2013-203248

Gierman, L. M., van der Ham, F., Koudijs, A., Wielinga, P. Y., Kleemann, R., Kooistra, T., . . . & Zuurmond, A. M. (2012). Metabolic stress–induced inflammation plays a major role in the development of osteoarthritis in mice. *Arthritis & Rheumatism, 64*(4), 1172-1181. doi: 10.1002/art.33443

Giltay, E., Hoogeveen, E., Elbers, J., Gooren, L., Asscheman, H., & Stehouwer, C. (1998). Insulin resistance is associated with elevated plasma total homocysteine levels in healthy, non-obese subjects. *Atherosclerosis, 139*(1), 197-198.

Glasson, S. S., Askew, R., Sheppard, B., Carito, B., Blanchet, T., Ma, H. L., . . . & Yang, Z. (2005). Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature, 434*(7033), 644-648.

Goker, B., Sumner, D. R., Hurwitz, D. E., & Block, J. A. (2000). Bone mineral density varies as a function of the rate of joint space narrowing in the hip. *The Journal of Rheumatology, 27*(3), 735-738.

Goldenberg, D. L., Egan, M. S., & Cohen, A. S. (1982). Inflammatory synovitis in degenerative joint disease. *Journal of Rheumatology, 9*(2), 204-209.

Goldring, M. B. (2012). Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. *Therapeutic Advances in Musculoskeletal Disease, 4*(4), 269-285. doi: 10.1177/1759720X12448454

Goldring, M. B., & Goldring, S. R. (2010). Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. *Annals of the New York Academy of Sciences, 1192*(1), 230-237. doi: 10.1111/j.1749-6632.2009.05240.x

Goldring, M. B., & Marcu, K. B. (2009). Cartilage homeostasis in health and rheumatic diseases. *Arthritis Research & Therapy, 11*(3), 224.

Goldring, S. R. (2012). Cross-talk between subchondral bone and articular cartilage in osteoarthritis. *Arthritis Research & Therapy, 14*(2), 1-8.

Goldring, S. R., & Goldring, M. B. (2004). The role of cytokines in cartilage matrix degeneration in osteoarthritis. *Clinical orthopaedics and related research, 427*, S27-S36.

Goldstein, J. L., & Brown, M. S. (2001). The cholesterol quartet. *Science, 292*(5520), 1310.

Gomez, R., Conde, J., Scotece, M., Gomez-Reino, J. J., Lago, F., & Gualillo, O. (2011). What's new in our understanding of the role of adipokines in rheumatic diseases? *Nature Reviews Rheumatology, 7*(9), 528-536.

Gordon, D. J., Probstfield, J. L., Garrison, R. J., Neaton, J. D., Castelli, W. P., Knoke, J. D., . . . & Tyroler, H. A. (1989). High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation, 79*(1), 8-15.

Graf, G. A., Li, W.-P., Gerard, R. D., Gelissen, I., White, A., Cohen, J. C., & Hobbs, H. H. (2002). Coexpression of ATP-binding cassette proteins ABCG5 and ABCG8 permits their transport to the apical surface. *The Journal of Clinical Investigation, 110*(110 (5)), 659-669.

Green, D. R., & Reed, J. C. (1998). Mitochondria and apoptosis. *Science, 281*(5381), 1309.

Gregor, M. F., & Hotamisligil, G. S. (2011). Inflammatory mechanisms in obesity. *Annual review of immunology, 29*, 415-445. doi: 10.1146/annurev-immunol-031210-101322

Griffin, T. M., Fermor, B., Huebner, J. L., Kraus, V. B., Rodriguiz, R. M., Wetsel, W. C., . . . & Guilak, F. (2010). Research article Diet-induced obesity differentially regulates behavioral, biomechanical, and molecular risk factors for osteoarthritis in mice. *Arthritis Res Ther, 12*(4), R130.

Griffin, T. M., & Guilak, F. (2008). Why is obesity associated with osteoarthritis? Insights from mouse models of obesity. *Biorheology, 45*(3-4), 387.

Griffin, T. M., Huebner, J. L., Kraus, V. B., & Guilak, F. (2009). Extreme obesity due to impaired leptin signaling in mice does not cause knee osteoarthritis. *Arthritis & Rheumatism, 60*(10), 2935-2944.

Griffin, T. M., Huebner, J. L., Kraus, V. B., Yan, Z., & Guilak, F. (2012). Induction of osteoarthritis and metabolic inflammation by a very high-fat diet in mice: Effects of short-term exercise. *Arthritis & Rheumatism, 64*(2), 443-453.

Grotle, M., Hagen, K. B., Natvig, B., Dahl, F. A., & Kvien, T. K. (2008). Obesity and osteoarthritis in knee, hip and/or hand: an epidemiological study in the general population with 10 years follow-up. *BMC Musculoskeletal Disorders, 9*(1), 132.

Grundy Md, P. S. M. (1998). Hypertriglyceridemia, Atherogenic Dyslipidemia, and the Metabolic Syndrome. *The American Journal of Cardiology, 81*(4, Supplement 1), 18B-25B. doi: http://dx.doi.org/10.1016/S0002-9149(98)00033-2

Guilak, F., Fermor, B., Keefe, F. J., Kraus, V. B., Olson, S. A., Pisetsky, D. S., . . . & Weinberg, J. B. (2004). The role of biomechanics and inflammation in cartilage injury and repair. *Clinical orthopaedics and related research, 423*, 17-26.

Gushue, D. L., Houck, J., & Lerner, A. L. (2005). Effects of childhood obesity on three-dimensional knee joint biomechanics during walking. *Journal of Pediatric Orthopaedics, 25*(6), 763-768.

H Mabuchi, J. K., M Shimizu and R Takeda. (1989). Development of Coronary Heart Disease in Familial Hypercholesterolemia. *Circulation, 79*, 225-232. doi: 10.1161/01.CIR.79.2.225

Hall, E., Ren, S., Hylemon, P., Rodriguez-Agudo, D., Redford, K., Marques, D., . . . & Pandak, W. (2005). Detection of the steroidogenic acute regulatory protein, StAR, in human liver cells. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1733*(2), 111-119.

Hamrick, M. W., Pennington, C., Newton, D., Xie, D., & Isales, C. (2004). Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. *Bone, 34*(3), 376-383. doi: http://dx.doi.org/10.1016/j.bone.2003.11.020

Hansson, G. K. (2005). Inflammation, atherosclerosis, and coronary artery disease. *New England Journal of Medicine, 352*(16), 1685-1695. doi: 10.1056/NEJMra043430

Hansson, G. K. (2005). Inflammation, atherosclerosis, and coronary artery disease. *New England Journal of Medicine, 352*(16), 1685-1695.

Hanukoglu, I. (1992). Steroidogenic enzymes: Structure, function, and role in regulation of steroid hormone biosynthesis. *The Journal of Steroid Biochemistry and Molecular Biology, 43*(8), 779-804. doi: http://dx.doi.org/10.1016/0960-0760(92)90307-5

Harman, D. (1972). The Biologic Clock: The Mitochondria? *Journal of the American Geriatrics Society, 20*(4), 145-147. doi: 10.1111/j.1532-5415.1972.tb00787.x

Harris, M., & Thompson, C. (2000). The role of the Bcl-2 family in the regulation of outer mitochondrial membrane permeability. *Cell death and differentiation, 7*(12), 1182-1191.

Hart, D. J., Doyle, D. V., & Spector, T. D. (1995). Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. *Journal of rheumatology, 22*(6), 1118-1123.

Hart, D. J., Doyle, D. V., & Spector, T. D. (1995). Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. *The Journal of Rheumatology, 22*(6), 1118-1123.

Hartz, A. J., Fischer, M. E., Bril, G., Kelber, S., Rupley, D., Oken, B., & Rimm, A. A. (1986). The association of obesity with joint pain and osteoarthritis in the HANES data. *Journal of Chronic Diseases, 39*(4), 311-319.

Hatano, H., Maruo, A., Bolander, M. E., & Sarkar, G. (2003). Statin stimulates bone morphogenetic protein-2, aggrecan, and type 2 collagen gene expression and proteoglycan synthesis in rat chondrocytes. *Journal of orthopaedic science, 8*(6), 842-848.

Hattori, K., Ikeuchi, K., Morita, Y., & Takakura, Y. (2005). Quantitative ultrasonic assessment for detecting microscopic cartilage damage in osteoarthritis. *Arthritis Res Ther, 7*(1), R38-R46.

Havel, R. J. (1986). Functional activities of hepatic lipoprotein receptors. *Annual review of physiology, 48*(1), 119-134.

Hayami, T., Pickarski, M., Wesolowski, G. A., McLane, J., Bone, A., Destefano, J., . . . & Duong, L. T. (2004). The role of subchondral bone remodeling in osteoarthritis: Reduction of cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate ligament transection model. *Arthritis & Rheumatism, 50*(4), 1193-1206. doi: 10.1002/art.20124

Haynes, M. K., Hume, E. L., & Smith, J. B. (2002). Phenotypic Characterization of Inflammatory Cells from Osteoarthritic Synovium and Synovial Fluids. *Clinical Immunology, 105*(3), 315-325. doi: http://dx.doi.org/10.1006/clim.2002.5283

Haywood, L., McWilliams, D., Pearson, C., Gill, S., Ganesan, A., Wilson, D., & Walsh, D. (2003). Inflammation and angiogenesis in osteoarthritis. *Arthritis & Rheumatism, 48*(8), 2173-2177.

Hebert, S. L., Lanza, I. R., & Nair, K. S. (2010). Mitochondrial DNA alterations and reduced mitochondrial function in aging. *Mechanisms of ageing and development, 131*(7), 451-462.

Hedbom, E., & Häuselmann, H. (2002). Molecular aspects of pathogenesis in osteoarthritis: the role of inflammation. *Cellular and Molecular Life Sciences CMLS, 59*(1), 45-53Hegde, R., Srinivasula, S. M., Zhang, Z., Wassell, R., Mukattash, R., Cilenti, L., . . . & Fernandes-Alnemri, T. (2002). Identification of Omi/HtrA2 as a mitochondrial apoptotic serine protease that disrupts inhibitor of apoptosis protein-caspase interaction. *Journal of Biological Chemistry, 277*(1), 432-438.

Heike A. Wieland, M. M., Bernhard J. Kirschbaum & Karl A. Rudolphi. (2005). Osteoarthritis — an untreatable disease? *Nature Reviews Drug Discovery, 4*, 331-344. doi: 10.1038/nrd1693

Heliövaara, M., Mäkelä, M., Impivaara, O., Knekt, P., Aromaa, A., & Sievers, K. (1993). Association of overweight, trauma and workload with coxarthrosis: a health survey of 7,217 persons. *Acta Orthopaedica, 64*(5), 513-518.

Hendrich, L., Pons, J., Ribera, I., & Balke, M. (2010). Mitochondrial cox1 sequence data reliably uncover patterns of insect diversity but suffer from high lineage-idiosyncratic error rates. *PLoS ONE, 5*(12), e14448. doi: 10.1371/journal.pone.0014448

Herrero-Beaumont, G., Roman-Blas, J. A., Castañeda, S., & Jimenez, S. A. (2009). Primary Osteoarthritis No Longer Primary: Three Subsets with Distinct Etiological, Clinical, and Therapeutic Characteristics. *Seminars in Arthritis and Rheumatism, 39*(2), 71-80. doi: http://dx.doi.org/10.1016/j.semarthrit.2009.03.006

Hilal, G., Martel-Pelletier, J., Pelletier, J. P., Ranger, P., & Lajeunesse, D. (1998). Osteoblast-like cells from human subchondral osteoarthritic bone demonstrate

an altered phenotype in vitro: possible role in subchondral bone sclerosis. *Arthritis & Rheumatism, 41*(5), 891-899.

Hilal, G., Massicotte, F., Martel-Pelletier, J., Fernandes, J. C., Pelletier, J. P., & Lajeunesse, D. (2001). Endogenous prostaglandin E2 and insulin-like growth factor 1 can modulate the levels of parathyroid hormone receptor in human osteoarthritic osteoblasts. *Journal of Bone and Mineral Research, 16*(4), 713-721.

Hochberg, M., Lethbridge-Cejku, M., Scott Jr, W., Reichle, R., Plato, C., & Tobin, J. (1995). The association of body weight, body fatness and body fat distribution with osteoarthritis of the knee: data from the Baltimore Longitudinal Study of Aging. *The Journal of Rheumatology, 22*(3), 488-493.

Hochberg, M. C., Lethbridge-Cejku, M., Scott, W. W., Plato, C. C., & Tobin, J. D. (1993). Obesity and osteoarthritis of the hands in women. *Osteoarthritis and Cartilage, 1*(2), 129-135.

Holliday, K., McWilliams, D., Maciewicz, R. A., Muir, K., Zhang, W., & Doherty, M. (2011). Lifetime body mass index, other anthropometric measures of obesity and risk of knee or hip osteoarthritis in the GOAL case-control study. *Osteoarthritis and Cartilage, 19*(1), 37-43.

Horton, J. D., Goldstein, J. L., & Brown, M. S. (2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *The Journal of Clinical Investigation, 109*(9), 1125-1131. doi: 10.1172/JCI15593

Howell, D. S. (2002). Articular cartilage calcification and matrix vesicles. *Current rheumatology reports, 4*(3), 265-269.

Hozoji-Inada, M., Munehira, Y., Nagao, K., Kioka, N., & Ueda, K. (2011). Liver X receptor beta (LXRbeta) interacts directly with ATP-binding cassette A1 (ABCA1) to promote high density lipoprotein formation during acute cholesterol accumulation. *Journal of Biological Chemistry, 286*(22), 20117-20124. doi: M111.235846 [pii] 10.1074/jbc.M111.235846

Hsin-Chen, L., Pen-Hui, Y., Ching-You, L., Chin-Wen, C., & Yau-Huei, W. (2000). Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells. *Biochemical journal, 348*(2), 425-432.

Hudelmaier, M., Wirth, W., Nevitt, M., & Eckstein, F. (2013). Longitudinal rates of change in subchondral bone size in healthy knees and knees with radiographic osteoarthritis. *Osteoarthritis and Cartilage, 21*, S242.

Hui, D. Y., & Howles, P. N. (2005). *Molecular mechanisms of cholesterol absorption and transport in the intestine.* Paper presented at the Seminars in cell & developmental biology.

Hui, W., Barksby, H. E., Young, D. A., Cawston, T. E., Mckie, N., & Rowan, A. D. (2005). Oncostatin M in combination with tumour necrosis factor α induces a chondrocyte membrane associated aggrecanase that is distinct from ADAMTS aggrecanase-1 or-2. *Annals of the rheumatic diseases, 64*(11), 1624-1632.

Hulth A, L. L., Telhag H. (1970). Experimental osteoarthritis in rabbits. Preliminary report. *Acta Orthop Scand, 41*, 522-530.

Hunter, D. J., & Spector, T. D. (2003). The role of bone metabolism in osteoarthritis. *Current rheumatology reports, 5*(1), 15-19.

Iannone, F., & Lapadula, G. (2003). The pathophysiology of osteoarthritis. *Aging Clin Exp Res, 15*(5), 364-372.

Iannone, F., & Lapadula, G. (2008). Phenotype of chondrocytes in osteoarthritis. *Biorheology, 45*(3-4), 411-413.

IDF. International Diabetes Federation. *The IDF consensus worldwide definition of the metabolic syndrome, http://www.idf.org/metabolic-syndrome*.

Igney, F. H., & Krammer, P. H. (2002). Death and anti-death: tumour resistance to apoptosis. *Nature Reviews Cancer, 2*(4), 277-288.

Ikonen, E. (2006). Mechanisms for cellular cholesterol transport: defects and human disease. *Physiological reviews, 86*(4), 1237-1261. doi: 86/4/1237 [pii] 10.1152/physrev.00022.2005

Iliopoulos, D., Malizos, K. N., Oikonomou, P., & Tsezou, A. (2008). Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One, 3*(11), e3740.

Iwamoto, M., Kitagaki, J., Tamamura, Y., Gentili, C., Koyama, E., Enomoto, H., . . . & Enomoto-Iwamoto, M. (2003). Runx2 expression and action in chondrocytes are regulated by retinoid signaling and parathyroid hormone-related peptide (PTHrP). *Osteoarthritis Cartilage, 11*(1), 6-15.

Jaeger, K., Selent, C., Jaehme, W., Mahr, S., Goebel, U., Ibrahim, S., . . . & Mueller-Hilke, B. (2008). The genetics of osteoarthritis in STR/ort mice. Osteoarthritis and Cartilage, 16(5), 607-614.

Jaiprakash, A., Prasadam, I., Feng, J. Q., Liu, Y., Crawford, R., & Xiao, Y. (2012). Phenotypic characterization of osteoarthritic osteocytes from the sclerotic zones: a possible pathological role in subchondral bone sclerosis. International Journal of Biological Sciences, 8(3), 406-417.

Järvholm, B., Lewold, S., Malchau, H., & Vingård, E. (2005). Age, bodyweight, smoking habits and the risk of severe osteoarthritis in the hip and knee in men. European journal of epidemiology, 20(6), 537-542.

Jiang, Y., & Tuan, R. S. (2015). Origin and function of cartilage stem/progenitor cells in osteoarthritis. *Nature Reviews Rheumatology, 11*(4), 206-212.

Johnson, K., Svensson, C. I., Etten, D. V., Ghosh, S. S., Murphy, A. N., Powell, H. C., & Terkeltaub, R. (2004). Mediation of spontaneous knee osteoarthritis by progressive chondrocyte ATP depletion in Hartley guinea pigs. *Arthritis & Rheumatism, 50*(4), 1216-1225. doi: 10.1002/art.20149

Kamata, H., Honda, S.I., Maeda, S., Chang, L., Hirata, H., & Karin, M. (2005). Reactive Oxygen Species Promote TNFα-Induced Death and Sustained JNK

Activation by Inhibiting MAP Kinase Phosphatases. *Cell, 120*(5), 649-661. doi: http://dx.doi.org/10.1016/j.cell.2004.12.041

Karsdal, M., Bay-Jensen, A., Lories, R., Abramson, S., Spector, T., Pastoureau, P., . . . & Goldring, S. (2013). The coupling of bone and cartilage turnover in osteoarthritis: opportunities for bone antiresorptives and anabolics as potential treatments? *Annals of the rheumatic diseases*, annrheumdis-2013-204111.

Katz, J. D., Agrawal, S., & Velasquez, M. (2010). Getting to the heart of the matter: osteoarthritis takes its place as part of the metabolic syndrome. *Current opinion in rheumatology, 22*(5), 512-519. doi: 10.1097/BOR.0b013e32833bfb4b

Kelekar, A., & Thompson, C. B. (1998). Bcl-2-family proteins: the role of the BH3 domain in apoptosis. *Trends in cell biology, 8*(8), 324-330.

Kellgren, J. (1961). Osteoarthrosis in patients and populations. *British Medical Journal, 2*(5243), 1.

Kellner-Weibel, G., Geng, Y., & Rothblat, G. (1999). Cytotoxic cholesterol is generated by the hydrolysis of cytoplasmic cholesteryl ester and transported to the plasma membrane. *Atherosclerosis, 146*(2), 309-319.

Kerr, J. F., Wyllie, A. H., & Currie, A. R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *British journal of cancer, 26*(4), 239.

Khurana, S., Gupta, S., Bhalla, H., Nandwani, S., & Gupta, V. (2015). Comparison of anti-inflammatory effect of atorvastatin with rosuvastatin in patients of acute coronary syndrome. *Journal of pharmacology & pharmacotherapeutics, 6*(3), 130-135. doi: 10.4103/0976-500x.162011

Kim, J. H., Lee, J. N., & Paik, Y. K. (2001). Cholesterol biosynthesis from lanosterol A concerted role for Sp1 and NF-Y-binding sites for sterol-mediated regulation of rat 7-dehydrocholesterol reductase gene expression. *Journal of Biological Chemistry*, *276*(21), 18153-18160.

King, L. K., Birmingham, T. B., Kean, C. O., Jones, I. C., Bryant, D. M., & Giffin, J. R. (2008). Resistance training for medial compartment knee osteoarthritis and malalignment. *Medicine and science in sports and exercise, 40*(8), 1376-1384.

Kishida, Y., Hirao, M., Tamai, N., Nampei, A., Fujimoto, T., Nakase, T., . . . & Myoui, A. (2005). Leptin regulates chondrocyte differentiation and matrix maturation during endochondral ossification. *Bone, 37*(5), 607-621.

Kolovou, G. D., Anagnostopoulou, K. K., Salpea, K. D., & Mikhailidis, D. P. (2007). The prevalence of metabolic syndrome in various populations. *The American journal of the medical sciences, 333*(6), 362-371.

Koskinen, J., Magnussen, C. G., Würtz, P., Soininen, P., Kangas, A. J., Viikari, J. S., . . . & Ahotupa, M. (2012). Apolipoprotein B, oxidized low-density lipoprotein, and LDL particle size in predicting the incidence of metabolic syndrome: the Cardiovascular Risk in Young Finns study. *European journal of preventive cardiology, 19*(6), 1296-1303.

Kostopoulou, F., Gkretsi, V., Malizos, K. N., Iliopoulos, D., Oikonomou, P., Poultsides, L., & Tsezou, A. (2012). Central role of SREBP-2 in the pathogenesis of osteoarthritis. *PLoS One, 7*(5), e35753.

Krieger, M. (1999). Charting the fate of the "good cholesterol": identification and characterization of the high-density lipoprotein receptor SR-BI. *Annual review of biochemistry, 68*, 523-558. doi: 10.1146/annurev.biochem.68.1.523

Kroemer, G., Zamzami, N., & Susin, S. A. (1997). Mitochondrial control of apoptosis. *Immunology Today, 18*(1), 44-51. doi: http://dx.doi.org/10.1016/S0167-5699(97)80014-X

Laakmann, S., Auel, H., & Kochzius, M. (2012). Evolution in the deep sea: biological traits, ecology and phylogenetics of pelagic copepods. *Molecular phylogenetics and evolution, 65*(2), 535-546. doi: 10.1016/j.ympev.2012.07.007

Lane, N. E., & Nevitt, M. C. (2002). Osteoarthritis, bone mass, and fractures: how are they related?. *Arthritis & Rheumatism, 46*(1), 1-4.

Lajeunesse, D., & Reboul, P. (2003). Subchondral bone in osteoarthritis: a biologic link with articular cartilage leading to abnormal remodeling. , *15*(5), 628-633.

Lakka, H. M., Laaksonen, D. E., Lakka, T. A., Niskanen, L. K., Kumpusalo, E., Tuomilehto, J., & Salonen, J. T. (2002). The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *Jama, 288*(21), 2709-2716.

Lange, Y., Ye, J., & Steck, T. L. (2004). How cholesterol homeostasis is regulated by plasma membrane cholesterol in excess of phospholipids. *Proceedings of the National Academy of Sciences of the United States of America, 101*(32), 11664-11667.

Lanza, I., & Nair, K. S. (2010). Mitochondrial function as a determinant of life span. *Pflügers Archive - European Journal of Physiology, 459*(2), 277-289. doi: 10.1007/s00424-009-0724-5

Lawrence, J. S., Bremner, J. M., & Bier, F. (1966). Osteo-arthrosis. Prevalence in the population and relationship between symptoms and x-ray changes. *Ann Rheum Dis, 25*(1), 1-24.

Lee, H. C., & Wei, Y. H. (2012). Mitochondria and aging. *Advances in Mitochondrial Medicine, 942*, 311-327. doi: 10.1007/978-94-007-2869-1_14

Leung, B. P., Sattar, N., Crilly, A., Prach, M., McCarey, D. W., Payne, H., . . . & Liew, F. Y. (2003). A novel anti-inflammatory role for simvastatin in inflammatory arthritis. *The Journal of Immunology, 170*(3), 1524-1530.

Levick, J. R., & McDonald, J. N. (1995). Fluid movement across synovium in healthy joints: role of synovial fluid macromolecules. *Annals of the rheumatic diseases, 54*(5), 417-423.

Lewis, G. F., & Rader, D. J. (2005). New insights into the regulation of HDL metabolism and reverse cholesterol transport. *Circulation research, 96*(12), 1221-1232.

Li, B., & Aspden, R. M. (1997). Mechanical and material properties of the subchondral bone plate from the femoral head of patients with osteoarthritis or osteoporosis. *Annals of the rheumatic diseases, 56*(4), 247-254.

Li, C., Wang, W., Guo, X., Zhang, F., Ma, W., Zhang, Y., . . . & Lammi, M. (2012). Pathways related to mitochondrial dysfunction in cartilage of endemic osteoarthritis patients in China. *Science China Life Sciences, 55*(12), 1057-1063. doi: 10.1007/s11427-012-4418-4

Li, H., Cybulsky, M. I., Gimbrone, M. A., Jr., & Libby, P. (1993). An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. *Arteriosclerosis, Thrombosis, and Vascular Biology, 13*(2), 197-204.

Li, L. Y., Luo, X., & Wang, X. (2001). Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature, 412*(6842), 95-99.

Libby, P., Lichtman, A. H., & Hansson, G. K. (2013). Immune effector mechanisms implicated in atherosclerosis: from mice to humans. *Immunity, 38*(6), 1092-1104.

Libby, P., Ridker, P. M., & Hansson, G. K. (2011). Progress and challenges in translating the biology of atherosclerosis. *Nature, 473*(7347), 317-325. doi: 10.1038/nature10146

Libby, P., Ridker, P. M., & Maseri, A. (2002). Inflammation and atherosclerosis. *Circulation, 105*(9), 1135-1143.

Lim, S., & Barter, P. (2014). Antioxidant effects of statins in the management of cardiometabolic disorders. *Journal of atherosclerosis and thrombosis, 21*(10), 997-1010.

Lin, M. T., & Beal, M. F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature, 443*(7113), 787-795.

Lin, Z., Willers, C., Xu, J., & Zheng, M.-H. (2006). The chondrocyte: biology and clinical application. *Tissue engineering, 12*(7), 1971-1984.

Lindblad, S., & Hedfors, E. (1987). Arthroscopic and immunohistologic characterization of knee joint synovitis in osteoarthritis. *Arthritis & Rheumatism, 30*(10), 1081-1088.

Linnane, A., Ozawa, T., Marzuki, S., & Tanaka, M. (1989). Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *The Lancet, 333*(8639), 642-645. doi: http://dx.doi.org/10.1016/S0140-6736(89)92145-4

Lippiello, L., Hall, D., & Mankin, H. J. (1977). Collagen synthesis in normal and osteoarthritic human cartilage. *Journal of Clinical Investigation*, 59(4), 593.

Lippiello, L., Walsh, T., & Fienhold, M. (1991). The association of lipid abnormalities with tissue pathology in human osteoarthritic articular cartilage. *Metabolism, 40*(6), 571-576.

Liscum, L. (2008). Chapter 14 - Cholesterol biosynthesis. In D. E. V. E. Vance (Ed.), *Biochemistry of Lipids, Lipoproteins and Membranes (Fifth Edition)* (pp. 399-XI). San Diego: Elsevier.

Lisignoli, G., Toneguzzi, S., Pozzi, C., Piacentini, A., Riccio, M., Ferruzzi, A., . . . & Facchini, A. (1999). Proinflammatory cytokines and chemokine production and expression by human osteoblasts isolated from patients with rheumatoid arthritis and osteoarthritis. *The Journal of rheumatology, 26*(4), 791-799.

Little, C. B., Hughes, C. E., Curtis, C. L., Janusz, M. J., Bohne, R., Wang-Weigand, S., . . . & Flannery, C. R. (2002). Matrix metalloproteinases are involved in C-terminal and interglobular domain processing of cartilage aggrecan in late stage cartilage degradation. *Matrix Biology*, 21(3), 271-288.

Liu, M., Liu, H., & Dudley, S. C. (2010). Reactive oxygen species originating from mitochondria regulate the cardiac sodium channel. Circulation research, 107(8), 967-974.

Liu, J. T., Guo, X., Ma, W. J., Zhang, Y. G., Xu, P., Yao, J. F., & Bai, Y. D. (2010). Mitochondrial function is altered in articular chondrocytes of an endemic osteoarthritis, Kashin–Beck disease. *Osteoarthritis and Cartilage, 18*(9), 1218-1226. doi: http://dx.doi.org/10.1016/j.joca.2010.07.003

Liu, X., Kim, C. N., Yang, J., Jemmerson, R., & Wang, X. (1996). Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell, 86*(1), 147-157.

Liyanage, K. E., Burnett, J. R., Hooper, A. J., & van Bockxmeer, F. M. (2011). Familial hypercholesterolemia: epidemiology, Neolithic origins and modern geographic distribution. *Critical reviews in clinical laboratory sciences, 48*(1), 1-18.

Loeser, R. F., Yammani, R. R., Carlson, C. S., Chen, H., Cole, A., Im, H. J., . . . & Yan, S. D. (2005). Articular chondrocytes express the receptor for advanced glycation end products: potential role in osteoarthritis. *Arthritis & Rheumatism, 52*(8), 2376-2385.

Loeuille, D., Chary-Valckenaere, I., Champigneulle, J., Rat, A. C., Toussaint, F., Pinzano-Watrin, A., . . . & Gillet, P. (2005). Macroscopic and microscopic features of synovial membrane inflammation in the osteoarthritic knee: correlating magnetic resonance imaging findings with disease severity. *Arthritis & Rheumatism, 52*(11), 3492-3501. doi: 10.1002/art.21373

Loeuille, D., Chary-Valckenaere, I., Champigneulle, J., Rat, A. C., Toussaint, F., Pinzano-Watrin, A., . . . & Pourel, J. (2005). Macroscopic and microscopic features of synovial membrane inflammation in the osteoarthritic knee: correlating magnetic resonance imaging findings with disease severity. *Arthritis & Rheumatism, 52*(11), 3492-3501.

Lohmander, L. S., de Verdier, M. G., Rollof, J., Nilsson, P. M., & Engström, G. (2009). Incidence of severe knee and hip osteoarthritis in relation to different measures of body mass: a population-based prospective cohort study. *Annals of the rheumatic diseases, 68*(4), 490-496.

Lordan, S., Mackrill, J. J., & O'Brien, N. M. (2009). Oxysterols and mechanisms of apoptotic signaling: implications in the pathology of degenerative diseases. *The Journal of nutritional biochemistry, 20*(5), 321-336. doi: S0955-2863(09)00008-4 [pii] 10.1016/j.jnutbio.2009.01.001

Lowman, E. W. (1955). Osteoarthritis. *Journal of the American Medical Association, 157*(6), 487-488.

Lyons, T. J., Stoddart, R. W., McClure, S. F., & McClure, J. (2005). The tidemark of the chondro-osseous junction of the normal human knee joint. Journal of molecular histology, 36(3), 207-215.

MacGregor, A., Li, Q., Spector, T., & Williams, F. (2009). The genetic influence on radiographic osteoarthritis is site specific at the hand, hip and knee. Rheumatology, ken475.

Maly, M. R., Costigan, P. A., & Olney, S. J (2005). Contribution of Psychosocial and Mechanical Variables to Physical Performance Measures in Knee Osteoarthritis. Physical Therapy, 85, 13-18-1328.

Maneiro, E., de Andres, M., Martin, M., Lopez-Armada, M., Pinto, J., Fernandez-Sueiro, J., . . . & Blanco, F. (2001). Mitochondrial respiratory activity is altered in OA human articular chondrocytes: A new approach to understand OA. Paper presented at the Arthritis and Rheumatism Conference.

Mankin, H. J., Buckwalter, J.A. (2000). Articular cartilage structure, composition and function (2 ed.): Rosemont: American Academy of Orthopaedic Surgeons.

Mankin, H. J., Dorfman, H., Lippiello, L., & ZARINS, A. (1971). Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. *The Journal of Bone & Joint Surgery, 53*(3), 523-537.

Manuel T. Velasquez , M. D., and James D. Katz , M.D. (2010). Osteoarthritis: Another Component of Metabolic Syndrome? *Metabolic syndrome and related disorders, 8*(4), 295–305. doi: 10.1089/met.2009.0110

Marcil, V., Delvin, E., Sane, A. T., Tremblay, A., & Levy, E. (2006). Oxidative stress influences cholesterol efflux in THP-1 macrophages: role of ATP-binding cassette A1 and nuclear factors. *Cardiovasc Research, 72*(3), 473-482. doi: S0008-6363(06)00399-3 [pii] 10.1016/j.cardiores.2006.08.024

Martin, K., Lethbridge-Cejku, M., Muller, D. C., Elahi, D., Andres, R., Tobin, J. D., & Hochberg, M. C. (1997). Metabolic correlates of obesity and radiographic features of knee osteoarthritis: data from the Baltimore Longitudinal Study of Aging. *Journal of Rheumatology, 24*(4), 702-707.

Martin, R. (2007). Targeted bone remodeling involves BMU steering as well as activation. *Bone, 40*(6), 1574-1580.

Martins, I., Hone, E., Foster, J., Sünram-Lea, S., Gnjec, A., Fuller, S., . . . & Martins, R. (2006). Apolipoprotein E, cholesterol metabolism, diabetes, and the convergence of risk factors for Alzheimer's disease and cardiovascular disease. *Molecular psychiatry, 11*(8), 721-736.

Mason, R., Chambers, M., Flannelly, J., Gaffen, J., Dudhia, J., & Bayliss, M. (2001). The STR/ort mouse and its use as a model of osteoarthritis. *Osteoarthritis and Cartilage, 9*(2), 85-91.

Massicotte, F., Lajeunesse, D., Benderdour, M., Pelletier, J. P., Hilal, G., Duval, N., & Martel-Pelletier, J. (2002). Can altered production of interleukin-1beta, interleukin-6, transforming growth factor-beta and prostaglandin E(2) by isolated human subchondral osteoblasts identify two subgroups of osteoarthritic patients. *Osteoarthritis Cartilage, 10*(6), 491-500.

Matsuda, M., & Shimomura, I. (2013). Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. *Obesity Research & Clinical Practice, 7*(5), e330-e341.

Maxfield, F. R., & Wüstner, D. (2002). Intracellular cholesterol transport. *The Journal of Clinical Investigation, 110*(110 (7)), 891-898.

Meireles, S., De Groote, F., Reeves, N.D., Verschueren, S., Maganaris, C., Luyten, F., Jonkers, I., 2016. Knee contact forces are not altered in early knee osteoarthritis. Gait & posture 45, 115-120.

Meir, K. S., & Leitersdorf, E. (2004). Atherosclerosis in the Apolipoprotein E–Deficient Mouse A Decade of Progress. *Arteriosclerosis, Thrombosis, and Vascular Biology, 24*(6), 1006-1014.

Messier, S. P., Gutekunst, D. J., Davis, C., & DeVita, P. (2005). Weight loss reduces knee-joint loads in overweight and obese older adults with knee osteoarthritis. *Arthritis & Rheumatism, 52*(7), 2026-2032. doi: 10.1002/art.21139

Meunier, P., & Boivin, G. (1997). Bone mineral density reflects bone mass but also the degree of mineralization of bone: therapeutic implications. *Bone, 21*(5), 373-377.

Miller, L. M., Novatt, J. T., Hamerman, D., & Carlson, C. S. (2004). Alterations in mineral composition observed in osteoarthritic joints of cynomolgus monkeys. *Bone, 35*(2), 498-506.

Mitchell, P. (1979). Compartmentation and Communication in Living Systems. Ligand Conduction: a General Catalytic Principle in Chemical, Osmotic and Chemiosmotic Reaction Systems. *European Journal of Biochemistry, 95*(1), 1-20. doi: 10.1111/j.1432-1033.1979.tb12934.x

Morgan, T. G., Rowan, A. D., Dickinson, S. C., Jones, D., Hollander, A. P., Deehan, D., & Cawston, T. E. (2006). Human nasal cartilage responds to oncostatin M in combination with interleukin 1 or tumour necrosis factor α by the release of collagen fragments via collagenases. *Annals of the rheumatic diseases, 65*(2), 184-190.

Moyer, R. F., Birmingham, T. B., Chesworth, B. M., Kean, C., & Giffin, J. R. (2010). Alignment, body mass and their interaction on dynamic knee joint load in patients with knee osteoarthritis. *Osteoarthritis and Cartilage, 18*(7), 888-893.

Mündermann, A., Dyrby, C. O., & Andriacchi, T. P. (2005). Secondary gait changes in patients with medial compartment knee osteoarthritis: increased load at the ankle, knee, and hip during walking. *Arthritis & Rheumatism, 52*(9), 2835-2844.

Mündermann, A., Payer, N., Felmet, G., & Riehle, H. (2015). Comparison of volumetric bone mineral density in the operated and contralateral knee after anterior cruciate ligament and reconstruction: A 1-year follow-up study using peripheral quantitative computed tomography. *Journal of Orthopaedic Research,* 33(12), 1804-1810.

Murphy, G., & Nagase, H. (2008). Reappraising metalloproteinases in rheumatoid arthritis and osteoarthritis: destruction or repair? *Nature Clinical Practice Rheumatology, 4*(2), 128-135.

Murphy, M. P., & Smith, R. A. (2007). Targeting antioxidants to mitochondria by conjugation to lipophilic cations. *Annu. Rev. Pharmacol. Toxicol., 47*, 629-656.

Mwale, F., Tchetina, E., Wu, C. W., & Poole, A. R. (2002). The Assembly and Remodeling of the Extracellular Matrix in the Growth Plate in Relationship to Mineral Deposition and Cellular Hypertrophy: An In Situ Study of Collagens II and IX and Proteoglycan. *Journal of Bone and Mineral Research, 17*(2), 275-283. doi: 10.1359/jbmr.2002.17.2.275

Myant, N. B. (1990). Chapter 2 - Cholesterol in Animal Tissues. In N. B. Myant (Ed.), *Cholesterol Metabolism, Ldl, and the Ldl Receptor* (pp. 4-8): Academic Press.

Nazarewicz, R. R., Dikalova, A., Bikineyeva, A., Ivanov, S., Kirilyuk, I. A., Grigor'ev, I. A., & Dikalov, S. I. (2013). Does scavenging of mitochondrial superoxide attenuate cancer prosurvival signaling pathways? *Antioxid Redox Signal, 19*(4), 344-349. doi: 10.1089/ars.2013.5185

Nechushtan, A., Smith, C. L., Lamensdorf, I., Yoon, S. H., & Youle, R. J. (2001). Bax and Bak coalesce into novel mitochondria-associated clusters during apoptosis. *The Journal of cell biology, 153*(6), 1265-1276.

Neufeld, E. B. (1998). What the Niemann-Pick type C gene has taught us about cholesterol transport *Intracellular Cholesterol Trafficking* (pp. 93-107): Springer.

Neuhold, L. A., Killar, L., Zhao, W., Sung, M. L. A., Warner, L., Kulik, J., . . . & Meijers, T. (2001). Postnatal expression in hyaline cartilage of constitutively active human collagenase-3 (MMP-13) induces osteoarthritis in mice. *Journal of Clinical Investigation, 107*(1), 35.

Nezic, L., Skrbic, R., Dobric, S., Stojiljkovic, M. P., Jacevic, V., Satara, S. S., . . . & Stojakovic, N. (2009). Simvastatin and indomethacin have similar anti-inflammatory activity in a rat model of acute local inflammation. *Basic &*

clinical pharmacology & toxicology, *104*(3), 185-191. doi: 10.1111/j.1742-7843.2008.00302.x

Nicholls, D., & Åkerman, K. (1982). Mitochondrial calcium transport. *Biochimica et Biophysica Acta (BBA) - Reviews on Bioenergetics*, *683*(1), 57-88. doi: http://dx.doi.org/10.1016/0304-4173(82)90013-1

Nicholls, S. J., Tuzcu, E. M., Sipahi, I., Grasso, A. W., Schoenhagen, P., Hu, T., . . . & Hazen, S. L. (2007). Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis. *Jama*, *297*(5), 499-508.

Nissen, S. E., Nicholls, S. J., Sipahi, I., Libby, P., Raichlen, J. S., Ballantyne, C. M., . . . & Tardif, J. C. (2006). Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. *Jama*, *295*(13), 1556-1565.

Okamoto, K., & Shaw, J. M. (2005). Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. *Annu. Rev. Genet.*, *39*, 503-536.

Oliviero, F., Sfriso, P., Baldo, G., Dayer, J. M., Giunco, S., Scanu, A., ... & Punzi, L. (2009). Apolipoprotein AI and cholesterol in synovial fluid of patients with rheumatoid arthritis, psoriatic arthritis and osteoarthritis. *Clinical & Experimental Rheumatology*, *27*(1), 79.

Oliveira, H. C., Cosso, R. G., Alberici, L. C., Maciel, E. N., Salerno, A. G., Dorighello, G. G., . . . & Vercesi, A. E. (2005). Oxidative stress in atherosclerosis-prone mouse is due to low antioxidant capacity of mitochondria. *FASEB Journal*, *19*(2), 278-280. doi: 04-2095fje [pii] 10.1096/fj.04-2095fje

Oliveria, S. A., Felson, D. T., Cirillo, P. A., Reed, J. I., & Walker, A. M. (1999). Body weight, body mass index, and incident symptomatic osteoarthritis of the hand, hip, and knee. *Epidemiology*, *10*(2), 161-166.

Oliviero, F., Lo Nigro, A., Bernardi, D., Giunco, S., Baldo, G., Scanu, A., . . . & Punzi, L. (2012). A comparative study of serum and synovial fluid lipoprotein levels in patients with various arthritides. *Clinica chimica acta; international journal of clinical chemistry*, *413*(1-2), 303-307. doi: 10.1016/j.cca.2011.10.019

Oliviero, F., Sfriso, P., Baldo, G., Dayer, J. M., Giunco, S., Scanu, A., . . . & Punzi, L. (2009). Apolipoprotein A-I and cholesterol in synovial fluid of patients with rheumatoid arthritis, psoriatic arthritis and osteoarthritis. *Clinical & Experimental Rheumatology*, *27*(1), 79-83. doi: 2561 [pii]

Oram, J. F. (2002). ATP-binding cassette transporter A1 and cholesterol trafficking. *Current Opinion in Lipidology*, *13*(4), 373-381.

Ostalowska, A., Birkner, E., Wiecha, M., Kasperczyk, S., Kasperczyk, A., Kapolka, D., & Zon-Giebel, A. (2006). Lipid peroxidation and antioxidant enzymes in synovial fluid of patients with primary and secondary osteoarthritis of the knee joint. *Osteoarthritis Cartilage*, *14*(2), 139-145. doi: S1063-4584(05)00235-9 [pii] 10.1016/j.joca.2005.08.009

Ouvrier, A., Cadet, R., Vernet, P., Laillet, B., Chardigny, J. M., Lobaccaro, J. M., . . . & Saez, F. (2009). LXR and ABCA1 control cholesterol homeostasis in the

proximal mouse epididymis in a cell-specific manner. *Journal of lipid research, 50*(9), 1766-1775. doi: M800657-JLR200 [pii] 10.1194/jlr.M800657-JLR200

Pagler, T. A., Rhode, S., Neuhofer, A., Laggner, H., Strobl, W., Hinterndorfer, C., . . . & Stangl, H. (2006). SR-BI-mediated high density lipoprotein (HDL) endocytosis leads to HDL resecretion facilitating cholesterol efflux. *Journal of Biological Chemistry, 281*(16), 11193-11204. doi: M510261200 [pii] 10.1074/jbc.M510261200

Palade, G. E. (1952). The fine structure of mitochondria. *The Anatomical Record, 114*(3), 427-451. doi: 10.1002/ar.1091140304

Pallu, S., Francin, P. J., Guillaume, C., Gegout-Pottie, P., Netter, P., Mainard, D., ... & Presle, N. (2010). Obesity affects the chondrocyte responsiveness to leptin in patients with osteoarthritis. *Arthritis research & therapy, 12*(3), 1-9.

Panini, H. R. a. S. R. (1993). Cholesterol biosynthesis *Current Opinion in Lipidology, 4*, 230-237.

Pastoureau, P., Leduc, S., Chomel, A., & De Ceuninck, F. (2003). Quantitative assessment of articular cartilage and subchondral bone histology in the meniscectomized guinea pig model of osteoarthritis. *Osteoarthritis and Cartilage, 11*(6), 412-423.

Pavlov, E. V., Priault, M., Pietkiewicz, D., Cheng, E. H.-Y., Antonsson, B., Manon, S., . . . & Kinnally, K. W. (2001). A novel, high conductance channel of mitochondria linked to apoptosis in mammalian cells and Bax expression in yeast. *The Journal of cell biology, 155*(5), 725-732.

Pearson, G., Robinson, F., Beers Gibson, T., Xu, B.-e., Karandikar, M., Berman, K., & Cobb, M. H. (2001). Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions 1. *Endocrine reviews, 22*(2), 153-183.

Pelletier, J. P., Boileau, C., Brunet, J., Boily, M., Lajeunesse, D., Reboul, P., . . . & Martel-Pelletier, J. (2004). The inhibition of subchondral bone resorption in the early phase of experimental dog osteoarthritis by licofelone is associated with a reduction in the synthesis of MMP-13 and cathepsin K. *Bone, 34*(3), 527-538.

Perkins, G. A., & Frey, T. G. (2000). Recent structural insight into mitochondria gained by microscopy. *Micron, 31*(1), 97-111. doi: http://dx.doi.org/10.1016/S0968-4328(99)00065-7

Phillips, M. C. (1972). The physical state of phospholipids and cholesterol in monolayers, bilayers, and membranes. *Progress in surface and membrane science, 5*, 139-221.

Pohl, A., Devaux, P. F., & Herrmann, A. (2005). Function of prokaryotic and eukaryotic ABC proteins in lipid transport. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1733*(1), 29-52.

Poole, A. R., Kobayashi, M., Yasuda, T., Laverty, S., Mwale, F., Kojima, T., ... & Tchetina, E. (2002). Type II collagen degradation and its regulation in articular cartilage in osteoarthritis. *Annals of the rheumatic diseases, 61*(suppl 2), ii78-ii81.

Poole, A. R., Kojima, T., Yasuda, T., Mwale, F., Kobayashi, M., & Laverty, S. (2001). Composition and structure of articular cartilage: a template for tissue repair. Clinical orthopaedics and related research (391 Suppl), S26-33.

Poole, A. R., Rosenberg, L. C., Reiner, A., Ionescu, M., Bogoch, E., & Roughley, P. J. (1996). Contents and distributions of the proteoglycans decorin and biglycan in normal and osteoarthritic human articular cartilage. Journal of Orthopaedic Research, 14(5), 681-689. doi: 10.1002/jor.1100140502

Pottie, P., Presle, N., Terlain, B., Netter, P., Mainard, D., & Berenbaum, F. (2006). Obesity and osteoarthritis: more complex than predicted! Annals of the rheumatic diseases, 65(11), 1403-1405.

Prasadam, I., Crawford, R., & Xiao, Y. (2010). Cell interactions of osteoarthritic subchondral bone osteoblasts and articular chondrocytes aggravate MMP-2 and MMP-9 production through the mediation of ERK1/2 and JNK phosphorylation. Cross-talk of subchondral bone osteoblasts and articular cartilage chondrocytes: a new insight in understanding osteoarthritis pathogenesis, 118.

Prasadam, I., Mao, X., Shi, W., Crawford, R., & Xiao, Y. (2013). Combination of MEK-ERK inhibitor and hyaluronic acid has a synergistic effect on anti-hypertrophic and pro-chondrogenic activities in osteoarthritis treatment. Journal of Molecular Medicine, 91(3), 369-380.

Prasadam, I., Mao, X., Wang, Y., Shi, W., Crawford, R., & Xiao, Y. (2012). Inhibition of p38 pathway leads to OA-like changes in a rat animal model. Rheumatology, 51(5), 813-823.

Prasadam, I., van Gennip, S., Friis, T., Shi, W., Crawford, R., & Xiao, Y. (2010). ERK-1/2 and p38 in the regulation of hypertrophic changes of normal articular cartilage chondrocytes induced by osteoarthritic subchondral osteoblasts. Arthritis & Rheumatism, 62(5), 1349-1360.

Presle, N., Pottie, P., Dumond, H., Guillaume, C., Lapicque, F., Pallu, S., . . . & Terlain, B. (2006). Differential distribution of adipokines between serum and synovial fluid in patients with osteoarthritis. Contribution of joint tissues to their articular production. Osteoarthritis and Cartilage, 14(7), 690-695. doi: http://dx.doi.org/10.1016/j.joca.2006.01.009

Prinz, W. (2002). *Cholesterol trafficking in the secretory and endocytic systems.* Paper presented at the Seminars in cell & developmental biology.

Pritchett, K., & Taft, R. (2006). The Mouse in Biomedical Research: Normative Biology, Husbandry, and Models. *Fox, JG., et al., editors, 3*, 91-121.

Pritzker, K. P. H., Gay, S., Jimenez, S. A., Ostergaard, K., Pelletier, J. P., Revell, P. A., . . . & van den Berg, W. B. (2006). Osteoarthritis cartilage histopathology: grading and staging. *Osteoarthritis and Cartilage, 14*(1), 13-29. doi: http://dx.doi.org/10.1016/j.joca.2005.07.014

Pucadyil, T. J., & Chattopadhyay, A. (2004). Cholesterol modulates ligand binding and G-protein coupling to serotonin 1A receptors from bovine hippocampus. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1663*(1), 188-200.

Puddu, P., Puddu, G. M., Galletti, L., Cravero, E., & Muscari, A. (2005). Mitochondrial dysfunction as an initiating event in atherogenesis: a plausible hypothesis. *Cardiology, 103*(3), 137-141. doi: 83440 [pii] 10.1159/000083440

Puenpatom RA, V. T. (2009). Increased prevalence of metabolic syndrome in individuals with osteoarthritis: an analysis of NHANES III data. *Postgraduate medicine, 121*, 9-20. doi: 10.3810/pgm.2009.11.2073

Puglielli, L., Rigotti, A., Amigo, L., Nunez, L., Greco, A., Santos, M., & Nervi, F. (1996). Modulation of intrahepatic cholesterol trafficking: evidence by in vivo antisense treatment for the involvement of sterol carrier protein-2 in newly synthesized cholesterol transport into rat bile. *Biochemical Journal, 317*, 681-687.

Pullig, O., Weseloh, G., Gauer, S., & Swoboda, B. (2000). Osteopontin is expressed by adult human osteoarthritic chondrocytes: protein and mRNA analysis of normal and osteoarthritic cartilage. *Matrix Biology, 19*(3), 245-255.

Pullig, O., Weseloh, G., Ronneberger, D. L., Käkönen, S. M., & Swoboda, B. (2000). Chondrocyte differentiation in human osteoarthritis: expression of osteocalcin in normal and osteoarthritic cartilage and bone. *Calcified Tissue International, 67*(3), 230-240.

Pullig, O., Weseloh, G., Ronneberger, D., Kakonen, S., & Swoboda, B. (2000). Chondrocyte differentiation in human osteoarthritis: expression of osteocalcin in normal and osteoarthritic cartilage and bone. *Calcified Tissue International, 67*(3), 230-240.

Quasnichka, H. L., Anderson-MacKenzie, J. M., & Bailey, A. J. (2006). Subchondral bone and ligament changes precede cartilage degradation in guinea pig osteoarthritis. *Biorheology, 43*(3), 389-400.

Quasnichka, H. L., Anderson-MacKenzie, J. M., & Bailey, A. J. (2006). Subchondral bone and ligament changes precede cartilage degradation in guinea pig osteoarthritis. *Biorheology, 43*(3-4), 389-397.

Radin, E. L., & Rose, R. M. (1986). Role of subchondral bone in the initiation and progression of cartilage damage. *Clinical orthopaedics and related research, 213*, 34-40.

Rajamaki, K., Lappalainen, J., Oorni, K., Valimaki, E., Matikainen, S., Kovanen, P. T., & Eklund, K. K. (2010). Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. *PLoS One, 5*(7), e11765.

Ratcliffe, A., & Seibel, M. J. (1990). Biochemical markers of osteoarthritis. *Current Opinion in Rheumatology, 2*(5), 770-776.

Regan, E., Flannelly, J., Bowler, R., Tran, K., Nicks, M., Carbone, B. D., . . . & Crapo, J. (2005). Extracellular superoxide dismutase and oxidant damage in osteoarthritis. *Arthritis & Rheumatism, 52*(11), 3479-3491. doi: 10.1002/art.21387

Reijman, M., Pols, H., Bergink, A., Hazes, J., Belo, J., Lievense, A., & Bierma-Zeinstra, S. (2007). Body mass index associated with onset and progression of osteoarthritis of the knee but not of the hip: the Rotterdam Study. *Annals of the rheumatic diseases, 66*(2), 158-162.

Rejeski, W. J., Craven, T., Ettinger, W. H., McFarlane, M., & Shumaker, S. (1996). Self-efficacy and pain in disability with osteoarthritis of the knee. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences, 51*(1), P24-P29.

Rengel, Y., Ospelt, C., & Gay, S. (2007). Proteinases in the joint: clinical relevance of proteinases in joint destruction. *Arthritis Research and Therapy, 9*(5), 221.

Revell, P. A., Mayston, V., Lalor, P., & Mapp, P. (1988). The synovial membrane in osteoarthritis: a histological study including the characterisation of the cellular infiltrate present in inflammatory osteoarthritis using monoclonal antibodies. *Annals of the rheumatic diseases, 47*(4), 300-307.

Richette, P., Poitou, C., Garnero, P., Vicaut, E., Bouillot, J. L., Lacorte, J. M., . . . & Chevalier, X. (2011). Benefits of massive weight loss on symptoms, systemic inflammation and cartilage turnover in obese patients with knee osteoarthritis. *Annals of the rheumatic diseases, 70*(1), 139-144. doi: 10.1136/ard.2010.134015

Rinninger, F., Brundert, M., Budzinski, R. M., Fruchart, J. C., Greten, H., & Castro, G. R. (2003). Scavenger receptor BI (SR-BI) mediates a higher selective cholesteryl ester uptake from LpA-I compared with LpA-I:A-II lipoprotein particles. *Atherosclerosis, 166*(1), 31-40. doi: S0021915002003118 [pii]

Rizzuto, R., Simpson, A., Brini, M., & Pozzan, T. (1992). Rapid changes of mitochondrial Ca2+ revealed by specifically targeted recombinant aequorin. *Nature, 358*(6384), 325-327.

Roach, H., Aigner, T., Soder, S., Haag, J., & Welkerling, H. (2007). Pathobiology of osteoarthritis: pathomechanisms and potential therapeutic targets. *Current Drug Targets, 8*(2), 271-282.

Roberts, C. K., Barnard, R. J., Sindhu, R. K., Jurczak, M., Ehdaie, A., & Vaziri, N. D. (2006). Oxidative stress and dysregulation of NAD (P) H oxidase and antioxidant enzymes in diet-induced metabolic syndrome. *Metabolism, 55*(7), 928-934.

Rothblat, G. H., de la Llera-Moya, M., Favari, E., Yancey, P. G., & Kellner-Weibel, G. (2002). Cellular cholesterol flux studies: methodological considerations. *Atherosclerosis, 163*(1), 1-8. doi: S0021915001007134 [pii]

Rouslin, W., MacGee, J., Gupte, S., Wesselman, A., & Epps, D. (1982). Mitochondrial cholesterol content and membrane properties in porcine myocardial ischemia. *American Journal of Physiology-Heart and Circulatory Physiology, 242*(2), H254-H259.

Runhaar, J., Koes, B. W., Clockaerts, S., & Bierma-Zeinstra, S. M. A. (2011). A systematic review on changed biomechanics of lower extremities in obese individuals: a possible role in development of osteoarthritis. *Obesity Reviews, 12*(12), 1071-1082. doi: 10.1111/j.1467-789X.2011.00916.x

Ruocco, M. G., Maeda, S., Park, J. M., Lawrence, T., Hsu, L.-C., Cao, Y., . . . & Karin, M. (2005). IκB kinase (IKK) β, but not IKKα, is a critical mediator of osteoclast survival and is required for inflammation-induced bone loss. *The Journal of experimental medicine, 201*(10), 1677-1687.

Saikumar, P., Dong, Z., Mikhailov, V., Denton, M., Weinberg, J. M., & Venkatachalam, M. A. (1999). Apoptosis: definition, mechanisms, and relevance to disease. *The American Journal of Medicine, 107*(5), 489-506.

Saleh, A. S., Najjar, S. S., Muller, D. C., Shetty, V., Ferrucci, L., Gelber, A. C., & Ling, S. M. (2007). Arterial stiffness and hand osteoarthritis: a novel relationship? *Osteoarthritis Cartilage, 15*(3), 357-361. doi: S1063-4584(06)00273-1 [pii] 10.1016/j.joca.2006.09.008

Salvesen, G. S., & Dixit, V. M. (1997). Caspases: intracellular signaling by proteolysis. *Cell, 91*(4), 443-446.

Sampath, H., & Ntambi, J. M. (2005). Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Annu. Rev. Nutr., 25*, 317-340.

Sanchez, C., Deberg, M. A., Bellahcene, A., Castronovo, V., Msika, P., Delcour, J. P., . . . & Henrotin, Y. E. (2008). Phenotypic characterization of osteoblasts from the sclerotic zones of osteoarthritic subchondral bone. *Arthritis & Rheumatism, 58*(2), 442-455.

Schnabel, M., Marlovits, S., Eckhoff, G., Fichtel, I., Gotzen, L., Vécsei, V., & Schlegel, J. (2002). Dedifferentiation-associated changes in morphology and gene expression in primary human articular chondrocytes in cell culture. *Osteoarthritis and Cartilage, 10*(1), 62-70. doi: http://dx.doi.org/10.1053/joca.2001.0482

Sandell, L. J., & Aigner, T. (2001). Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. *Arthritis Res, 3*(2), 107-113.

Sarwar, N., Danesh, J., Eiriksdottir, G., Sigurdsson, G., Wareham, N., Bingham, S., ... & Gudnason, V. (2007). Triglycerides and the risk of coronary heart disease 10 158 incident cases among 262 525 participants in 29 western prospective studies. *Circulation, 115*(4), 450-458.

Scanzello, C. R., Umoh, E., Pessler, F., Diaz-Torne, C., Miles, T., DiCarlo, E., . . . & Crow, M. K. (2009). Local cytokine profiles in knee osteoarthritis: elevated synovial fluid interleukin-15 differentiates early from end-stage disease. *Osteoarthritis and Cartilage, 17*(8), 1040-1048. doi: http://dx.doi.org/10.1016/j.joca.2009.02.011

Schroepfer, G. J. (2000). Oxysterols: modulators of cholesterol metabolism and other processes. *Physiological reviews, 80*(1), 361-554.

Schwenke, D. C., & St Clair, R. W. (1993). Influx, efflux, and accumulation of LDL in normal arterial areas and atherosclerotic lesions of white Carneau pigeons with naturally occurring and cholesterol-aggravated aortic atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology, 13*(9), 1368-1381.

Segal, N. A., Zimmerman, M. B., Brubaker, M., & Torner, J. C. (2011). Obesity and knee osteoarthritis are not associated with impaired quadriceps specific strength in adults. PM&R, 3(4), 314-323.

Sellam, J., & Berenbaum, F. (2013). Is osteoarthritis a metabolic disease? Joint Bone Spine, 80(6), 568-573. doi: http://dx.doi.org/10.1016/j.jbspin.2013.09.007

Seol, D., Yu, Y., Choe, H., Jang, K., Brouillette, M. J., Zheng, H., . . . & Martin, J. A. (2014). Effect of short-term enzymatic treatment on cell migration and cartilage regeneration: in vitro organ culture of bovine articular cartilage. Tissue Engineering Part A, 20(13-14), 1807-1814.

Sevin, G., Yasa, M., Akcay, D. Y., Kirkali, G., & Kerry, Z. (2013). Different responses of fluvastatin to cholesterol-induced oxidative modifications in rabbits: evidence for preventive effect against DNA damage. Cell biochemistry and function, 31(4), 325-332. doi: 10.1002/cbf.2903

Shingleton, W., Jones, D., Xu, X., Cawston, T., & Rowan, A. (2006). Retinoic acid and oncostatin M combine to promote cartilage degradation via matrix metalloproteinase-13 expression in bovine but not human chondrocytes. *Rheumatology*, 45(8), 958-965.

SILBERBERG, R., & SILBERBERG, M. (1950a). Growth and articular changes in slowly and rauidly developing mice fed a high-fat diet. *Growth*, 14, 213-230.

Silberberg, M., & Silberberg, R. (1950b). Effects of a high fat diet on the joints of aging mice. *Arch. Pathol.*, 50, 828-846.

Silberberg, M., & Silberberg, R. (1960). Osteoarthrosis in mice fed diets enriched with animal or vegetable fat. *Arch. Pathol.*, *70*, 385-390.

Simons, K., & Toomre, D. (2000). Lipid rafts and signal transduction. *Nature reviews Molecular cell biology*, *1*(1), 31-39.

Simopoulou, T., Malizos, K., & Tsezou, A. (2007). Lectin-like oxidized low density lipoprotein receptor 1 (LOX-1) expression in human articular chondrocytes. *Clinical and experimental rheumatology*, *25*(4), 605.

Simopoulou, T., Malizos, K. N., Iliopoulos, D., Stefanou, N., Papatheodorou, L., Ioannou, M., & Tsezou, A. (2007). Differential expression of leptin and leptin's receptor isoform (Ob-Rb) mRNA between advanced and minimally affected osteoarthritic cartilage; effect on cartilage metabolism. *Osteoarthritis and Cartilage*, *15*(8), 872-883. doi: http://dx.doi.org/10.1016/j.joca.2007.01.018

Simopoulou, T., Malizos, K. N., Poultsides, L., & Tsezou, A. (2010). Protective effect of atorvastatin in cultured osteoarthritic chondrocytes. *Journal of Orthopaedic Research*, *28*(1), 110-115. doi: 10.1002/jor.20953

Sjöstrand, F. S. (1956). The ultrastructure of cells as revealed by the electron microscope. *International Review of Cytology*, *5*, 455-533.

Smith, M., Barg, E., Weedon, H., Papengelis, V., Smeets, T., Tak, P., . . . & Ahern, M. (2003). Microarchitecture and protective mechanisms in synovial tissue from

clinically and arthroscopically normal knee joints. *Annals of the rheumatic diseases, 62*(4), 303-307.

Smith, M. D., Triantafillou, S., Parker, A., Youssef, P. P., & Coleman, M. (1997). Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. *The Journal of Rheumatology, 24*(2), 365-371.

Soccio, R. E., & Breslow, J. L. (2003). StAR-related lipid transfer (START) proteins: mediators of intracellular lipid metabolism. *Journal of Biological Chemistry, 278*(25), 22183-22186.

Sokoloff, L., Mickelsen, O., Silverstein, E., Jay, G. E., & Yamamoto, R. S. (1960). Experimental obesity and osteoarthritis. *American Journal of Physiology--Legacy Content, 198*(4), 765-770.

Soumian, S., Albrecht, C., Davies, A. H., & Gibbs, R. G. (2005). ABCA1 and atherosclerosis. *Vascular Medicine, 10*(2), 109-119.

Sowers, M., Karvonen-Gutierrez, C. A., Palmieri-Smith, R., Jacobson, J. A., Jiang, Y., & Ashton-Miller, J. A. (2009). Knee osteoarthritis in obese women with cardiometabolic clustering. *Arthritis Care & Research, 61*(10), 1328-1336.

Sowers, M. R., & Karvonen-Gutierrez, C. A. (2010). The evolving role of obesity in knee osteoarthritis. *Current Opinion in Rheumatology, 22*(5), 533.

Spector, T. D., Hart, D. J., & Doyle, D. V. (1994). Incidence and progression of osteoarthritis in women with unilateral knee disease in the general population: the effect of obesity. *Annals of the rheumatic diseases, 53*(9), 565-568.

Stemme, S., Faber, B., Holm, J., Wiklund, O., Witztum, J. L., & Hansson, G. K. (1995). T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proceedings of the National Academy of Sciences, 92*(9), 3893-3897. doi: 10.1073/pnas.92.9.3893

Stürmer, T., Sun, Y., Sauerland, S., Zeissig, I., Günther, K., Puhl, W., & Brenner, H. (1998). Serum cholesterol and osteoarthritis. The baseline examination of the Ulm Osteoarthritis Study. *The Journal of Rheumatology, 25*(9), 1827-1832.

Sturmer, T., Sun, Y., Sauerland, S., Zeissig, I., Gunther, K. P., Puhl, W., & Brenner, H. (1998). Serum cholesterol and osteoarthritis. The baseline examination of the Ulm Osteoarthritis Study. *The Journal of Rheumatology, 25*(9), 1827-1832.

Stürmer, T., Sun, Y., Sauerland, S., Zeissig, I., Günther, K. P., Puhl, W., & Brenner, H. (1998). Serum cholesterol and osteoarthritis. The baseline examination of the Ulm Osteoarthritis Study. *The Journal of Rheumatology, 25*(9), 1827-1832.

Sugii, S., Reid, P. C., Ohgami, N., Du, H., & Chang, T. Y. (2003). Distinct endosomal compartments in early trafficking of low density lipoprotein-derived cholesterol. *Journal of Biological Chemistry, 278*(29), 27180-27189.

Sun, Y., & Kandel, R. (1999). Deep zone articular chondrocytes in vitro express genes that show specific changes with mineralization. *Journal of Bone and Mineral Research, 14*(11), 1916-1925.

Susin, S. A., Lorenzo, H. K., Zamzami, N., Marzo, I., Snow, B. E., Brothers, G. M., . . . & Loeffler, M. (1999). Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature, 397*(6718), 441-446.

Tabas, I. (2002). Consequences of cellular cholesterol accumulation: basic concepts and physiological implications. *Journal of Clinical Investigation, 110*(7), 905-912.

Tall, A. R., Costet, P., & Wang, N. (2002). Regulation and mechanisms of macrophage cholesterol efflux. *Journal of Clinical Investigation, 110*(7), 899-904.

Tamakoshi, K., Yatsuya, H., Kondo, T., Hori, Y., Ishikawa, M., Zhang, H., . . . & Toyoshima, H. (2003). The metabolic syndrome is associated with elevated circulating C-reactive protein in healthy reference range, a systemic low-grade inflammatory state. *International journal of obesity, 27*(4), 443-449.

Teeple, E., Jay, G. D., Elsaid, K. A., & Fleming, B. C. (2013). Animal models of osteoarthritis: challenges of model selection and analysis. *The AAPS journal, 15*(2), 438-446.

Teichtahl, A. J., Wluka, A. E., Wang, Y., Hanna, F., English, D. R., Giles, G. G., & Cicuttini, F. M. (2009). Obesity and adiposity are associated with the rate of patella cartilage volume loss over 2 years in adults without knee osteoarthritis. *Annals of the rheumatic diseases, 68*(6), 909-913.

Teichtahl, A. J., Wang, Y., Wluka, A. E., & Cicuttini, F. M. (2008). Obesity and knee osteoarthritis: new insights provided by body composition studies. *Obesity, 16*(2), 232-240.

Tepper, S., & Hochberg, M. C. (1993). Factors associated with hip osteoarthritis: data from the first national health and nutrition examination survey (NHANES-1). *American Journal of Epidemiology, 137*(10), 1081-1088.

Tilg, H., & Moschen, A. R. (2006). Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nature Reviews Immunology, 6*(10), 772-783.

Toivanen, A. T., Heliövaara, M., Impivaara, O., Arokoski, J. P., Knekt, P., Lauren, H., & Kröger, H. (2010). Obesity, physically demanding work and traumatic knee injury are major risk factors for knee osteoarthritis—a population-based study with a follow-up of 22 years. *Rheumatology, 49*(2), 308-314.

Tremoulet, A. H. (2015). The role of statins in inflammatory vasculitides. *Autoimmunity, 48*(3), 177-180. doi: 10.3109/08916934.2015.1027818

Triantaphyllidou, I. E., Kalyvioti, E., Karavia, E., Lilis, I., Kypreos, K. E., & Papachristou, D. J. (2013). Perturbations in the HDL metabolic pathway predispose to the development of osteoarthritis in mice following long-term exposure to western-type diet. *Osteoarthritis and Cartilage, 21*(2), 322-330.

Truong, L. H., Kuliwaba, J. S., Tsangari, H., & Fazzalari, N. L. (2006). Differential gene expression of bone anabolic factors and trabecular bone architectural

changes in the proximal femoral shaft of primary hip osteoarthritis patients. *Arthritis Research & Therapy, 8*(6), R188.

Truong, T. Q., Aubin, D., Falstrault, L., Brodeur, M. R., & Brissette, L. (2010). SR-BI, CD36, and caveolin-1 contribute positively to cholesterol efflux in hepatic cells. *Cell biochemistry and function, 28*(6), 480-489. doi: 10.1002/cbf.1680

Tsezou, A., Iliopoulos, D., Malizos, K. N., & Simopoulou, T. (2010). Impaired expression of genes regulating cholesterol efflux in human osteoarthritic chondrocytes. *Journal of Orthopaedic Research, 28*(8), 1033-1039.

Tsezou, A., Iliopoulos, D., Malizos, K. N., & Simopoulou, T. (2010). Impaired expression of genes regulating cholesterol efflux in human osteoarthritic chondrocytes. *Journal of Orthopaedic Research, 28*(8), 1033-1039. doi: 10.1002/jor.21084

Turley, S. D., & Dietschy, J. M. (2003). Sterol absorption by the small intestine. *Current Opinion in Lipidology, 14*(3), 233-240.

Urbani, L., & Simoni, R. D. (1990). Cholesterol and vesicular stomatitis virus G protein take separate routes from the endoplasmic reticulum to the plasma membrane. *Journal of Biological Chemistry, 265*(4), 1919-1923.

Vakkilainen, J., Steiner, G., Ansquer, J. C., Aubin, F., Rattier, S., Foucher, C., . . . & Taskinen, M. R. (2003). Relationships Between Low-Density Lipoprotein Particle Size, Plasma Lipoproteins, and Progression of Coronary Artery Disease The Diabetes Atherosclerosis Intervention Study (DAIS). *Circulation, 107*(13), 1733-1737.

Van der Kraan, P. M., & van den Berg, W. B. (2000). Anabolic and destructive mediators in osteoarthritis. *Current Opinion in Clinical Nutrition & Metabolic Care, 3*(3), 205-211.

Van Eck, M., Twisk, J., Hoekstra, M., Van Rij, B. T., Van der Lans, C. A., Bos, I. S., . . . & Van Berkel, T. J. (2003). Differential effects of scavenger receptor BI deficiency on lipid metabolism in cells of the arterial wall and in the liver. *Journal of Biological Chemistry, 278*(26), 23699-23705. doi: 10.1074/jbc.M211233200 M211233200 [pii]

Vance, D., & Vance, J. (2002). Cholesterol biosynthesis. *Biochemistry of lipids, lipoproteins and membranes*, 399-421.

Velasquez, M. T., & Katz, J. D. (2010). Osteoarthritis: another component of metabolic syndrome? *Metabolic syndrome and related disorders, 8*(4), 295-305.

Vikram, N. K., Pandey, R. M., Misra, A., Sharma, R., Devi, J. R., & Khanna, N. (2003). Non-obese (body mass index< 25 kg/m 2) Asian Indians with normal waist circumference have high cardiovascular risk. *Nutrition, 19*(6), 503-509.

Vingård, E. (1991). Overweight predisposes to coxarthrosis: body-mass index studied in 239 males with hip arthroplasty. *Acta Orthopaedica, 62*(2), 106-109.

Vingård, E., Alfredsson, L., & Malchau, H. (1997). Lifestyle factors and hip arthrosis: a case referent study of body mass index, smoking and hormone therapy in 503 Swedish women. *Acta Orthopaedica, 68*(3), 216-220.

Vlad, S. C., Neogi, T., Aliabadi, P., Fontes, J. D., & Felson, D. T. (2011). No association between markers of inflammation and osteoarthritis of the hands and knees. *The Journal of rheumatology*, *38*(8), 1665-1670.

von der Mark, K., Gauss, V., von der Mark, H., & Müller, P. (1977). Relationship between cell shape and type of collagen synthesised as chondrocytes lose their cartilage phenotype in culture.

von Eckardstein, A., Huang, Y., Wu, S., Funke, H., Noseda, G., & Assmann, G. (1995). Reverse cholesterol transport in plasma of patients with different forms of familial HDL deficiency. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *15*(5), 691-703.

Wang, L., Park, P., La Marca, F., Than, K., Rahman, S., & Lin, C. Y. (2013). Bone formation induced by BMP-2 in human osteosarcoma cells. *International journal of oncology*, *43*(4), 1095-1102. doi: 10.3892/ijo.2013.2030

Wang, N., Silver, D. L., Costet, P., & Tall, A. R. (2000). Specific binding of ApoA-I, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. *Journal of Biological Chemistry*, *275*(42), 33053-33058. doi: 10.1074/jbc.M005438200M005438200 [pii]

Wang, W., Yang, L., & Huang, H. W. (2007). Evidence of cholesterol accumulated in high curvature regions: implication to the curvature elastic energy for lipid mixtures. *Biophysical journal*, *92*(8), 2819-2830.

Weisberg, S. P., Hunter, D., Huber, R., Lemieux, J., Slaymaker, S., Vaddi, K., . . . & Ferrante Jr, A. W. (2006). CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *Journal of Clinical Investigation*, *116*(1), 115.

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante Jr, A. W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation*, *112*(12), 1796.

Wojtanik, K. M., & Liscum, L. (2003). The transport of low density lipoprotein-derived cholesterol to the plasma membrane is defective in NPC1 cells. *Journal of Biological Chemistry*, *278*(17), 14850-14856.

Woolf, A., Breedveld, F., & Kvien, T. (2006). Controlling the obesity epidemic is important for maintaining musculoskeletal health. *Annals of the rheumatic diseases*, *65*(11), 1401-1402.

Wu, J., Liu, W., Bemis, A., Wang, E., Qiu, Y., Morris, E. A., . . . & Yang, Z. (2007). Comparative proteomic characterization of articular cartilage tissue from normal donors and patients with osteoarthritis. *Arthritis & Rheumatism*, *56*(11), 3675-3684.

Wüstner, D., Mondal, M., Tabas, I., & Maxfield, F. R. (2005). Direct observation of rapid internalization and intracellular transport of sterol by macrophage foam cells. *Traffic*, *6*(5), 396-412.

Xia, X., Jung, D., Webb, P., Zhang, A., Zhang, B., Li, L., . . . & Lesage, G. D. (2012). Liver X receptor beta and peroxisome proliferator-activated receptor

delta regulate cholesterol transport in murine cholangiocytes. *Hepatology, 56*(6), 2288-2296. doi: 10.1002/hep.25919

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., ... & Chen, H. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *The Journal of clinical investigation, 112*(12), 1821-1830.

Yamada, K., Healey, R., Amiel, D., Lotz, M., & Coutts, R. (2002). Subchondral bone of the human knee joint in aging and osteoarthritis. *Osteoarthritis and Cartilage, 10*(5), 360-369.

Yang, S., Kim, J., Ryu, J. H., Oh, H., Chun, C. H., Kim, B. J., . . . & Chun, J. S. (2010). Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. *Nature medicine, 16*(6), 687-693. doi: 10.1038/nm.2153

Yao, P. M., & Tabas, I. (2001). Free cholesterol loading of macrophages is associated with widespread mitochondrial dysfunction and activation of the mitochondrial apoptosis pathway. *Journal of Biological Chemistry, 276*(45), 42468-42476.

Yoshimura, N., Muraki, S., Oka, H., Tanaka, S., Kawaguchi, H., Nakamura, K., & Akune, T. (2012). Accumulation of metabolic risk factors such as overweight, hypertension, dyslipidaemia, and impaired glucose tolerance raises the risk of occurrence and progression of knee osteoarthritis: a 3-year follow-up of the ROAD study. *Osteoarthritis and Cartilage, 20*(11), 1217-1226. doi: http://dx.doi.org/10.1016/j.joca.2012.06.006

Yu, L., Bharadwaj, S., Brown, J. M., Ma, Y., Du, W., Davis, M. A., . . . & Rudel, L. L. (2006). Cholesterol-regulated translocation of NPC1L1 to the cell surface facilitates free cholesterol uptake. *Journal of Biological Chemistry, 281*(10), 6616-6624.

Yu, W., Gong, J. S., Ko, M., Garver, W. S., Yanagisawa, K., & Michikawa, M. (2005). Altered cholesterol metabolism in Niemann-Pick type C1 mouse brains affects mitochondrial function. *Journal of Biological Chemistry, 280*(12), 11731-11739.

Yudoh, K., & Karasawa, R. (2010). Statin prevents chondrocyte aging and degeneration of articular cartilage in osteoarthritis (OA). *Aging (Albany NY), 2*(12), 990.

Zamzami, N., Marchetti, P., Castedo, M., Zanin, C., Vayssiere, J. L., Petit, P. X., & Kroemer, G. (1995). Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death in vivo. *The Journal of experimental medicine, 181*(5), 1661-1672.

Zerega, B., Pagano, A., Pianezzi, A., Ulivi, V., Camardella, L., Cancedda, R., & Cancedda, F. D. (2004). Expression of serum amyloid A in chondrocytes and myoblasts differentiation and inflammation: possible role in cholesterol homeostasis. *Matrix Biol, 23*(1), 35-46. doi: 10.1016/j.matbio.2004.02.002S0945053X04000150 [pii]

Zhang, Y., Yu, C., Liu, J., Spencer, T. A., Chang, C. C., & Chang, T. Y. (2003). Cholesterol is superior to 7-ketocholesterol or 7α-hydroxycholesterol as an allosteric activator for acyl-coenzyme A: cholesterol acyltransferase 1. *Journal of Biological Chemistry, 278*(13), 11642-11647.

Zhao, X., Liu, Y., Zhong, Y., Liu, B., Yu, K., Shi, H., . . . & Zeng, Q. (2015). Atorvastatin Improves Inflammatory Response in Atherosclerosis by Upregulating the Expression of GARP. *Mediators Inflamm, 2015*, 841472. doi: 10.1155/2015/841472

Zhuo, Q., Yang, W., Chen, J., & Wang, Y. (2012). Metabolic syndrome meets osteoarthritis. *Nature Reviews Rheumatology, 8*(12), 729-737. doi: 10.1038/nrrheum.2012.135

Zhuo, Q., Yang, W., Chen, J., & Wang, Y. (2012). Metabolic syndrome meets osteoarthritis. *Nature Reviews Rheumatology, 8*(12), 729-737.

Acehan, D., Jiang, X., Morgan, D. G., Heuser, J. E., Wang, X., & Akey, C. W. (2002). Three-Dimensional Structure of the Apoptosome: Implications for Assembly, Procaspase-9 Binding, and Activation. *Molecular Cell, 9*(2), 423-432. doi: http://dx.doi.org/10.1016/S1097-2765(02)00442-2

Adams, J. M. (2003). Ways of dying: multiple pathways to apoptosis. *Genes & development, 17*(20), 2481-2495.

Adorni, M. P., Zimetti, F., Puntoni, M., Bigazzi, F., Sbrana, F., Minichilli, F., . . . Sampietro, T. (2012). Cellular cholesterol efflux and cholesterol loading capacity of serum: effects of LDL-apheresis. *J Lipid Res, 53*(5), 984-989. doi: jlr.P024810 [pii]
10.1194/jlr.P024810

Afara, I. O., Prasadam, I., Crawford, R., Xiao, Y., & Oloyede, A. (2013). Near infrared (NIR) absorption spectra correlates with subchondral bone micro-CT parameters in osteoarthritic rat models. *Bone, 53*(2), 350-357. doi: http://dx.doi.org/10.1016/j.bone.2012.12.042

Afonso, V., Champy, R., Mitrovic, D., Collin, P., & Lomri, A. (2007). Reactive oxygen species and superoxide dismutases: role in joint diseases. *Joint Bone Spine, 74*(4), 324-329. doi: S1297-319X(07)00159-5 [pii]
10.1016/j.jbspin.2007.02.002

Ai, M., Tanaka, A., & Schaefer, E. (2014). Apolipoprotein B-48: a unique marker of chylomicron metabolism. *Advances in clinical chemistry, 64*, 117.

Aiello, R. J., Brees, D., & Francone, O. L. (2003). ABCA1-Deficient Mice Insights Into the Role of Monocyte Lipid Efflux in HDL Formation and Inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology, 23*(6), 972-980.

Aigner, T., & Schmitz, N. (2011). Pathogenesis and pathology of osteoarthritis. *Rheumatology*, 1741-1759.

Akasaki, Y., Matsuda, S., & Iwamoto, Y. (2009). [Progress of research in osteoarthritis. The anti-inflammatory effects of intra-articular injected statin on experimental osteoarthritis]. *Clin Calcium, 19*(11), 1653-1662. doi: 091116531662 [pii]
CliCa091116531662
Akasaki, Y., Matsuda, S., Nakayama, K., Fukagawa, S., Miura, H., & Iwamoto, Y. (2009). Mevastatin reduces cartilage degradation in rabbit experimental osteoarthritis through inhibition of synovial inflammation. *Osteoarthritis Cartilage, 17*(2), 235-243. doi: S1063-4584(08)00210-0 [pii]
10.1016/j.joca.2008.06.012
Al-Arfaj, A. S. (2003). Radiographic osteoarthritis and serum cholesterol. *Saudi Med J, 24*(7), 745-747.
Alshehri, A. M. (2010). Metabolic syndrome and cardiovascular risk. *J Family Community Med, 17*(2), 73-78. doi: 10.4103/1319-1683.71987
Altman, R., Asch, E., Bloch, D., Bole, G., Borenstein, D., Brandt, K., . . . & Hochberg, M. . (1986). Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. *Arthritis & Rheumatism, 29*(8), 1039-1049.
Amin, S., Baker, K., Niu, J., Clancy, M., Goggins, J., Guermazi, A., . . . & Felson, D. T. . (2009). Quadriceps strength and the risk of cartilage loss and symptom progression in knee osteoarthritis. *Arthritis & Rheumatism, 60*(1), 189-198.
Ananth, L., Prete, P. E., & Kashyap, M. L. (1993). Apolipoproteins A-I and B and cholesterol in synovial fluid of patients with rheumatoid arthritis. *Metabolism, 42*(7), 803-806.
Andriacchi, T. P., Mündermann, A., Smith, R. L., Alexander, E. J., Dyrby, C. O., & Koo, S. (2004). A framework for the in vivo pathomechanics of osteoarthritis at the knee. *Annals of biomedical engineering, 32*(3), 447-457.
Andujar, C., Serrano, J., & Gomez-Zurita, J. (2012). Winding up the molecular clock in the genus Carabus (Coleoptera: Carabidae): assessment of methodological decisions on rate and node age estimation. *BMC Evol Biol, 12*, 40. doi: 10.1186/1471-2148-12-40
Arkill, K. P., & Winlove, C. P. (2006). Fatty acid transport in articular cartilage. *Arch Biochem Biophys, 456*(1), 71-78. doi: S0003-9861(06)00345-6 [pii]
10.1016/j.abb.2006.09.014
Arner, E. C. (2002). Aggrecanase-mediated cartilage degradation. *Current opinion in pharmacology, 2*(3), 322-329.
Arthritis research foundation. *Australia, www.arthritisfoundation.com.au*
Bagge, E., Bjelle, A., Eden, S., & Svanborg, A. (1991). Factors associated with radiographic osteoarthritis: results from the population study 70-year-old people in Goteborg. *J Rheumatol, 18*(8), 1218-1222.
Baigent, C., Landray, M. J., Reith, C., Emberson, J., Wheeler, D. C., Tomson, C., . . . Craig, J. (2011). The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. *The Lancet, 377*(9784), 2181-2192.
Balaban, R. S., Nemoto, S., & Finkel, T. (2005). Mitochondria, Oxidants, and Aging. *Cell, 120*(4), 483-495. doi: http://dx.doi.org/10.1016/j.cell.2005.02.001
Baliga, B., & Kumar, S. (2003). Apaf-1//cytochrome c apoptosome: an essential initiatorof caspase activation or just a sideshow? *Cell Death Differ, 10*(1), 16-18.

Bao, Q., & Shi, Y. (2007). Apoptosome: a platform for the activation of initiator caspases. *Cell Death & Differentiation, 14*(1), 56-65.

Barbier, O., Torra, I. P., Duguay, Y., Blanquart, C., Fruchart, J. C., Glineur, C., & Staels, B. (2002). Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis. *Arterioscler Thromb Vasc Biol, 22*(5), 717-726.

Bascoul-Colombo, C., Garaiova, I., Plummer, S. F., Harwood, J. L., Caterson, B., & Hughes, C. E. (2015). Glucosamine Hydrochloride but Not Chondroitin Sulfate Prevents Cartilage Degradation and Inflammation Induced by Interleukin-1α in Bovine Cartilage Explants. *Cartilage*, 1947603515603762.

Bastiaansen-Jenniskens, Y. M., Clockaerts, S., Feijt, C., Zuurmond, A. M., Stojanovic-Susulic, V., Bridts, C., . . . van Osch, G. J. (2012). Infrapatellar fat pad of patients with end-stage osteoarthritis inhibits catabolic mediators in cartilage. *Ann Rheum Dis, 71*(2), 288-294. doi: 10.1136/ard.2011.153858

Bendele, A. (2001). Animal models of osteoarthritis. *J Musculoskelet Neuronal Interact, 1*(4), 363-376.

Bendele, A. M., & Hulman, J. F. (1991). Effects of body weight restriction on the development and progression of spontaneous osteoarthritis in guinea pigs. *Arthritis & Rheumatism, 34*(9), 1180-1184.

Benito, M. J., Veale, D. J., FitzGerald, O., van den Berg, W. B., & Bresnihan, B. (2005). Synovial tissue inflammation in early and late osteoarthritis. *Ann Rheum Dis, 64*(9), 1263-1267. doi: 10.1136/ard.2004.025270

Benya, P. D., Padilla, S. R., & Nimni, M. E. (1978). Independent regulation of collagen types by chondrocytes during the loss of differentiated function in culture. *Cell, 15*(4), 1313-1321.

Bereiter-Hahn, J., & Vöth, M. (1994). Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. *Microscopy research and technique, 27*(3), 198-219.

Berenbaum, F. (2012). Diabetes-induced osteoarthritis: from a new paradigm to a new phenotype. *Postgraduate medical journal, 88*(1038), 240-242.

Bernardi, P. (1996). The permeability transition pore. Control points of a cyclosporin A-sensitive mitochondrial channel involved in cell death. *Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1275*(1–2), 5-9. doi: http://dx.doi.org/10.1016/0005-2728(96)00041-2

Bernardi, P., Krauskopf, A., Basso, E., Petronilli, V., Blalchy-Dyson, E., Di Lisa, F., & Forte, M. A. (2006). The mitochondrial permeability transition from in vitro artifact to disease target. *FEBS Journal, 273*(10), 2077-2099. doi: 10.1111/j.1742-4658.2006.05213.x

Bhandari, S., Gupta, P., Quinn, P., Sandhu, J., Hakimi, A., Jones, D., & Ng, L. (2015). Pleiotropic effects of statins in hypercholesterolaemia: a prospective observational study using a lipoproteomic based approach. *Lancet, 385 Suppl 1*, S21. doi: 10.1016/s0140-6736(15)60336-1

Bierma-Zeinstra, S. M., & Koes, B. W. (2007). Risk factors and prognostic factors of hip and knee osteoarthritis. *Nat Clin Pract Rheumatol, 3*(2), 78-85. doi: ncprheum0423 [pii]
10.1038/ncprheum0423

Blagojevic, M., Jinks, C., Jeffery, A., & Jordan, K. (2010). Risk factors for onset of osteoarthritis of the knee in older adults: a systematic review and meta-analysis. *Osteoarthritis and Cartilage, 18*(1), 24-33.

Blanchette-Mackie, E. J. (2000). Intracellular cholesterol trafficking: role of the NPC1 protein. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1486*(1), 171-183.

Blanco, F. J., Guitian, R., Vázquez-Martul, E., de Toro, F. J., & Galdo, F. (1998). Osteoarthritis chondrocytes die by apoptosis: A possible pathway for osteoarthritis pathology. *Arthritis & Rheumatism, 41*(2), 284-289. doi: 10.1002/1529-0131(199802)41:2<284::AID-ART12>3.0.CO;2-T

Blanco, F. J., López-Armada, M. J., & Maneiro, E. (2004). Mitochondrial dysfunction in osteoarthritis. *Mitochondrion, 4*(5–6), 715-728. doi: http://dx.doi.org/10.1016/j.mito.2004.07.022

Blanco, F. J., Rego, I., & Ruiz-Romero, C. (2011). The role of mitochondria in osteoarthritis. *Nat Rev Rheumatol, 7*(3), 161-169.

Bondeson, J. (2010). Activated synovial macrophages as targets for osteoarthritis drug therapy. *Curr Drug Targets, 11*(5), 576-585.

Bondeson, J., Wainwright, S. D., Lauder, S., Amos, N., & Hughes, C. E. (2006). The role of synovial macrophages and macrophage-produced cytokines in driving aggrecanases, matrix metalloproteinases, and other destructive and inflammatory responses in osteoarthritis. *Arthritis Research & Therapy, 8*(6), R187.

Bonora, E., Kiechl, S., Willeit, J., Oberhollenzer, F., Egger, G., Bonadonna, R., & Muggeo, M. (2003). Metabolic syndrome: epidemiology and more extensive phenotypic description. Cross-sectional data from the Bruneck Study. *International journal of obesity, 27*(10), 1283-1289.

Bosch, M., Marí, M., Herms, A., Fernández, A., Fajardo, A., Kassan, A., . . . Pol, A. (2011). Caveolin-1 Deficiency Causes Cholesterol-Dependent Mitochondrial Dysfunction and Apoptotic Susceptibility. *Current Biology, 21*(8), 681-686. doi: http://dx.doi.org/10.1016/j.cub.2011.03.030

Bossy-Wetzel, E., Newmeyer, D. D., & Green, D. R. (1998). Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarization. *The EMBO Journal, 17*(1), 37-49.

Brandt, K., Schauwecker, D., Dansereau, S., Meyer, J., O'Connor, B., & Myers, S. (1997). Bone scintigraphy in the canine cruciate deficiency model of osteoarthritis. Comparison of the unstable and contralateral knee. *The Journal of Rheumatology, 24*(1), 140-145.

Brandt, K. D., Dieppe, P., & Radin, E. L. (2008). Etiopathogenesis of osteoarthritis. *Rheumatic Disease Clinics of North America, 34*(3), 531-559.

Brown, M. S., & Goldstein, J. L. (1986). A receptor-mediated pathway for cholesterol homeostasis. *Science, 232*(4746), 34-47.

Brown, M. S., & Goldstein, J. L. (1999). A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proceedings of the National Academy of Sciences, 96*(20), 11041-11048.

Brown, M. S., Kovanen, P. T., & Goldstein, J. L. (1981). Regulation of plasma cholesterol by lipoprotein receptors. *Science, 212*(4495), 628-635.

Buckland-Wright, C. (2004). Subchondral bone changes in hand and knee osteoarthritis detected by radiography. *Osteoarthritis and Cartilage, 12, Supplement*, 10-19. doi: http://dx.doi.org/10.1016/j.joca.2003.09.007

Buckland-Wright, J., Lynch, J., & Dave, B. (2000). Early radiographic features in patients with anterior cruciate ligament rupture. *Annals of the rheumatic diseases, 59*(8), 641-646.

Buckland-Wright, J. C., Macfarlane, D. G., & Lynch, J. A. . (1992). Relationship between joint space width and subchondral sclerosis in the osteoarthritic hand: a quantitative microfocal radiographic study. *The Journal of Rheumatology, 19*(5), 788-795.

Buckwalter, J. A., & Mankin, H. J. . (1997). *Instructional Course Lectures, The American Academy of Orthopaedic Surgeons-Articular Cartilage. Part I: Tissue Design and Chondrocyte-Matrix Interactions*†.* (Vol. 79(4)): Journal Bone Joint Surgery.

Bultel, S., Helin, L., Clavey, V., Chinetti-Gbaguidi, G., Rigamonti, E., Colin, M., . . . Lestavel, S. (2008). Liver X receptor activation induces the uptake of cholesteryl esters from high density lipoproteins in primary human macrophages. *Arterioscler Thromb Vasc Biol, 28*(12), 2288-2295. doi: ATVBAHA.108.175042 [pii]
10.1161/ATVBAHA.108.175042

Burr, D. B. (1998). The importance of subchondral bone in osteoarthrosis. *Current Opinion in Rheumatology, 10*(3), 256-262.

Burr, D. B. (2004). Anatomy and physiology of the mineralized tissues: role in the pathogenesis of osteoarthrosis. *Osteoarthritis and Cartilage, 12*, 20-30.

Burr, D. B., & Gallant, M. A. (2012). Bone remodelling in osteoarthritis. *Nat Rev Rheumatol, 8*(11), 665-673.

Burr, D. B., & Radin, E. L. (2003). Microfractures and microcracks in subchondral bone: are they relevant to osteoarthrosis? *Rheum Dis Clin North Am, 29*(4), 675-685.

Cadenas, E., & Davies, K. J. A. (2000). Mitochondrial free radical generation, oxidative stress, and aging1. *Free Radical Biology and Medicine, 29*(3–4), 222-230. doi: http://dx.doi.org/10.1016/S0891-5849(00)00317-8

Carman, W. J., Sowers, M., Hawthorne, V. M., & Weissfeld, L. A. (1994). Obesity as a risk factor for osteoarthritis of the hand and wrist: a prospective study. *American Journal of Epidemiology, 139*(2), 119-129.

Caron, M. M. J., Emans, P. J., Coolsen, M. M. E., Voss, L., Surtel, D. A. M., Cremers, A., . . . Welting, T. J. M. (2012). Redifferentiation of dedifferentiated human articular chondrocytes: comparison of 2D and 3D cultures. *Osteoarthritis and Cartilage, 20*(10), 1170-1178. doi: http://dx.doi.org/10.1016/j.joca.2012.06.016

Castañeda, S., Roman-Blas, J. A., Largo, R., & Herrero-Beaumont, G. (2013). Osteoarthritis: a progressive disease with changing phenotypes. *Rheumatology*, ket247.

Cawston, T. E., & Wilson, A. J. (2006). Understanding the role of tissue degrading enzymes and their inhibitors in development and disease. *Best Practice & Research Clinical Rheumatology, 20*(5), 983-1002.

Chan, D. C. (2006). Mitochondrial fusion and fission in mammals. *Annu. Rev. Cell Dev. Biol., 22*, 79-99.

Chang, T. Y., Chang, C. C., & Cheng, D. . (1997). Acyl-coenzyme A: cholesterol acyltransferase. *Annual review of biochemistry, 66*, 613-638.

Chawla, A., Repa, J. J., Evans, R. M., & Mangelsdorf, D. J. (2001). Nuclear receptors and lipid physiology: opening the X-files. *Science, 294*(5548), 1866-1870.

Chen, F., Frenkel, S., & Di Cesare, P. (1999). Repair of articular cartilage defects: part I. Basic Science of cartilage healing. *American journal of orthopedics (Belle Mead, NJ), 28*(1), 31-33.

Chen, H., Detmer, S. A., Ewald, A. J., Griffin, E. E., Fraser, S. E., & Chan, D. C. (2003). Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *The Journal of cell biology, 160*(2), 189-200.

Chen, L. B. (1988). Mitochondrial membrane potential in living cells. *Annual review of cell biology, 4*(1), 155-181.

Cheung, H. S. (2005). Role of calcium-containing crystals in osteoarthritis. *Front Biosci, 10*, 1336-1340.

Choi, Y.-H., & Toyoda, Y. (1998). Cyclodextrin removes cholesterol from mouse sperm and induces capacitation in a protein-free medium. *Biology of reproduction, 59*(6), 1328-1333.

Chowdhury, T., Akanji, O., Salter, D., Bader, D., & Lee, D. (2008). Dynamic compression influences interleukin-1β-induced nitric oxide and prostaglandin E2 release by articular chondrocytes via alterations in iNOS and COX-2 expression. *Biorheology, 45*(3-4), 257-274.

Christian, A., Haynes, M., Phillips, M., & Rothblat, G. (1997). Use of cyclodextrins for manipulating cellular cholesterol content. *Journal of lipid research, 38*(11), 2264-2272.

Christie, W. W., & Han, X. (2010). Lipid analysis: isolation, separation, identification and lipidomic analysis. *Oily Press, an imprint of PJ Barnes & Associates, 24*, xviii, 428.

Chua, K., Aminuddin, B., Fuzina, N., & Ruszymah, B. (2005). Insulin-transferrin-selenium prevent human chondrocyte dedifferentiation and promote the formation of high quality tissue engineered human hyaline cartilage. *Eur Cell Mater, 9*(9), 58-67.

Clayton, P. T. (1998). Disorders of cholesterol biosynthesis. *Arch Dis Child, 78*, 185–189.

Clockaerts, S., Van Osch, G. J., Bastiaansen-Jenniskens, Y. M., Verhaar, J. A., Van Glabbeek, F., Van Meurs, J. B., . . . Bierma-Zeinstra, S. M. (2012). Statin use is associated with reduced incidence and progression of knee osteoarthritis in the Rotterdam study. *Ann Rheum Dis, 71*(5), 642-647. doi: annrheumdis-2011-200092 [pii]
10.1136/annrheumdis-2011-200092

Cohen-Solal, M., Funck-Brentano, T., & Hay, E. (2013). Animal models of osteoarthritis for the understanding of the bone contribution. *BoneKEy Rep, 2*. doi: 10.1038/bonekey.2013.156

Colell, A., García-Ruiz, C., Lluis, J. M., Coll, O., Mari, M., & Fernández-Checa, J. C. (2003). Cholesterol impairs the adenine nucleotide translocator-mediated mitochondrial permeability transition through altered membrane fluidity. *Journal of Biological Chemistry, 278*(36), 33928-33935.

Collins, S., Martin, T. L., Surwit, R. S., & Robidoux, J. (2004). Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. *Physiology & behavior, 81*(2), 243-248.

Collins, T. J., & Bootman, M. D. (2003). Mitochondria are morphologically heterogeneous within cells. *Journal of experimental biology, 206*(12), 1993-2000.

Conaghan, P. G., Vanharanta, H., & Dieppe, P. A. (2005). Is progressive osteoarthritis an atheromatous vascular disease? *Ann Rheum Dis, 64*(11), 1539-1541. doi: ard.2005.039263 [pii]
10.1136/ard.2005.039263

Cooper, C., Inskip, H., Croft, P., Campbell, L., Smith, G., McLearn, M., & Coggon, D. (1998). Individual risk factors for hip osteoarthritis: obesity, hip injury and physical activity. *American Journal of Epidemiology, 147*(6), 516-522.

Cooper, C., Snow, S., McAlindon, T. E., Kellingray, S., Stuart, B., Coggon, D., & Dieppe, P. A. (2000). Risk factors for the incidence and progression of radiographic knee osteoarthritis. *Arthritis & Rheumatism, 43*(5), 995.

Cooper, G. M. (2000). The Cell: A Molecular Approach. 2nd edition. *Sunderland (MA): Sinauer Associates*.

Costet, P., Lalanne, F., Gerbod-Giannone, M. C., Molina, J. R., Fu, X., Lund, E. G., . . . Tall, A. R. (2003). Retinoic acid receptor-mediated induction of ABCA1 in macrophages. *Molecular and cellular biology, 23*(21), 7756-7766.

Crain, R. C., Clark, R. W., & Harvey, B. E. (1983). Role of lipid transfer proteins in the abnormal lipid content of Morris hepatoma mitochondria and microsomes. *Cancer research, 43*(7), 3197-3202.

Crompton, M. (1999). The mitochondrial permeability transition pore and its role in cell death. *Biochemical journal, 341*(2), 233-249.

Dahaghin, S., Bierma-Zeinstra, S. M., Koes, B. W., Hazes, J. M., & Pols, H. A. (2007). Do metabolic factors add to the effect of overweight on hand osteoarthritis? The Rotterdam Study. *Ann Rheum Dis, 66*(7), 916-920. doi: ard.2005.045724 [pii]

10.1136/ard.2005.045724

Dahaghin, S., Bierma-Zeinstra, S. M., Koes, B. W., Hazes, J. M., & Pols, H. A. (2007). Do metabolic factors add to the effect of overweight on hand osteoarthritis? The Rotterdam Study. *Ann Rheum Dis, 66*, 916–920. doi: 10.1136/ard.2005.045724

Daniels, S. R., & Greer, F. R. (2008). Lipid screening and cardiovascular health in childhood. *Pediatrics, 122*(1), 198-208.

Davies-Tuck, M. L., Hanna, F., Davis, S. R., Bell, R. J., Davison, S. L., Wluka, A. E., . . . Cicuttini, F. M. (2009). Total cholesterol and triglycerides are associated with the development of new bone marrow lesions in asymptomatic middle-aged women—a prospective cohort study. *Arthritis Research and Therapy, 11*(6), 181-187.

Davies-Tuck, M. L., Hanna, F., Davis, S. R., Bell, R. J., Davison, S. L., Wluka, A. E., . . . Cicuttini, F. M. (2009). Total cholesterol and triglycerides are associated with the development of new bone marrow lesions in asymptomatic middle-aged women - a prospective cohort study. *Arthritis Res Ther, 11*(6), R181. doi: ar2873 [pii]

10.1186/ar2873

Davis, H. R., Zhu, L. J., Hoos, L. M., Tetzloff, G., Maguire, M., Liu, J., . . . & Lund, E. G. (2004). "Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis". *Journal of Biological Chemistry, 279*(32), 33586-33592.

Davis, M. A., Ettinger, W. H., & Neuhaus, J. M. (1988). The role of metabolic factors and blood pressure in the association of obesity with osteoarthritis of the knee. *J Rheumatol, 15*(12), 1827-1832.

Davis, M. A., Neuhaus, J. M., Ettinger, W. H., & Mueller, W. H. (1990). Body fat distribution and osteoarthritis. *American Journal of Epidemiology, 132*(4), 701-707.

DAY, C. (2007). Metabolic syndrome, or What you will: definitions and epidemiology. *Diabetes Vasc Dis Res, 4*, 32-38. doi: 10.3132/dvdr.2007.003

Day, J., Ding, M., Van der Linden, J., Hvid, I., Sumner, D., & Weinans, H. (2001). A decreased subchondral trabecular bone tissue elastic modulus is associated with pre-arthritic cartilage damage. *Journal of Orthopaedic Research, 19*(5), 914-918.

de Boer, T. N., van Spil, W. E., Huisman, A. M., Polak, A. A., Bijlsma, J. W. J., Lafeber, F. P. J. G., & Mastbergen, S. C. (2012). Serum adipokines in osteoarthritis; comparison with controls and relationship with local parameters of synovial inflammation and cartilage damage. *Osteoarthritis and Cartilage, 20*(8), 846-853. doi: http://dx.doi.org/10.1016/j.joca.2012.05.002

de Haart, M., Marijnissen, W. J., van Osch, G. J., & Verhaar, J. A. (1999). Optimization of chondrocyte expansion in culture: Effect of TGFβ-2, bFGF and L-ascorbic acid on bovine articular chondrocytes. *Acta Orthopaedica Scandinavica, 70*(1), 55-61.

de Munter, W., Blom, A. B., Helsen, M. M., Walgreen, B., van der Kraan, P. M., Joosten, L. A., . . . van Lent, P. L. (2013). Cholesterol accumulation caused by low density lipoprotein receptor deficiency or a cholesterol-rich diet results in ectopic bone formation during experimental osteoarthritis. *Arthritis Research & Therapy, 15*(6), R178.

de Seny, D., Cobraiville, G., Charlier, E., Neuville, S., Lutteri, L., Le Goff, C., . . . Malaise, M. G. (2015). Apolipoprotein-A1 as a damage-associated molecular patterns protein in osteoarthritis: ex vivo and in vitro pro-inflammatory properties. *PloS one, 10*(4), e0122904. doi: 10.1371/journal.pone.0122904

Dedrick, D., Goulet, R., Huston, L., Goldstein, S., & Bole, G. (1991). Early bone changes in experimental osteoarthritis using microscopic computed tomography. *The Journal of rheumatology. Supplement, 27*, 44-45.

Dedrick, D. K., Goldstein, S. A., Brandt, K., O'Connor, B., Goulet, R. W., & Albrecht, M. (1993). A longitudinal study of subchondral plate and trabecular bone in cruciate-deficient dogs with osteoarthritis followed up for 54 months. *Arthritis & Rheumatism, 36*(10), 1460-1467.

DeGrella, R., & Simoni, R. (1982). Intracellular transport of cholesterol to the plasma membrane. *Journal of Biological Chemistry, 257*(23), 14256-14262.

DeGroot, J. (2004). The AGE of the matrix: chemistry, consequence and cure. *Current opinion in pharmacology, 4*(3), 301-305.

Desroches, S., & Lamarche, B. (2007). The evolving definitions and increasing prevalence of the metabolic syndrome. *Applied Physiology, Nutrition, and Metabolism, 32*(1), 23-32.

Di, D., Wang, Z., Liu, Y., Luo, G., Shi, Y., Berggren-Soderlund, M., . . . Xu, N. (2012). ABCA1 upregulating apolipoproein M expression mediates via the RXR/LXR pathway in HepG2 cells. *Biochem Biophys Res Commun, 421*(1), 152-156. doi: S0006-291X(12)00658-4 [pii]
10.1016/j.bbrc.2012.04.022

Dietschy, J. M., & Turley, S. D. (2001). Cholesterol metabolism in the brain. *Current Opinion in Lipidology, 12*(2), 105-112.

Dikalova, A. E., Bikineyeva, A. T., Budzyn, K., Nazarewicz, R. R., McCann, L., Lewis, W., . . . Dikalov, S. I. (2010). Therapeutic targeting of mitochondrial superoxide in hypertension. *Circ Res, 107*(1), 106-116. doi: 10.1161/CIRCRESAHA.109.214601

Donovan, M., & Cotter, T. G. (2004). Control of mitochondrial integrity by Bcl-2 family members and caspase-independent cell death. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1644*(2), 133-147.

Doulabi, A. H., Mequanint, K., & Mohammadi, H. (2014). Blends and Nanocomposite Biomaterials for Articular Cartilage Tissue Engineering. *Materials, 7*, 5327-5355. doi: 10.3390/ma7075327

Driban, J. B., Sitler, M. R., Barbe, M. F., & Balasubramanian, E. (2010). Is osteoarthritis a heterogeneous disease that can be stratified into subsets? *Clinical rheumatology, 29*(2), 123-131.

Du, C., Fang, M., Li, Y., Li, L., & Wang, X. (2000). Smac, a mitochondrial protein that promotes cytochrome c–dependent caspase activation by eliminating IAP inhibition. *Cell, 102*(1), 33-42.

Ducy, P., Amling, M., Takeda, S., Priemel, M., Schilling, A. F., Beil, F. T., . . . Karsenty, G. (2000). Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell, 100*(2), 197-207.

Dumond, H., Presle, N., Terlain, B., Mainard, D., Loeuille, D., Netter, P., & Pottie, P. (2003). Evidence for a key role of leptin in osteoarthritis. *Arthritis & Rheumatism, 48*(11), 3118-3129.

Echegoyen, S., Oliva, E., Sepulveda, J., Diaz-Zagoya, J., Espinosa-Garcia, M., Pardo, J., & Martinez, F. (1993). Cholesterol increase in mitochondria: its effect on inner-membrane functions, submitochondrial localization and ultrastructural morphology. *Biochem. J, 289*, 703-708.

Erb, N., Pace, A. V., Douglas, K. M., Banks, M. J., & Kitas, G. D. (2004). Risk assessment for coronary heart disease in rheumatoid arthritis and osteoarthritis. *Scand J Rheumatol, 33*(5), 293-299. doi: 10.1080/03009740410006899

Eva Hurt-Camejo, U. O., Olov Wiklund, Göran Bondjers, Germán Camejo, & (1997). Cellular Consequences of the Association of ApoB Lipoproteins With Proteoglycans. *Arteriosclerosis, Thrombosis, and Vascular Biology, 17*, 1011-1017. doi: 10.1161/01.ATV.17.6.1011

Faggiotto, A., & Ross, R. (1984). Studies of hypercholesterolemia in the nonhuman primate. II. Fatty streak conversion to fibrous plaque. *Arteriosclerosis, 4*(4), 341-356.

Faggiotto, A., Ross, R., & Harker, L. (1984). Studies of hypercholesterolemia in the nonhuman primate. I. Changes that lead to fatty streak formation. *Arteriosclerosis, 4*(4), 323-340.

Felson, D. T. (1996). Weight and osteoarthritis. *The American journal of clinical nutrition, 63*(3), 430S-432S.

Felson, D. T. (2010). Identifying different osteoarthritis phenotypes through epidemiology. *Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society, 18*(5), 601.

Felson, D. T., & Chaisson, C. E. (1997). 2 Understanding the relationship between body weight and osteoarthritis. *Bailliere's clinical rheumatology, 11*(4), 671-681.

Felson, D. T., Chaisson, C. E., Hill, C. L., Totterman, S. M., Gale, M. E., Skinner, K. M., ... & Gale, D. R. (2001). The Association of Bone Marrow Lesions with Pain in Knee Osteoarthritis. *Annal International Medicine, 134*(7), 541-549. doi: 10.7326/0003-4819-134-7-200104030-00007

Felson, D. T., Zhang, Y., Hannan, M. T., Naimark, A., Weissman, B., Aliabadi, P., & Levy, D. (1997). Risk factors for incident radiographic knee osteoarthritis in the elderly. The Framingham Study. *Arthritis & Rheumatism, 40*(4), 728-733.

Feng, B., Yao, P. M., Li, Y., Devlin, C. M., Zhang, D., Harding, H. P., . . . Fisher, E. A. (2003). The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. *Nature cell biology, 5*(9), 781-792.

Feo, F., Canuto, R., Garcea, R., & Gabriel, L. (1975). Effect of cholesterol content on some physical and functional properties of mitochondria isolated from adult rat liver, fetal liver, cholesterol-enriched liver and hepatomas AH-130, 3924A and 5123. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 413*(1), 116-134.

Field, F. J., Born, E., Murthy, S., & Mathur, S. N. (1998). Transport of cholesterol from the endoplasmic reticulum to the plasma membrane is constitutive in CaCo-2 cells and differs from the transport of plasma membrane cholesterol to the endoplasmic reticulum. *Journal of lipid research, 39*(2), 333-343.

Fielding, C. J., & Fielding, P. E. (2001). Cellular cholesterol efflux. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1533*(3), 175-189.

Findlay, D. M. (2007). Vascular pathology and osteoarthritis. *Rheumatology (Oxford), 46*(12), 1763-1768. doi: kem191 [pii]
10.1093/rheumatology/kem191

Firestein, G. S., Budd, R., O'Dell, J. R., Gabriel, S. E., & McInnes, I. B. (2012). *Kelley's textbook of rheumatology*: Saunders.

Fleury, C., Mignotte, B., & Vayssière, J. L. (2002). Mitochondrial reactive oxygen species in cell death signaling. *Biochimie, 84*(2), 131-141.Fosslien, E. (2001). Mitochondrial medicine--molecular pathology of defective oxidative phosphorylation. *Ann Clin Lab Sci, 31*(1), 25-67.Freeman, M. A. R. (1973). *Adult articular cartilage*. London: Pitman.

Frey, T. G., & Mannella, C. A. (2000). The internal structure of mitochondria. *Trends in Biochemical Sciences, 25*(7), 319-324. doi: http://dx.doi.org/10.1016/S0968-0004(00)01609-1

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., . . . Shimomura, I. (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of Clinical Investigation, 114*(12), 1752-1761.

Galluzzi, L., Larochette, N., Zamzami, N., & Kroemer, G. (2006). Mitochondria as therapeutic targets for cancer chemotherapy. *Oncogene, 25*(34), 4812-4830.

Garcia-Calvo, M., Lisnock, J., Bull, H. G., Hawes, B. E., Burnett, D. A., Braun, M. P., . . . Detmers, P. A. (2005). The target of ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). *Proceedings of the National Academy of Sciences of the United States of America, 102*(23), 8132-8137.

Gavriilidis, C., Miwa, S., von Zglinicki, T., Taylor, R. W., & Young, D. A. (2013). Mitochondrial dysfunction in osteoarthritis is associated with down-regulation of superoxide dismutase 2. *Arthritis & Rheumatism, 65*(2), 378-387. doi: 10.1002/art.37782

Gegout, P. P., Francin, P.-J., Mainard, D., & Presle, N. (2008). Adipokines in osteoarthritis: friends or foes of cartilage homeostasis? *Joint Bone Spine, 75*(6), 669-671. doi: http://dx.doi.org/10.1016/j.jbspin.2008.07.008

Gelber, A. C., Hochberg, M. C., Mead, L. A., Wang, N.-Y., Wigley, F. M., & Klag, M. J. (1999). Body mass index in young men and the risk of subsequent knee and hip osteoarthritis. *The American Journal of Medicine, 107*(6), 542-548.

Gentili, C., Tutolo, G., Pianezzi, A., Cancedda, R., & Descalzi Cancedda, F. (2005). Cholesterol secretion and homeostasis in chondrocytes: a liver X receptor and retinoid X receptor heterodimer mediates apolipoprotein A1 expression. *Matrix Biol, 24*(1), 35-44. doi: S0945-053X(04)00157-X [pii]
10.1016/j.matbio.2004.12.003

Gevers, G., Dequeker, J., Martens, M., Van Audekercke, R., Nyssen-Behets, C., & Dhem, A. (1989). Biomechanical characteristics of iliac crest bone in elderly women according to osteoarthritis grade at the hand joints. *J Rheumatol, 16*(5), 660-663.

Ghadially, F. N., Mehta, P. N., & Kirkaldy-Willis, W. H. (1970). Ultrastructure of articular cartilage in experimentally produced lipoarthrosis. *J Bone Joint Surg Am, 52*(6), 1147-1158.

Gibson, B. W. (2005). The human mitochondrial proteome: oxidative stress, protein modifications and oxidative phosphorylation. *The International Journal of Biochemistry & Cell Biology, 37*(5), 927-934. doi: http://dx.doi.org/10.1016/j.biocel.2004.11.013

Gierman, L., Kühnast, S., Koudijs, A., Pieterman, E., Kloppenburg, M., van Osch, G., . . . Zuurmond, A.-M. (2014). Osteoarthritis development is induced by increased dietary cholesterol and can be inhibited by atorvastatin in APOE* 3Leiden. CETP mice—a translational model for atherosclerosis. *Annals of the rheumatic diseases, 73*(5), 921-927.

Gierman, L. M., Kuhnast, S., Koudijs, A., Pieterman, E. J., Kloppenburg, M., van Osch, G. J., . . . Zuurmond, A. M. (2013). Osteoarthritis development is induced by increased dietary cholesterol and can be inhibited by atorvastatin in APOE*3Leiden.CETP mice--a translational model for atherosclerosis. *Ann Rheum Dis*. doi: annrheumdis-2013-203248 [pii]
10.1136/annrheumdis-2013-203248

Gierman, L. M., van der Ham, F., Koudijs, A., Wielinga, P. Y., Kleemann, R., Kooistra, T., . . . Zuurmond, A. M. (2012). Metabolic stress–induced inflammation plays a major role in the development of osteoarthritis in mice. *Arthritis & Rheumatism, 64*(4), 1172-1181. doi: 10.1002/art.33443

Giltay, E., Hoogeveen, E., Elbers, J., Gooren, L., Asscheman, H., & Stehouwer, C. (1998). Insulin resistance is associated with elevated plasma total homocysteine levels in healthy, non-obese subjects. *Atherosclerosis, 139*(1), 197-198.

Glasson, S. S., Askew, R., Sheppard, B., Carito, B., Blanchet, T., Ma, H.-L., . . . Yang, Z. (2005). Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature, 434*(7033), 644-648.

Goker, B., Sumner, D. R., Hurwitz, D. E., & Block, J. A. (2000). Bone mineral density varies as a function of the rate of joint space narrowing in the hip. *The Journal of Rheumatology, 27*(3), 735-738.

Goldenberg, D. L., Egan, M. S., & Cohen, A. S. (1982). Inflammatory synovitis in degenerative joint disease. *J Rheumatol, 9*(2), 204-209.

Goldring, M. B. (2012). Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. *Therapeutic Advances in Musculoskeletal Disease, 4*(4), 269-285. doi: 10.1177/1759720X12448454

Goldring, M. B., & Goldring, S. R. (2010). Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. *Annals of the New York Academy of Sciences, 1192*(1), 230-237. doi: 10.1111/j.1749-6632.2009.05240.x
Goldring, M. B., & Marcu, K. B. (2009). Cartilage homeostasis in health and rheumatic diseases. *Arthritis Res Ther, 11*(3), 224.
Goldring, S. R. (2012). Cross-talk between subchondral bone and articular cartilage in osteoarthritis. *Arthritis Research & Therapy, 14*(2), 1-8.
Goldring, S. R., & Goldring, M. B. (2004). The role of cytokines in cartilage matrix degeneration in osteoarthritis. *Clinical orthopaedics and related research, 427*, S27-S36.
Goldstein, J. L., & Brown, M. S. (2001). The cholesterol quartet. *Science, 292*(5520), 1310.
Gomez, R., Conde, J., Scotece, M., Gomez-Reino, J. J., Lago, F., & Gualillo, O. (2011). What's new in our understanding of the role of adipokines in rheumatic diseases? *Nat Rev Rheumatol, 7*(9), 528-536.
Gordon, D. J., Probstfield, J. L., Garrison, R. J., Neaton, J. D., Castelli, W. P., Knoke, J. D., . . . Tyroler, H. A. (1989). High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation, 79*(1), 8-15.
Graf, G. A., Li, W.-P., Gerard, R. D., Gelissen, I., White, A., Cohen, J. C., & Hobbs, H. H. (2002). Coexpression of ATP-binding cassette proteins ABCG5 and ABCG8 permits their transport to the apical surface. *The Journal of Clinical Investigation, 110*(110 (5)), 659-669.
Green, D. R., & Reed, J. C. (1998). Mitochondria and Apoptosis. *Science, 281*(5381), 1309. doi: 10.1126/science.281.5381.1309
Gregor, M. F., & Hotamisligil, G. S. (2011). Inflammatory mechanisms in obesity. *Annu Rev Immunol, 29*, 415-445. doi: 10.1146/annurev-immunol-031210-101322
Griffin, T. M., Fermor, B., Huebner, J. L., Kraus, V. B., Rodriguiz, R. M., Wetsel, W. C., . . . Guilak, F. (2010). Research article Diet-induced obesity differentially regulates behavioral, biomechanical, and molecular risk factors for osteoarthritis in mice. *Arthritis Res Ther, 12*(4), R130.
Griffin, T. M., & Guilak, F. (2008). Why is obesity associated with osteoarthritis? Insights from mouse models of obesity. *Biorheology, 45*(3-4), 387.
Griffin, T. M., Huebner, J. L., Kraus, V. B., & Guilak, F. (2009). Extreme obesity due to impaired leptin signaling in mice does not cause knee osteoarthritis. *Arthritis & Rheumatism, 60*(10), 2935-2944.
Griffin, T. M., Huebner, J. L., Kraus, V. B., Yan, Z., & Guilak, F. (2012). Induction of osteoarthritis and metabolic inflammation by a very high-fat diet in mice: Effects of short-term exercise. *Arthritis & Rheumatism, 64*(2), 443-453.
Grotle, M., Hagen, K. B., Natvig, B., Dahl, F. A., & Kvien, T. K. (2008). Obesity and osteoarthritis in knee, hip and/or hand: an epidemiological study in the general population with 10 years follow-up. *BMC Musculoskeletal Disorders, 9*(1), 132.
Grotle, M., Hagen, K. B., Natvig, B., Dahl, F. A., & Kvien, T. K. (2008a). Obesity and osteoarthritis in knee, hip and/or hand: An epidemiological study in the general population with 10 years follow-up. *BMC Musculoskeletal Disorders, 9*. doi: 10.1186/1471-2474-9-132
Grotle, M., Hagen, K. B., Natvig, B., Dahl, F. A., & Kvien, T. K. (2008b). Obesity and osteoarthritis in knee, hip and/or hand: An epidemiological study in the

general population with 10 years follow-up. *BMC Musculoskeletal Disorders, 9*(1). doi: 10.1186/1471-2474-9-132
Grundy Md, P. S. M. (1998). Hypertriglyceridemia, Atherogenic Dyslipidemia, and the Metabolic Syndrome. *The American Journal of Cardiology, 81*(4, Supplement 1), 18B-25B. doi: http://dx.doi.org/10.1016/S0002-9149(98)00033-2
Guilak, F., Fermor, B., Keefe, F. J., Kraus, V. B., Olson, S. A., Pisetsky, D. S., . . . Weinberg, J. B. (2004). The role of biomechanics and inflammation in cartilage injury and repair. *Clinical orthopaedics and related research, 423*, 17-26.
Gushue, D. L., Houck, J., & Lerner, A. L. (2005). Effects of childhood obesity on three-dimensional knee joint biomechanics during walking. *Journal of Pediatric Orthopaedics, 25*(6), 763-768.
H Mabuchi, J. K., M Shimizu and R Takeda. (1989). Development of Coronary Heart Disease in Familial Hypercholesterolemia. *Circulation, 79*, 225-232. doi: 10.1161/01.CIR.79.2.225
Hall, E., Ren, S., Hylemon, P., Rodriguez-Agudo, D., Redford, K., Marques, D., . . . Pandak, W. (2005). Detection of the steroidogenic acute regulatory protein, StAR, in human liver cells. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1733*(2), 111-119.
Hamrick, M. W., Pennington, C., Newton, D., Xie, D., & Isales, C. (2004). Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. *Bone, 34*(3), 376-383. doi: http://dx.doi.org/10.1016/j.bone.2003.11.020
Hansson, G. K. (2005). Inflammation, atherosclerosis, and coronary artery disease. *New England Journal of Medicine, 352*(16), 1685-1695.
Hansson, G. K. (2005). Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med, 352*(16), 1685-1695. doi: 10.1056/NEJMra043430
Hanukoglu, I. (1992). Steroidogenic enzymes: Structure, function, and role in regulation of steroid hormone biosynthesis. *The Journal of Steroid Biochemistry and Molecular Biology, 43*(8), 779-804. doi: http://dx.doi.org/10.1016/0960-0760(92)90307-5
Harman, D. (1972). The Biologic Clock: The Mitochondria? *Journal of the American Geriatrics Society, 20*(4), 145-147. doi: 10.1111/j.1532-5415.1972.tb00787.x
Harris, M., & Thompson, C. (2000). The role of the Bcl-2 family in the regulation of outer mitochondrial membrane permeability. *Cell death and differentiation, 7*(12), 1182-1191.
Hart, D. J., Doyle, D. V., & Spector, T. D. (1995). Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. *J Rheumatol, 22*(6), 1118-1123.
Hart, D. J., Doyle, D. V., & Spector, T. D. (1995). Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. *The Journal of Rheumatology, 22*(6), 1118-1123.
Hartz, A. J., Fischer, M. E., Bril, G., Kelber, S., Rupley, D., Oken, B., & Rimm, A. A. (1986). The association of obesity with joint pain and osteoarthritis in the HANES data. *Journal of Chronic Diseases, 39*(4), 311-319.
Hatano, H., Maruo, A., Bolander, M. E., & Sarkar, G. (2003). Statin stimulates bone morphogenetic protein-2, aggrecan, and type 2 collagen gene expression and proteoglycan synthesis in rat chondrocytes. *Journal of orthopaedic science, 8*(6), 842-848.

Hattori, K., Ikeuchi, K., Morita, Y., & Takakura, Y. (2005). Quantitative ultrasonic assessment for detecting microscopic cartilage damage in osteoarthritis. *Arthritis Res Ther, 7*(1), R38-R46.

Havel, R. J. (1986). Functional activities of hepatic lipoprotein receptors. *Annual review of physiology, 48*(1), 119-134.

Hayami, T., Pickarski, M., Wesolowski, G. A., McLane, J., Bone, A., Destefano, J., . . . Duong, L. T. (2004). The role of subchondral bone remodeling in osteoarthritis: Reduction of cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate ligament transection model. *Arthritis & Rheumatism, 50*(4), 1193-1206. doi: 10.1002/art.20124

Hayami, T., Pickarski, M., Zhuo, Y., Wesolowski, G. A., Rodan, G. A., & Duong, L. T. (2006). Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. *Bone, 38*(2), 234-243. doi: http://dx.doi.org/10.1016/j.bone.2005.08.007

Haynes, M. K., Hume, E. L., & Smith, J. B. (2002). Phenotypic Characterization of Inflammatory Cells from Osteoarthritic Synovium and Synovial Fluids. *Clinical Immunology, 105*(3), 315-325. doi: http://dx.doi.org/10.1006/clim.2002.5283

Haywood, L., McWilliams, D., Pearson, C., Gill, S., Ganesan, A., Wilson, D., & Walsh, D. (2003). Inflammation and angiogenesis in osteoarthritis. *Arthritis & Rheumatism, 48*(8), 2173-2177.

Hebert, S. L., Lanza, I. R., & Nair, K. S. (2010). Mitochondrial DNA alterations and reduced mitochondrial function in aging. *Mechanisms of ageing and development, 131*(7), 451-462.

Hedbom, E., & Häuselmann, H. (2002). Molecular aspects of pathogenesis in osteoarthritis: the role of inflammation. *Cellular and Molecular Life Sciences CMLS, 59*(1), 45-53.

Hegde, R., Srinivasula, S. M., Zhang, Z., Wassell, R., Mukattash, R., Cilenti, L., . . . Fernandes-Alnemri, T. (2002). Identification of Omi/HtrA2 as a mitochondrial apoptotic serine protease that disrupts inhibitor of apoptosis protein-caspase interaction. *Journal of Biological Chemistry, 277*(1), 432-438.

Heike A. Wieland, M. M., Bernhard J. Kirschbaum & Karl A. Rudolphi. (2005). Osteoarthritis — an untreatable disease? *Nature Reviews Drug Discovery, 4*, 331-344. doi: 10.1038/nrd1693

Heliövaara, M., Mäkelä, M., Impivaara, O., Knekt, P., Aromaa, A., & Sievers, K. (1993). Association of overweight, trauma and workload with coxarthrosis: a health survey of 7,217 persons. *Acta Orthopaedica, 64*(5), 513-518.

Hendrich, L., Pons, J., Ribera, I., & Balke, M. (2010). Mitochondrial cox1 sequence data reliably uncover patterns of insect diversity but suffer from high lineage-idiosyncratic error rates. *PLoS ONE, 5*(12), e14448. doi: 10.1371/journal.pone.0014448

Herrero-Beaumont, G., Roman-Blas, J. A., Castañeda, S., & Jimenez, S. A. (2009). Primary Osteoarthritis No Longer Primary: Three Subsets with Distinct Etiological, Clinical, and Therapeutic Characteristics. *Seminars in Arthritis and Rheumatism, 39*(2), 71-80. doi: http://dx.doi.org/10.1016/j.semarthrit.2009.03.006

Hilal, G., Martel-Pelletier, J., Pelletier, J. P., Ranger, P., & Lajeunesse, D. (1998). Osteoblast-like cells from human subchondral osteoarthritic bone demonstrate an altered phenotype in vitro: possible role in subchondral bone sclerosis. *Arthritis Rheum, 41*(5), 891-899.

Hilal, G., Massicotte, F., Martel-Pelletier, J., Fernandes, J. C., Pelletier, J. P., & Lajeunesse, D. (2001). Endogenous prostaglandin E2 and insulin-like growth factor 1 can modulate the levels of parathyroid hormone receptor in human osteoarthritic osteoblasts. *J Bone Miner Res, 16*(4), 713-721.

Hochberg, M., Lethbridge-Cejku, M., Scott Jr, W., Reichle, R., Plato, C., & Tobin, J. (1995). The association of body weight, body fatness and body fat distribution with osteoarthritis of the knee: data from the Baltimore Longitudinal Study of Aging. *The Journal of Rheumatology, 22*(3), 488-493.

Hochberg, M. C., Lethbridge-Cejku, M., Scott, W. W., Plato, C. C., & Tobin, J. D. (1993). Obesity and osteoarthritis of the hands in women. *Osteoarthritis and Cartilage, 1*(2), 129-135.

Holliday, K., McWilliams, D., Maciewicz, R. A., Muir, K., Zhang, W., & Doherty, M. (2011). Lifetime body mass index, other anthropometric measures of obesity and risk of knee or hip osteoarthritis in the GOAL case-control study. *Osteoarthritis and Cartilage, 19*(1), 37-43.

Horton, J. D., Goldstein, J. L., & Brown, M. S. (2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *The Journal of Clinical Investigation, 109*(9), 1125-1131. doi: 10.1172/JCI15593

Howell, D. S. (2002). Articular cartilage calcification and matrix vesicles. *Current rheumatology reports, 4*(3), 265-269.

Hozoji-Inada, M., Munehira, Y., Nagao, K., Kioka, N., & Ueda, K. (2011). Liver X receptor beta (LXRbeta) interacts directly with ATP-binding cassette A1 (ABCA1) to promote high density lipoprotein formation during acute cholesterol accumulation. *J Biol Chem, 286*(22), 20117-20124. doi: M111.235846 [pii]

10.1074/jbc.M111.235846

Hsin-Chen, L., Pen-Hui, Y., Ching-You, L., Chin-Wen, C., & Yau-Huei, W. (2000). Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells. *Biochemical journal, 348*(2), 425-432.

Hudelmaier, M., Wirth, W., Nevitt, M., & Eckstein, F. (2013). Longitudinal rates of change in subchondral bone size in healthy knees and knees with radiographic osteoarthritis. *Osteoarthritis and Cartilage, 21*, S242.

Hui, D. Y., & Howles, P. N. (2005). *Molecular mechanisms of cholesterol absorption and transport in the intestine.* Paper presented at the Seminars in cell & developmental biology.

Hui, W., Barksby, H. E., Young, D. A., Cawston, T. E., Mckie, N., & Rowan, A. D. (2005). Oncostatin M in combination with tumour necrosis factor a induces a chondrocyte membrane associated aggrecanase that is distinct from ADAMTS aggrecanase-1 or -2. *Annal of rheumatic diseases, 64*(11), 1624–1632. doi: 10.1136/ard.2004.028191

Hulth A, L. L., Telhag H. (1970). Experimental osteoarthritis in rabbits. Preliminary report. *Acta Orthop Scand, 41*, 522-530.

Hunter, D. J., & Spector, T. D. (2003). The role of bone metabolism in osteoarthritis. *Current rheumatology reports, 5*(1), 15-19.

Iannone, F., & Lapadula, G. (2003). The pathophysiology of osteoarthritis. *Aging Clin Exp Res, 15*(5), 364-372.

Iannone, F., & Lapadula, G. (2008). Phenotype of chondrocytes in osteoarthritis. *Biorheology, 45*(3-4), 411-413.
IDF. International Diabetes Federation. *The IDF consensus worldwide definition of the metabolic syndrome, http://www.idf.org/metabolic-syndrome*.
Igney, F. H., & Krammer, P. H. (2002). Death and anti-death: tumour resistance to apoptosis. *Nature Reviews Cancer, 2*(4), 277-288.
Ikonen, E. (2006). Mechanisms for cellular cholesterol transport: defects and human disease. *Physiol Rev, 86*(4), 1237-1261. doi: 86/4/1237 [pii]
10.1152/physrev.00022.2005
Iliopoulos, D., Malizos, K. N., Oikonomou, P., & Tsezou, A. (2008). Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One, 3*(11), e3740.
Iwamoto, M., Kitagaki, J., Tamamura, Y., Gentili, C., Koyama, E., Enomoto, H., . . . Enomoto-Iwamoto, M. (2003). Runx2 expression and action in chondrocytes are regulated by retinoid signaling and parathyroid hormone-related peptide (PTHrP). *Osteoarthritis Cartilage, 11*(1), 6-15.
Jaeger, K., Selent, C., Jaehme, W., Mahr, S., Goebel, U., Ibrahim, S., . . . Mueller-Hilke, B. (2008). The genetics of osteoarthritis in STR/ort mice. *Osteoarthritis and Cartilage, 16*(5), 607-614.
Järvholm, B., Lewold, S., Malchau, H., & Vingård, E. (2005). Age, bodyweight, smoking habits and the risk of severe osteoarthritis in the hip and knee in men. *European journal of epidemiology, 20*(6), 537-542.
Jiang, Y., & Tuan, R. S. (2015). Origin and function of cartilage stem/progenitor cells in osteoarthritis. *Nature Reviews Rheumatology, 11*(4), 206-212.
Johnson, K., Svensson, C. I., Etten, D. V., Ghosh, S. S., Murphy, A. N., Powell, H. C., & Terkeltaub, R. (2004). Mediation of spontaneous knee osteoarthritis by progressive chondrocyte ATP depletion in Hartley guinea pigs. *Arthritis Rheum, 50*(4), 1216-1225. doi: 10.1002/art.20149
Kamata, H., Honda, S.I., Maeda, S., Chang, L., Hirata, H., & Karin, M. (2005). Reactive Oxygen Species Promote TNFα-Induced Death and Sustained JNK Activation by Inhibiting MAP Kinase Phosphatases. *Cell, 120*(5), 649-661. doi: http://dx.doi.org/10.1016/j.cell.2004.12.041
Karsdal, M., Bay-Jensen, A., Lories, R., Abramson, S., Spector, T., Pastoureau, P., . . . Goldring, S. (2013). The coupling of bone and cartilage turnover in osteoarthritis: opportunities for bone antiresorptives and anabolics as potential treatments? *Annals of the rheumatic diseases*, annrheumdis-2013-204111.
Katz, J. D., Agrawal, S., & Velasquez, M. (2010). Getting to the heart of the matter: osteoarthritis takes its place as part of the metabolic syndrome. *Curr Opin Rheumatol, 22*(5), 512-519. doi: 10.1097/BOR.0b013e32833bfb4b
Kelekar, A., & Thompson, C. B. (1998). Bcl-2-family proteins: the role of the BH3 domain in apoptosis. *Trends in cell biology, 8*(8), 324-330.
Kellgren, J. (1961). Osteoarthrosis in patients and populations. *British Medical Journal, 2*(5243), 1.
Kellner-Weibel, G., Geng, Y., & Rothblat, G. (1999). Cytotoxic cholesterol is generated by the hydrolysis of cytoplasmic cholesteryl ester and transported to the plasma membrane. *Atherosclerosis, 146*(2), 309-319.

Kerr, J. F., Wyllie, A. H., & Currie, A. R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *British journal of cancer, 26*(4), 239.

Khurana, S., Gupta, S., Bhalla, H., Nandwani, S., & Gupta, V. (2015). Comparison of anti-inflammatory effect of atorvastatin with rosuvastatin in patients of acute coronary syndrome. *J Pharmacol Pharmacother, 6*(3), 130-135. doi: 10.4103/0976-500x.162011

Kim, J. H., Lee, J. N., & Paik, Y. K. . (2001). Cholesterol Biosynthesis from Lanosterol. *The Journal of Biological Chemistry, 276*, 18153-18160. doi: 10.1074/jbc.M101661200

King, L. K., Birmingham, T. B., Kean, C. O., Jones, I. C., Bryant, D. M., & Giffin, J. R. (2008). Resistance training for medial compartment knee osteoarthritis and malalignment. *Medicine and science in sports and exercise, 40*(8), 1376-1384.

Kishida, Y., Hirao, M., Tamai, N., Nampei, A., Fujimoto, T., Nakase, T., . . . & Myoui, A. . (2005). Leptin regulates chondrocyte differentiation and matrix maturation during endochondral ossification. *Bone, 37*(5), 607-621.

Kolovou, G. D., Anagnostopoulou, K. K., Salpea, K. D., & Mikhailidis, D. P. (2007). The prevalence of metabolic syndrome in various populations. *The American journal of the medical sciences, 333*(6), 362-371.

Koskinen, J., Magnussen, C. G., Würtz, P., Soininen, P., Kangas, A. J., Viikari, J. S., . . . Ahotupa, M. (2012). Apolipoprotein B, oxidized low-density lipoprotein, and LDL particle size in predicting the incidence of metabolic syndrome: the Cardiovascular Risk in Young Finns study. *European journal of preventive cardiology, 19*(6), 1296-1303.

Kostopoulou, F., Gkretsi, V., Malizos, K. N., Iliopoulos, D., Oikonomou, P., Poultsides, L., & Tsezou, A. (2012). Central role of SREBP-2 in the pathogenesis of osteoarthritis. *PLoS One, 7*(5), e35753.

Krieger, M. (1999). Charting the fate of the "good cholesterol": identification and characterization of the high-density lipoprotein receptor SR-BI. *Annu Rev Biochem, 68*, 523-558. doi: 10.1146/annurev.biochem.68.1.523

Kroemer, G., Zamzami, N., & Susin, S. A. (1997). Mitochondrial control of apoptosis. *Immunology Today, 18*(1), 44-51. doi: http://dx.doi.org/10.1016/S0167-5699(97)80014-X

Laakmann, S., Auel, H., & Kochzius, M. (2012). Evolution in the deep sea: biological traits, ecology and phylogenetics of pelagic copepods. *Molecular phylogenetics and evolution, 65*(2), 535-546. doi: 10.1016/j.ympev.2012.07.007

Lajeunesse, D., Hilal, G., Pelletier, J. P., & Martel-Pelletier, J. (1999). Subchondral bone morphological and biochemical alterations in osteoarthritis. *Osteoarthritis Cartilage, 7*(3), 321-322.

Lajeunesse, D., & Reboul, P. (2003). Subchondral bone in osteoarthritis: a biologic link with articular cartilage leading to abnormal remodeling. *Curr Opin Rheumatol, 15*(5), 628-633.

Lakka, H. M., Laaksonen, D. E., Lakka, T. A., Niskanen, L. K., Kumpusalo, E., Tuomilehto, J., & Salonen, J. T. (2002). The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *Jama, 288*(21), 2709-2716. doi: 10.1001/jama.288.21.2709

Lane, N. E., & Nevitt, M. C. (2002). Osteoarthritis, bone mass, and fractures: how are they related? *Arthritis Rheum, 46*(1), 1-4.

Lange, Y., Ye, J., & Steck, T. L. (2004). How cholesterol homeostasis is regulated by plasma membrane cholesterol in excess of phospholipids. *Proceedings of the National Academy of Sciences of the United States of America, 101*(32), 11664-11667.

Lanza, I., & Nair, K. S. (2010). Mitochondrial function as a determinant of life span. *Pflügers Archiv - European Journal of Physiology, 459*(2), 277-289. doi: 10.1007/s00424-009-0724-5

Lawrence, J. S., Bremner, J. M., & Bier, F. (1966). Osteo-arthrosis. Prevalence in the population and relationship between symptoms and x-ray changes. *Ann Rheum Dis, 25*(1), 1-24.

Lee, H. C., & Wei, Y. H. (2012). Mitochondria and aging. *Adv Exp Med Biol, 942*, 311-327. doi: 10.1007/978-94-007-2869-1_14

Leung, B. P., Sattar, N., Crilly, A., Prach, M., McCarey, D. W., Payne, H., . . . Liew, F. Y. (2003). A novel anti-inflammatory role for simvastatin in inflammatory arthritis. *The Journal of Immunology, 170*(3), 1524-1530.

Levick, J. R., & McDonald, J. N. (1995). Fluid movement across synovium in healthy joints: role of synovial fluid macromolecules. *Ann Rheum Dis, 54*(5), 417-423.

Lewis, G. F., & Rader, D. J. (2005). New insights into the regulation of HDL metabolism and reverse cholesterol transport. *Circulation research, 96*(12), 1221-1232.

Liang, H. L., Sedlic, F., Bosnjak, Z., & Nilakantan, V. (2010). SOD1 and MitoTEMPO partially prevent mitochondrial permeability transition pore opening, necrosis, and mitochondrial apoptosis after ATP depletion recovery. Free Radical Biology and Medicine, 49(10), 1550-1560.

Li, B., & Aspden, R. M. (1997). Mechanical and material properties of the subchondral bone plate from the femoral head of patients with osteoarthritis or osteoporosis. *Ann Rheum Dis, 56*(4), 247-254.

Li, C., Wang, W., Guo, X., Zhang, F., Ma, W., Zhang, Y., . . . Lammi, M. (2012). Pathways related to mitochondrial dysfunction in cartilage of endemic osteoarthritis patients in China. *Science China Life Sciences, 55*(12), 1057-1063. doi: 10.1007/s11427-012-4418-4

Li, H., Cybulsky, M. I., Gimbrone, M. A., Jr., & Libby, P. (1993). An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. *Arterioscler Thromb, 13*(2), 197-204.

Li, L. Y., Luo, X., & Wang, X. (2001). Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature, 412*(6842), 95-99.

Libby, P., Lichtman, A. H., & Hansson, G. K. (2013). Immune effector mechanisms implicated in atherosclerosis: from mice to humans. *Immunity, 38*(6), 1092-1104.

Libby, P., Ridker, P. M., & Hansson, G. K. (2011). Progress and challenges in translating the biology of atherosclerosis. *Nature, 473*(7347), 317-325. doi: 10.1038/nature10146

Libby, P., Ridker, P. M., & Maseri, A. (2002). Inflammation and atherosclerosis. *Circulation, 105*(9), 1135-1143.

Lim, S., & Barter, P. (2014). Antioxidant effects of statins in the management of cardiometabolic disorders. *J Atheroscler Thromb, 21*(10), 997-1010.

Lin, M. T., & Beal, M. F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature, 443*(7113), 787-795.
Lin, Z., Willers, C., Xu, J., & Zheng, M.-H. (2006). The chondrocyte: biology and clinical application. *Tissue engineering, 12*(7), 1971-1984.
Lindblad, S., & Hedfors, E. (1987). Arthroscopic and immunohistologic characterization of knee joint synovitis in osteoarthritis. *Arthritis Rheum, 30*(10), 1081-1088.
Linnane, A., Ozawa, T., Marzuki, S., & Tanaka, M. (1989). Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *The Lancet, 333*(8639), 642-645. doi: http://dx.doi.org/10.1016/S0140-6736(89)92145-4
Lippiello, L., Hall, D., & Mankin, H. J. (1977). Collagen synthesis in normal and osteoarthritic human cartilage. *Journal of Clinical Investigation, 59*(4), 593.
Lippiello, L., Walsh, T., & Fienhold, M. (1991). The association of lipid abnormalities with tissue pathology in human osteoarthritic articular cartilage. *Metabolism, 40*(6), 571-576.
Liscum, L. (2008). Chapter 14 - Cholesterol biosynthesis. In D. E. V. E. Vance (Ed.), *Biochemistry of Lipids, Lipoproteins and Membranes (Fifth Edition)* (pp. 399-XI). San Diego: Elsevier.
Lisignoli, G., Toneguzzi, S., Pozzi, C., Piacentini, A., Riccio, M., Ferruzzi, A., . . . Facchini, A. (1999). Proinflammatory cytokines and chemokine production and expression by human osteoblasts isolated from patients with rheumatoid arthritis and osteoarthritis. *J Rheumatol, 26*(4), 791-799.
Little, C. B., Hughes, C. E., Curtis, C. L., Janusz, M. J., Bohne, R., Wang-Weigand, S., . . . Flannery, C. R. (2002). Matrix metalloproteinases are involved in C-terminal and interglobular domain processing of cartilage aggrecan in late stage cartilage degradation. *Matrix Biology, 21*(3), 271-288.
Liu, J. T., Guo, X., Ma, W. J., Zhang, Y. G., Xu, P., Yao, J. F., & Bai, Y. D. (2010). Mitochondrial function is altered in articular chondrocytes of an endemic osteoarthritis, Kashin–Beck disease. *Osteoarthritis and Cartilage, 18*(9), 1218-1226. doi: http://dx.doi.org/10.1016/j.joca.2010.07.003
Liu, X., Kim, C. N., Yang, J., Jemmerson, R., & Wang, X. (1996). Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell, 86*(1), 147-157.
Liyanage, K. E., Burnett, J. R., Hooper, A. J., & van Bockxmeer, F. M. (2011). Familial hypercholesterolemia: epidemiology, Neolithic origins and modern geographic distribution. *Critical reviews in clinical laboratory sciences, 48*(1), 1-18.
Loeser, R. F., Yammani, R. R., Carlson, C. S., Chen, H., Cole, A., Im, H. J., . . . Yan, S. D. (2005). Articular chondrocytes express the receptor for advanced glycation end products: potential role in osteoarthritis. *Arthritis & Rheumatism, 52*(8), 2376-2385.
Loeuille, D., Chary-Valckenaere, I., Champigneulle, J., Rat, A. C., Toussaint, F., Pinzano-Watrin, A., . . . Gillet, P. (2005). Macroscopic and microscopic features of synovial membrane inflammation in the osteoarthritic knee: correlating magnetic resonance imaging findings with disease severity. *Arthritis Rheum, 52*(11), 3492-3501. doi: 10.1002/art.21373
Loeuille, D., Chary-Valckenaere, I., Champigneulle, J., Rat, A. C., Toussaint, F., Pinzano-Watrin, A., . . . Pourel, J. (2005). Macroscopic and microscopic features of synovial membrane inflammation in the osteoarthritic knee:

correlating magnetic resonance imaging findings with disease severity. *Arthritis & Rheumatism, 52*(11), 3492-3501.

Lohmander, L. S., de Verdier, M. G., Rollof, J., Nilsson, P. M., & Engström, G. (2009). Incidence of severe knee and hip osteoarthritis in relation to different measures of body mass: a population-based prospective cohort study. *Annals of the rheumatic diseases, 68*(4), 490-496.

Lohmander, L. S., de Verdier, M. G., Rollof, J., Nilsson, P. M., & Engström, G. (2009). Incidence of severe knee and hip osteoarthritis in relation to different measures of body mass: a population-based prospective cohort study. *Ann Rheum Dis, 68*, 490-496. doi: 10.1136/ard.2008.089748

Lordan, S., Mackrill, J. J., & O'Brien, N. M. (2009). Oxysterols and mechanisms of apoptotic signaling: implications in the pathology of degenerative diseases. *J Nutr Biochem, 20*(5), 321-336. doi: S0955-2863(09)00008-4 [pii]
10.1016/j.jnutbio.2009.01.001

Lowman, E. W. (1955). Osteoarthritis. *J Am Med Assoc, 157*(6), 487-488.

Lyons, T. J., Stoddart, R. W., McClure, S. F., & McClure, J. (2005). The tidemark of the chondro-osseous junction of the normal human knee joint. *J Mol Histol, 36*(3), 207-215.

MacGregor, A., Li, Q., Spector, T., & Williams, F. (2009). The genetic influence on radiographic osteoarthritis is site specific at the hand, hip and knee. *Rheumatology*, ken475.

Maly, M. R., Costigan, P. A., & Olney, S. J (2005). Contribution of Psychosocial and Mechanical Variables to Physical Performance Measures in Knee Osteoarthritis. *Physical Therapy, 85*, 13-18-1328.

Maneiro, E., de Andres, M., Martin, M., Lopez-Armada, M., Pinto, J., Fernandez-Sueiro, J., . . . Blanco, F. (2001). *Mitochondrial respiratory activity is altered in OA human articular chondrocytes: A new approach to understand OA.* Paper presented at the Arthritis and Rheumatism Conference.

Mankin, H. J., Buckwalter, J.A. (2000). *Articular cartilage structure, composition and function* (2 ed.): Rosemont: American Academy of Orthopaedic Surgeons.

Mankin, H. J., Dorfman, H., Lippiello, L., & ZARINS, A. (1971). Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. *The Journal of Bone & Joint Surgery, 53*(3), 523-537.

Manuel T. Velasquez , M. D., and James D. Katz , M.D. (2010). Osteoarthritis: Another Component of Metabolic Syndrome? *METABOLIC SYNDROME AND RELATED DISORDERS, 8*(4), 295–305. doi: 10.1089/met.2009.0110

Marcil, V., Delvin, E., Sane, A. T., Tremblay, A., & Levy, E. (2006). Oxidative stress influences cholesterol efflux in THP-1 macrophages: role of ATP-binding cassette A1 and nuclear factors. *Cardiovasc Res, 72*(3), 473-482. doi: S0008-6363(06)00399-3 [pii]
10.1016/j.cardiores.2006.08.024

Martin, K., Lethbridge-Cejku, M., Muller, D. C., Elahi, D., Andres, R., Tobin, J. D., & Hochberg, M. C. (1997). Metabolic correlates of obesity and radiographic features of knee osteoarthritis: data from the Baltimore Longitudinal Study of Aging. *J Rheumatol, 24*(4), 702-707.

Martin, R. (2007). Targeted bone remodeling involves BMU steering as well as activation. *Bone, 40*(6), 1574-1580.

Martins, I., Hone, E., Foster, J., Sünram-Lea, S., Gnjec, A., Fuller, S., . . . Martins, R. (2006). Apolipoprotein E, cholesterol metabolism, diabetes, and the

convergence of risk factors for Alzheimer's disease and cardiovascular disease. *Molecular psychiatry, 11*(8), 721-736.
Mason, R., Chambers, M., Flannelly, J., Gaffen, J., Dudhia, J., & Bayliss, M. (2001). The STR/ort mouse and its use as a model of osteoarthritis. *Osteoarthritis and Cartilage, 9*(2), 85-91.
Massicotte, F., Lajeunesse, D., Benderdour, M., Pelletier, J. P., Hilal, G., Duval, N., & Martel-Pelletier, J. (2002). Can altered production of interleukin-1beta, interleukin-6, transforming growth factor-beta and prostaglandin E(2) by isolated human subchondral osteoblasts identify two subgroups of osteoarthritic patients. *Osteoarthritis Cartilage, 10*(6), 491-500.
Matsuda, M., & Shimomura, I. (2013). Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. *Obesity Research & Clinical Practice, 7*(5), e330-e341.
Maxfield, F. R., & Wüstner, D. (2002). Intracellular cholesterol transport. *The Journal of Clinical Investigation, 110*(110 (7)), 891-898.
Meir, K. S., & Leitersdorf, E. (2004). Atherosclerosis in the Apolipoprotein E–Deficient Mouse A Decade of Progress. *Arteriosclerosis, Thrombosis, and Vascular Biology, 24*(6), 1006-1014.
Meireles, S., De Groote, F., Reeves, N., Verschueren, S., Maganaris, C., Luyten, F., & Jonkers, I. (2016). Knee contact forces are not altered in early knee osteoarthritis. *Gait & posture, 45*, 115-120.
Messier, S. P., Gutekunst, D. J., Davis, C., & DeVita, P. (2005). Weight loss reduces knee-joint loads in overweight and obese older adults with knee osteoarthritis. *Arthritis & Rheumatism, 52*(7), 2026-2032. doi: 10.1002/art.21139
Meunier, P., & Boivin, G. (1997). Bone mineral density reflects bone mass but also the degree of mineralization of bone: therapeutic implications. *Bone, 21*(5), 373-377.
Miller, L. M., Novatt, J. T., Hamerman, D., & Carlson, C. S. (2004). Alterations in mineral composition observed in osteoarthritic joints of cynomolgus monkeys. *Bone, 35*(2), 498-506.
Mitchell, P. (1979). Compartmentation and Communication in Living Systems. Ligand Conduction: a General Catalytic Principle in Chemical, Osmotic and Chemiosmotic Reaction Systems. *European Journal of Biochemistry, 95*(1), 1-20. doi: 10.1111/j.1432-1033.1979.tb12934.x
Montagne, K., Uchiyama, H., Furukawa, K.S., Ushida, T., 2014. Hydrostatic pressure decreases membrane fluidity and lipid desaturase expression in chondrocyte progenitor cells. Journal of biomechanics 47, 354-359.
Morgan, T. G., Rowan, A. D., Dickinson, S. C., Jones, D., Hollander, A. P., Deehan, D., & Cawston, T. E. (2006). Human nasal cartilage responds to oncostatin M in combination with interleukin 1 or tumour necrosis factor a by the release of collagen fragments via collagenases. *Ann Rheum Dis, 65*(2), 184–190. doi: 10.1136/ard.2004.033480
Moyer, R. F., Birmingham, T. B., Chesworth, B. M., Kean, C., & Giffin, J. R. (2010). Alignment, body mass and their interaction on dynamic knee joint load in patients with knee osteoarthritis. *Osteoarthritis and Cartilage, 18*(7), 888-893.
Mündermann, A., Dyrby, C. O., & Andriacchi, T. P. . (2005). Secondary gait changes in patients with medial compartment knee osteoarthritis: increased

load at the ankle, knee, and hip during walking. *Arthritis Rheum, 52*, 2835-2844.
Murphy, G., & Nagase, H. (2008). Reappraising metalloproteinases in rheumatoid arthritis and osteoarthritis: destruction or repair? *Nature Clinical Practice Rheumatology, 4*(2), 128-135.
Murphy, M. P., & Smith, R. A. (2007). Targeting antioxidants to mitochondria by conjugation to lipophilic cations. *Annu. Rev. Pharmacol. Toxicol., 47*, 629-656.
Mwale, F., Tchetina, E., Wu, C. W., & Poole, A. R. (2002). The Assembly and Remodeling of the Extracellular Matrix in the Growth Plate in Relationship to Mineral Deposition and Cellular Hypertrophy: An In Situ Study of Collagens II and IX and Proteoglycan. *Journal of Bone and Mineral Research, 17*(2), 275-283. doi: 10.1359/jbmr.2002.17.2.275
Myant, N. B. (1990). Chapter 2 - Cholesterol in Animal Tissues. In N. B. Myant (Ed.), *Cholesterol Metabolism, Ldl, and the Ldl Receptor* (pp. 4-8): Academic Press.
Nazarewicz, R. R., Dikalova, A., Bikineyeva, A., Ivanov, S., Kirilyuk, I. A., Grigor'ev, I. A., & Dikalov, S. I. (2013). Does scavenging of mitochondrial superoxide attenuate cancer prosurvival signaling pathways? *Antioxid Redox Signal, 19*(4), 344-349. doi: 10.1089/ars.2013.5185
Nechushtan, A., Smith, C. L., Lamensdorf, I., Yoon, S.-H., & Youle, R. J. (2001). Bax and Bak coalesce into novel mitochondria-associated clusters during apoptosis. *The Journal of cell biology, 153*(6), 1265-1276.
Neufeld, E. B. (1998). What the Niemann-Pick type C gene has taught us about cholesterol transport *Intracellular Cholesterol Trafficking* (pp. 93-107): Springer.
Neuhold, L. A., Killar, L., Zhao, W., Sung, M.-L. A., Warner, L., Kulik, J., . . . Meijers, T. (2001). Postnatal expression in hyaline cartilage of constitutively active human collagenase-3 (MMP-13) induces osteoarthritis in mice. *Journal of Clinical Investigation, 107*(1), 35.
Nezic, L., Skrbic, R., Dobric, S., Stojiljkovic, M. P., Jacevic, V., Satara, S. S., . . . Stojakovic, N. (2009). Simvastatin and indomethacin have similar anti-inflammatory activity in a rat model of acute local inflammation. *Basic Clin Pharmacol Toxicol, 104*(3), 185-191. doi: 10.1111/j.1742-7843.2008.00302.x
Nicholls, D., & Åkerman, K. (1982). Mitochondrial calcium transport. *Biochimica et Biophysica Acta (BBA) - Reviews on Bioenergetics, 683*(1), 57-88. doi: http://dx.doi.org/10.1016/0304-4173(82)90013-1
Nicholls, S. J., Tuzcu, E. M., Sipahi, I., Grasso, A. W., Schoenhagen, P., Hu, T., . . . Hazen, S. L. (2007). Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis. *Jama, 297*(5), 499-508.
Nissen, S. E., Nicholls, S. J., Sipahi, I., Libby, P., Raichlen, J. S., Ballantyne, C. M., . . . Tardif, J.-C. (2006). Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. *Jama, 295*(13), 1556-1565.
Okamoto, K., & Shaw, J. M. (2005). Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. *Annu. Rev. Genet., 39*, 503-536.
Oliveira, H. C., Cosso, R. G., Alberici, L. C., Maciel, E. N., Salerno, A. G., Dorighello, G. G., . . . Vercesi, A. E. (2005). Oxidative stress in atherosclerosis-prone mouse is due to low antioxidant capacity of mitochondria. *FASEB J, 19*(2), 278-280. doi: 04-2095fje [pii]

10.1096/fj.04-2095fje
Oliveria, S. A., Felson, D. T., Cirillo, P. A., Reed, J. I., & Walker, A. M. (1999). Body weight, body mass index, and incident symptomatic osteoarthritis of the hand, hip, and knee. *Epidemiology, 10*, 161-166.
Oliviero, F., Lo Nigro, A., Bernardi, D., Giunco, S., Baldo, G., Scanu, A., . . . Punzi, L. (2012). A comparative study of serum and synovial fluid lipoprotein levels in patients with various arthritides. *Clinica chimica acta; international journal of clinical chemistry, 413*(1-2), 303-307. doi: 10.1016/j.cca.2011.10.019
Oliviero, F., Sfriso, P., Baldo, G., Dayer, J. M., Giunco, S., Scanu, A., . . . Punzi, L. (2009). Apolipoprotein A-I and cholesterol in synovial fluid of patients with rheumatoid arthritis, psoriatic arthritis and osteoarthritis. *Clin Exp Rheumatol, 27*(1), 79-83. doi: 2561 [pii]
Oliviero, F., Sfriso, P., Baldo, G., Dayer, J. M., Giunco, S., Scanu, A., ... & Punzi, L. (2009). Apolipoprotein A-I and cholesterol in synovial fluid of patients with rheumatoid arthritis, psoriatic arthritis and osteoarthritis. *Clinical & Experimental Rheumatology, 27*(1), 79-83.
Oram, J. F. (2002). ATP-binding cassette transporter A1 and cholesterol trafficking. *Current Opinion in Lipidology, 13*(4), 373-381.
Ostalowska, A., Birkner, E., Wiecha, M., Kasperczyk, S., Kasperczyk, A., Kapolka, D., & Zon-Giebel, A. (2006). Lipid peroxidation and antioxidant enzymes in synovial fluid of patients with primary and secondary osteoarthritis of the knee joint. *Osteoarthritis Cartilage, 14*(2), 139-145. doi: S1063-4584(05)00235-9 [pii]
10.1016/j.joca.2005.08.009
Ouvrier, A., Cadet, R., Vernet, P., Laillet, B., Chardigny, J. M., Lobaccaro, J. M., . . . Saez, F. (2009). LXR and ABCA1 control cholesterol homeostasis in the proximal mouse epididymis in a cell-specific manner. *J Lipid Res, 50*(9), 1766-1775. doi: M800657-JLR200 [pii]
10.1194/jlr.M800657-JLR200
Pagler, T. A., Rhode, S., Neuhofer, A., Laggner, H., Strobl, W., Hinterndorfer, C., . . . Stangl, H. (2006). SR-BI-mediated high density lipoprotein (HDL) endocytosis leads to HDL resecretion facilitating cholesterol efflux. *J Biol Chem, 281*(16), 11193-11204. doi: M510261200 [pii]
10.1074/jbc.M510261200
Palade, G. E. (1952). The fine structure of mitochondria. *The Anatomical Record, 114*(3), 427-451. doi: 10.1002/ar.1091140304
Panini, H. R. a. S. R. (1993). Cholesterol biosynthesis *Current Opinion in Lipidology, 4*, 230-237.
Pastoureau, P., Leduc, S., Chomel, A., & De Ceuninck, F. (2003). Quantitative assessment of articular cartilage and subchondral bone histology in the meniscectomized guinea pig model of osteoarthritis. *Osteoarthritis and Cartilage, 11*(6), 412-423.
Pavlov, E. V., Priault, M., Pietkiewicz, D., Cheng, E. H.-Y., Antonsson, B., Manon, S., . . . Kinnally, K. W. (2001). A novel, high conductance channel of mitochondria linked to apoptosis in mammalian cells and Bax expression in yeast. *The Journal of cell biology, 155*(5), 725-732.
Pearson, G., Robinson, F., Beers Gibson, T., Xu, B.-e., Karandikar, M., Berman, K., & Cobb, M. H. (2001). Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions 1. *Endocrine reviews, 22*(2), 153-183.

Pelletier, J.-P., Boileau, C., Brunet, J., Boily, M., Lajeunesse, D., Reboul, P., . . . Martel-Pelletier, J. (2004). The inhibition of subchondral bone resorption in the early phase of experimental dog osteoarthritis by licofelone is associated with a reduction in the synthesis of MMP-13 and cathepsin K. *Bone, 34*(3), 527-538.

Perkins, G. A., & Frey, T. G. (2000). Recent structural insight into mitochondria gained by microscopy. *Micron, 31*(1), 97-111. doi: http://dx.doi.org/10.1016/S0968-4328(99)00065-7

Phillips, M. C. (1972). The physical state of phospholipids and cholesterol in monolayers, bilayers, and membranes. *Progress in surface and membrane science, 5*, 139-221.

Plump, A. S., Smith, J. D., Hayek, T., Aalto-Setälä, K., Walsh, A., Verstuyft, J. G., . . . Breslow, J. L. (1992). Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell, 71*(2), 343-353.

Pohl, A., Devaux, P. F., & Herrmann, A. (2005). Function of prokaryotic and eukaryotic ABC proteins in lipid transport. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1733*(1), 29-52.

Poole, A. R., Kobayashi, M., Yasuda, T., Laverty, S., Mwale, F., Kojima, T., ... & Tchetina, E. (2002). Type II collagen degradation and its regulation in articular cartilage in osteoarthritis. *Ann Rheum Dis, 61*. doi: 10.1136/ard.61.suppl_2.ii78

Poole, A. R., Kojima, T., Yasuda, T., Mwale, F., Kobayashi, M., & Laverty, S. (2001). Composition and structure of articular cartilage: a template for tissue repair. *Clin Orthop Relat Res*(391 Suppl), S26-33.

Poole, A. R., Rosenberg, L. C., Reiner, A., Ionescu, M., Bogoch, E., & Roughley, P. J. (1996). Contents and distributions of the proteoglycans decorin and biglycan in normal and osteoarthritic human articular cartilage. *Journal of Orthopaedic Research, 14*(5), 681-689. doi: 10.1002/jor.1100140502

Pottie, P., Presle, N., Terlain, B., Netter, P., Mainard, D., & Berenbaum, F. (2006). Obesity and osteoarthritis: more complex than predicted! *Annals of the rheumatic diseases, 65*(11), 1403-1405.

Prasadam, I., Crawford, R., & Xiao, Y. (2010). Cell interactions of osteoarthritic subchondral bone osteoblasts and articular chondrocytes aggravate MMP-2 and MMP-9 production through the mediation of ERK1/2 and JNK phosphorylation. *Cross-talk of subchondral bone osteoblasts and articular cartilage chondrocytes: a new insight in understanding osteoarthritis pathogenesis*, 118.

Prasadam, I., Mao, X., Shi, W., Crawford, R., & Xiao, Y. (2013). Combination of MEK-ERK inhibitor and hyaluronic acid has a synergistic effect on anti-hypertrophic and pro-chondrogenic activities in osteoarthritis treatment. *Journal of molecular medicine, 91*(3), 369-380. doi: 10.1007/s00109-012-0953-5

Prasadam, I., Mao, X., Shi, W., Crawford, R., & Xiao, Y. (2013). Combination of MEK-ERK inhibitor and hyaluronic acid has a synergistic effect on anti-hypertrophic and pro-chondrogenic activities in osteoarthritis treatment. *Journal of Molecular Medicine, 91*(3), 369-380.

Prasadam, I., Mao, X., Wang, Y., Shi, W., Crawford, R., & Xiao, Y. (2012). Inhibition of p38 pathway leads to OA-like changes in a rat animal model. *Rheumatology, 51*(5), 813-823.

Prasadam, I., Mao, X., Wang, Y., Shi, W., Crawford, R., & Xiao, Y. (2012). Inhibition of p38 pathway leads to OA-like changes in a rat animal model. *Rheumatology, 51*(5), 813-823. doi: 10.1093/rheumatology/ker360
Prasadam, I., van Gennip, S., Friis, T., Shi, W., Crawford, R., & Xiao, Y. (2010). ERK-1/2 and p38 in the regulation of hypertrophic changes of normal articular cartilage chondrocytes induced by osteoarthritic subchondral osteoblasts. *Arthritis & Rheumatism, 62*(5), 1349-1360.
Prasadam, I., Zhou, Y., Shi, W., Crawford, R., & Xiao, Y. (2014). Role of dentin matrix protein 1 in cartilage redifferentiation and osteoarthritis. *Rheumatology, 53*(12), 2280-2287. doi: 10.1093/rheumatology/keu262
Prasadam, I., Zhou, Y., Shi, W., Crawford, R., & Xiao, Y. (2014). Role of dentin matrix protein 1 in cartilage redifferentiation and osteoarthritis. *Rheumatology*, keu262.
Presle, N., Pottie, P., Dumond, H., Guillaume, C., Lapicque, F., Pallu, S., . . . Terlain, B. (2006). Differential distribution of adipokines between serum and synovial fluid in patients with osteoarthritis. Contribution of joint tissues to their articular production. *Osteoarthritis and Cartilage, 14*(7), 690-695. doi: http://dx.doi.org/10.1016/j.joca.2006.01.009
Prinz, W. (2002). *Cholesterol trafficking in the secretory and endocytic systems.* Paper presented at the Seminars in cell & developmental biology.
Pritchett, K., & Taft, R. (2006). The Mouse in Biomedical Research: Normative Biology, Husbandry, and Models. *Fox, JG., et al., editors, 3*, 91-121.
Pritzker, K. P. H., Gay, S., Jimenez, S. A., Ostergaard, K., Pelletier, J. P., Revell, P. A., . . . van den Berg, W. B. (2006). Osteoarthritis cartilage histopathology: grading and staging. *Osteoarthritis and Cartilage, 14*(1), 13-29. doi: http://dx.doi.org/10.1016/j.joca.2005.07.014
Pucadyil, T. J., & Chattopadhyay, A. (2004). Cholesterol modulates ligand binding and G-protein coupling to serotonin 1A receptors from bovine hippocampus. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1663*(1), 188-200.
Puddu, P., Puddu, G. M., Galletti, L., Cravero, E., & Muscari, A. (2005). Mitochondrial dysfunction as an initiating event in atherogenesis: a plausible hypothesis. *Cardiology, 103*(3), 137-141. doi: 83440 [pii]
10.1159/000083440
Puenpatom, R. A., & Victor, T. W. (2009). Increased prevalence of metabolic syndrome in individuals with osteoarthritis: an analysis of NHANES III data. *Postgrad Med, 121*(6), 9-20. doi: 10.3810/pgm.2009.11.2073
Puenpatom RA, V. T. (2009). Increased prevalence of metabolic syndrome in individuals with osteoarthritis: an analysis of NHANES III data. *Postgrad Med, 121*, 9-20. doi: 10.3810/pgm.2009.11.2073
Puglielli, L., Rigotti, A., Amigo, L., Nunez, L., Greco, A., Santos, M., & Nervi, F. (1996). Modulation of intrahepatic cholesterol trafficking: evidence by in vivo antisense treatment for the involvement of sterol carrier protein-2 in newly synthesized cholesterol transport into rat bile. *Biochem. J, 317*, 681-687.
Pullig, O., Weseloh, G., Gauer, S., & Swoboda, B. (2000). Osteopontin is expressed by adult human osteoarthritic chondrocytes: protein and mRNA analysis of normal and osteoarthritic cartilage. *Matrix Biol, 19*(3), 245-255.
Pullig, O., Weseloh, G., Ronneberger, D.-L., Käkönen, S.-M., & Swoboda, B. (2000). Chondrocyte differentiation in human osteoarthritis: expression of

osteocalcin in normal and osteoarthritic cartilage and bone. *Calcified Tissue International, 67*(3), 230-240.
Pullig, O., Weseloh, G., Ronneberger, D., Kakonen, S., & Swoboda, B. (2000). Chondrocyte differentiation in human osteoarthritis: expression of osteocalcin in normal and osteoarthritic cartilage and bone. *Calcif Tissue Int, 67*(3), 230-240.
Quasnichka, H. L., Anderson-MacKenzie, J. M., & Bailey, A. J. (2006). Subchondral bone and ligament changes precede cartilage degradation in guinea pig osteoarthritis. *Biorheology, 43*(3-4), 389-397.
Quasnichka, H. L., Anderson-MacKenzie, J. M., & Bailey, A. J. (2006). Subchondral bone and ligament changes precede cartilage degradation in guinea pig osteoarthritis. *Biorheology, 43*(3), 389-400.
Radin, E. L., & Rose, R. M. (1986). Role of subchondral bone in the initiation and progression of cartilage damage. *Clinical orthopaedics and related research, 213*, 34-40.
Rajamaki, K., Lappalainen, J., Oorni, K., Valimaki, E., Matikainen, S., Kovanen, P. T., & Eklund, K. K. (2010). Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. *PLoS One, 5*(7), e11765.
Ratcliffe, A., & Seibel, M. J. (1990). Biochemical markers of osteoarthritis. *Current Opinion in Rheumatology, 2*(5), 770-776.
Regan, E., Flannelly, J., Bowler, R., Tran, K., Nicks, M., Carbone, B. D., . . . Crapo, J. (2005). Extracellular superoxide dismutase and oxidant damage in osteoarthritis. *Arthritis Rheum, 52*(11), 3479-3491. doi: 10.1002/art.21387
Reijman, M., Pols, H., Bergink, A., Hazes, J., Belo, J., Lievense, A., & Bierma-Zeinstra, S. (2007). Body mass index associated with onset and progression of osteoarthritis of the knee but not of the hip: the Rotterdam Study. *Annals of the rheumatic diseases, 66*(2), 158-162.
Rejeski, W. J., Craven, T., Ettinger, W. H., McFarlane, M., & Shumaker, S. (1996). Self-efficacy and pain in disability with osteoarthritis of the knee. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences, 51*(1), P24-P29.
Rengel, Y., Ospelt, C., & Gay, S. (2007). Proteinases in the joint: clinical relevance of proteinases in joint destruction. *Arthritis Research and Therapy, 9*(5), 221.
Revell, P. A., Mayston, V., Lalor, P., & Mapp, P. (1988). The synovial membrane in osteoarthritis: a histological study including the characterisation of the cellular infiltrate present in inflammatory osteoarthritis using monoclonal antibodies. *Ann Rheum Dis, 47*(4), 300-307.
Richette, P., Poitou, C., Garnero, P., Vicaut, E., Bouillot, J.-L., Lacorte, J.-M., . . . Chevalier, X. (2011). Benefits of massive weight loss on symptoms, systemic inflammation and cartilage turnover in obese patients with knee osteoarthritis. *Annals of the rheumatic diseases, 70*(1), 139-144. doi: 10.1136/ard.2010.134015
Rinninger, F., Brundert, M., Budzinski, R. M., Fruchart, J. C., Greten, H., & Castro, G. R. (2003). Scavenger receptor BI (SR-BI) mediates a higher selective cholesteryl ester uptake from LpA-I compared with LpA-I:A-II lipoprotein particles. *Atherosclerosis, 166*(1), 31-40. doi: S0021915002003118 [pii]
Rizzuto, R., Simpson, A., Brini, M., & Pozzan, T. (1992). Rapid changes of mitochondrial Ca2+ revealed by specifically targeted recombinant aequorin. *Nature, 358*(6384), 325-327.

Roach, H., Aigner, T., Soder, S., Haag, J., & Welkerling, H. (2007). Pathobiology of osteoarthritis: pathomechanisms and potential therapeutic targets. *Current Drug Targets, 8*(2), 271-282.

Roberts, C. K., Barnard, R. J., Sindhu, R. K., Jurczak, M., Ehdaie, A., & Vaziri, N. D. (2006). Oxidative stress and dysregulation of NAD (P) H oxidase and antioxidant enzymes in diet-induced metabolic syndrome. *Metabolism, 55*(7), 928-934.

Rothblat, G. H., de la Llera-Moya, M., Favari, E., Yancey, P. G., & Kellner-Weibel, G. (2002). Cellular cholesterol flux studies: methodological considerations. *Atherosclerosis, 163*(1), 1-8. doi: S0021915001007134 [pii]

Rouslin, W., MacGee, J., Gupte, S., Wesselman, A., & Epps, D. (1982). Mitochondrial cholesterol content and membrane properties in porcine myocardial ischemia. *American Journal of Physiology-Heart and Circulatory Physiology, 242*(2), H254-H259.

Runhaar, J., Koes, B. W., Clockaerts, S., & Bierma-Zeinstra, S. M. A. (2011). A systematic review on changed biomechanics of lower extremities in obese individuals: a possible role in development of osteoarthritis. *Obesity Reviews, 12*(12), 1071-1082. doi: 10.1111/j.1467-789X.2011.00916.x

Ruocco, M. G., Maeda, S., Park, J. M., Lawrence, T., Hsu, L.-C., Cao, Y., . . . Karin, M. (2005). IκB kinase (IKK) β, but not IKKα, is a critical mediator of osteoclast survival and is required for inflammation-induced bone loss. *The Journal of experimental medicine, 201*(10), 1677-1687.

Saikumar, P., Dong, Z., Mikhailov, V., Denton, M., Weinberg, J. M., & Venkatachalam, M. A. (1999). Apoptosis: definition, mechanisms, and relevance to disease. *The American Journal of Medicine, 107*(5), 489-506.

Saleh, A. S., Najjar, S. S., Muller, D. C., Shetty, V., Ferrucci, L., Gelber, A. C., & Ling, S. M. (2007). Arterial stiffness and hand osteoarthritis: a novel relationship? *Osteoarthritis Cartilage, 15*(3), 357-361. doi: S1063-4584(06)00273-1 [pii]
10.1016/j.joca.2006.09.008

Salvesen, G. S., & Dixit, V. M. (1997). Caspases: intracellular signaling by proteolysis. *Cell, 91*(4), 443-446.

Sampath, H., & Ntambi, J. M. (2005). Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Annu. Rev. Nutr., 25*, 317-340.

Sanchez, C., Deberg, M. A., Bellahcene, A., Castronovo, V., Msika, P., Delcour, J. P., . . . Henrotin, Y. E. (2008). Phenotypic characterization of osteoblasts from the sclerotic zones of osteoarthritic subchondral bone. *Arthritis Rheum, 58*(2), 442-455.

Sandell, L. J., & Aigner, T. (2001). Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. *Arthritis Res, 3*(2), 107-113.

Sarwar, N., Danesh, J., Eiriksdottir, G., Sigurdsson, G., Wareham, N., Bingham, S., ... & Gudnason, V. (2007). Triglycerides and the Risk of Coronary Heart Disease. *Circulation, 115*(4), 450-458 doi: 10.1161/CIRCULATIONAHA.106.637793

Scanzello, C. R., Umoh, E., Pessler, F., Diaz-Torne, C., Miles, T., DiCarlo, E., . . . Crow, M. K. (2009). Local cytokine profiles in knee osteoarthritis: elevated synovial fluid interleukin-15 differentiates early from end-stage disease. *Osteoarthritis and Cartilage, 17*(8), 1040-1048. doi: http://dx.doi.org/10.1016/j.joca.2009.02.011

Schnabel, M., Marlovits, S., Eckhoff, G., Fichtel, I., Gotzen, L., Vécsei, V., & Schlegel, J. (2002). Dedifferentiation-associated changes in morphology and gene expression in primary human articular chondrocytes in cell culture. *Osteoarthritis and Cartilage, 10*(1), 62-70. doi: http://dx.doi.org/10.1053/joca.2001.0482

Schroepfer, G. J. (2000). Oxysterols: modulators of cholesterol metabolism and other processes. *Physiological reviews, 80*(1), 361-554.

Schwenke, D. C., & St Clair, R. W. (1993). Influx, efflux, and accumulation of LDL in normal arterial areas and atherosclerotic lesions of white Carneau pigeons with naturally occurring and cholesterol-aggravated aortic atherosclerosis. *Arterioscler Thromb, 13*(9), 1368-1381.

Segal, N. A., Zimmerman, M. B., Brubaker, M., & Torner, J. C. (2011). Obesity and knee osteoarthritis are not associated with impaired quadriceps specific strength in adults. *PM&R, 3*(4), 314-323.

Sellam, J., & Berenbaum, F. (2013). Is osteoarthritis a metabolic disease? *Joint Bone Spine, 80*(6), 568-573. doi: http://dx.doi.org/10.1016/j.jbspin.2013.09.007

Seol, D., Yu, Y., Choe, H., Jang, K., Brouillette, M. J., Zheng, H., . . . Martin, J. A. (2014). Effect of short-term enzymatic treatment on cell migration and cartilage regeneration: in vitro organ culture of bovine articular cartilage. *Tissue Engineering Part A, 20*(13-14), 1807-1814.

Sevin, G., Yasa, M., Akcay, D. Y., Kirkali, G., & Kerry, Z. (2013). Different responses of fluvastatin to cholesterol-induced oxidative modifications in rabbits: evidence for preventive effect against DNA damage. *Cell Biochem Funct, 31*(4), 325-332. doi: 10.1002/cbf.2903

Shingleton, W., Jones, D., Xu, X., Cawston, T., & Rowan, A. (2006). Retinoic acid and oncostatin M combine to promote cartilage degradation via matrix metalloproteinase-13 expression in bovine but not human chondrocytes. *Rheumatology, 45*(8), 958-965.

Silberberg, M., & Silberberg, R. (1950a). Effects of a high fat diet on the joints of aging mice. *AMA Arch Pathol, 50*, 828-846.

Silberberg, M., & Silberberg, R. (1950b). Effects of a high fat diet on the joints of aging mice. *Arch. Pathol., 50*, 828-846.

Silberberg, M., & Silberberg, R. (1960). Osteoarthrosis in mice fed diets enriched with animal or vegetable fat. *Pathol, 70*, 385-390.

Simons, K., & Toomre, D. (2000). Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol, 1*(1), 31-39.

Simopoulou, T., Malizos, K., & Tsezou, A. (2007). Lectin-like oxidized low density lipoprotein receptor 1 (LOX-1) expression in human articular chondrocytes. *Clinical and experimental rheumatology, 25*(4), 605.

Simopoulou, T., Malizos, K. N., Iliopoulos, D., Stefanou, N., Papatheodorou, L., Ioannou, M., & Tsezou, A. (2007). Differential expression of leptin and leptin's receptor isoform (Ob-Rb) mRNA between advanced and minimally affected osteoarthritic cartilage; effect on cartilage metabolism. *Osteoarthritis and Cartilage, 15*(8), 872-883. doi: http://dx.doi.org/10.1016/j.joca.2007.01.018

Simopoulou, T., Malizos, K. N., Poultsides, L., & Tsezou, A. (2010). Protective effect of atorvastatin in cultured osteoarthritic chondrocytes. *Journal of Orthopaedic Research, 28*(1), 110-115. doi: 10.1002/jor.20953

Sjöstrand, F. S. (1956). The ultrastructure of cells as revealed by the electron microscope. *International Review of Cytology, 5*, 455-533.

Smith, M., Barg, E., Weedon, H., Papengelis, V., Smeets, T., Tak, P., . . . Ahern, M. (2003). Microarchitecture and protective mechanisms in synovial tissue from clinically and arthroscopically normal knee joints. *Ann Rheum Dis, 62*(4), 303-307.

Smith, M. D., Triantafillou, S., Parker, A., Youssef, P. P., & Coleman, M. (1997). Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. *J Rheumatol, 24*(2), 365-371.

Soccio, R. E., & Breslow, J. L. (2003). StAR-related lipid transfer (START) proteins: mediators of intracellular lipid metabolism. *Journal of Biological Chemistry, 278*(25), 22183-22186.

Sokoloff, L., Mickelsen, O., Silverstein, E., Jay, G. E., & Yamamoto, R. S. (1960). Experimental obesity and osteoarthritis. *American Journal of Physiology--Legacy Content, 198*(4), 765-770.

Soumian, S., Albrecht, C., Davies, A. H., & Gibbs, R. G. (2005). ABCA1 and atherosclerosis. *Vasc Med, 10*(2), 109-119.

Sowers, M., Karvonen-Gutierrez, C. A., Palmieri-Smith, R., Jacobson, J. A., Jiang, Y., & Ashton-Miller, J. A. (2009). Knee osteoarthritis in obese women with cardiometabolic clustering. *Arthritis Care & Research, 61*(10), 1328-1336.

Sowers, M. R., & Karvonen-Gutierrez, C. A. (2010). The evolving role of obesity in knee osteoarthritis. *Current Opinion in Rheumatology, 22*(5), 533.

Spector, T. D., Hart, D. J., & Doyle, D. V. (1994). Incidence and progression of osteoarthritis in women with unilateral knee disease in the general population: the effect of obesity. *Annals of the rheumatic diseases, 53*(9), 565-568.

Stemme, S., Faber, B., Holm, J., Wiklund, O., Witztum, J. L., & Hansson, G. K. (1995). T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proceedings of the National Academy of Sciences, 92*(9), 3893-3897. doi: 10.1073/pnas.92.9.3893

Stéphane Pallu, P.-J. F., Cécile Guillaume, Pascale Gegout-Pottie, Patrick Netter, Didier Mainard, Bernard Terlain and Nathalie Preslecorresponding author2. (2010). Obesity affects the chondrocyte responsiveness to leptin in patients with osteoarthritis. *Arthritis Res Ther, 12*. doi: 10.1186/ar3048

Stürmer, T., Sun, Y., Sauerland, S., Zeissig, I., Günther, K., Puhl, W., & Brenner, H. (1998). Serum cholesterol and osteoarthritis. The baseline examination of the Ulm Osteoarthritis Study. *The Journal of rheumatology, 25*(9), 1827-1832.

Sturmer, T., Sun, Y., Sauerland, S., Zeissig, I., Gunther, K. P., Puhl, W., & Brenner, H. (1998). Serum cholesterol and osteoarthritis. The baseline examination of the Ulm Osteoarthritis Study. *J Rheumatol, 25*(9), 1827-1832.

Stürmer, T., Sun, Y., Sauerland, S., Zeissig, I., Günther, K. P., Puhl, W., & Brenner, H. (1998). Serum cholesterol and osteoarthritis. The baseline examination of the Ulm Osteoarthritis Study. *The Journal of Rheumatology, 25*, 1827-1832.

Sugii, S., Reid, P. C., Ohgami, N., Du, H., & Chang, T.-Y. (2003). Distinct endosomal compartments in early trafficking of low density lipoprotein-derived cholesterol. *Journal of Biological Chemistry, 278*(29), 27180-27189.

Sun, Y., & Kandel, R. (1999). Deep zone articular chondrocytes in vitro express genes that show specific changes with mineralization. *J Bone Miner Res, 14*(11), 1916-1925.

Susin, S. A., Lorenzo, H. K., Zamzami, N., Marzo, I., Snow, B. E., Brothers, G. M., . . . Loeffler, M. (1999). Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature, 397*(6718), 441-446.

Tabas, I. (2002). Consequences of cellular cholesterol accumulation: basic concepts and physiological implications. *Journal of Clinical Investigation, 110*(7), 905-912.

Tall, A. R., Costet, P., & Wang, N. (2002). Regulation and mechanisms of macrophage cholesterol efflux. *Journal of Clinical Investigation, 110*(7), 899-904.

Tamakoshi, K., Yatsuya, H., Kondo, T., Hori, Y., Ishikawa, M., Zhang, H., . . . Toyoshima, H. (2003). The metabolic syndrome is associated with elevated circulating C-reactive protein in healthy reference range, a systemic low-grade inflammatory state. *International journal of obesity, 27*(4), 443-449.

Teeple, E., Jay, G. D., Elsaid, K. A., & Fleming, B. C. (2013). Animal models of osteoarthritis: challenges of model selection and analysis. *The AAPS journal, 15*(2), 438-446.

Teichtahl, A. J., Wang, Y., Wluka, A. E., & Cicuttini, F. M. (2008). Obesity and knee osteoarthritis: new insights provided by body composition studies. *Obesity, 16*(2), 232-240.

Teichtahl, A. J., Wluka, A. E., Wang, Y., Hanna, F., English, D. R., Giles, G. G., & Cicuttini, F. M. (2009). Obesity and adiposity are associated with the rate of patella cartilage volume loss over 2 years in adults without knee osteoarthritis. *Ann Rheum Dis, 68*, 909-913. doi: 10.1136/ard.2008.093310

Tepper, S., & Hochberg, M. C. (1993). Factors associated with hip osteoarthritis: data from the first national health and nutrition examination survey (NHANES-1). *American Journal of Epidemiology, 137*(10), 1081-1088.

Tilg, H., & Moschen, A. R. (2006). Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nature Reviews Immunology, 6*(10), 772-783.

Toivanen, A. T., Heliövaara, M., Impivaara, O., Arokoski, J. P., Knekt, P., Lauren, H., & Kröger, H. (2010). Obesity, physically demanding work and traumatic knee injury are major risk factors for knee osteoarthritis—a population-based study with a follow-up of 22 years. *Rheumatology, 49*(2), 308-314.

Tremoulet, A. H. (2015). The role of statins in inflammatory vasculitides. *Autoimmunity, 48*(3), 177-180. doi: 10.3109/08916934.2015.1027818

Triantaphyllidou, I.-E., Kalyvioti, E., Karavia, E., Lilis, I., Kypreos, K. E., & Papachristou, D. J. (2013). Perturbations in the HDL metabolic pathway predispose to the development of osteoarthritis in mice following long-term exposure to western-type diet. *Osteoarthritis and Cartilage, 21*(2), 322-330.

Truong, L. H., Kuliwaba, J. S., Tsangari, H., & Fazzalari, N. L. (2006). Differential gene expression of bone anabolic factors and trabecular bone architectural changes in the proximal femoral shaft of primary hip osteoarthritis patients. *Arthritis Res Ther, 8*(6), R188.

Truong, T. Q., Aubin, D., Falstrault, L., Brodeur, M. R., & Brissette, L. (2010). SR-BI, CD36, and caveolin-1 contribute positively to cholesterol efflux in hepatic cells. *Cell Biochem Funct, 28*(6), 480-489. doi: 10.1002/cbf.1680

Tsezou, A., Iliopoulos, D., Malizos, K. N., & Simopoulou, T. (2010). Impaired expression of genes regulating cholesterol efflux in human osteoarthritic chondrocytes. *J Orthop Res, 28*(8), 1033-1039. doi: 10.1002/jor.21084

Tsezou, A., Iliopoulos, D., Malizos, K. N., & Simopoulou, T. (2010). Impaired expression of genes regulating cholesterol efflux in human osteoarthritic chondrocytes. *Journal of Orthopaedic Research, 28*(8), 1033-1039.

Turley, S. D., & Dietschy, J. M. (2003). Sterol absorption by the small intestine. *Current Opinion in Lipidology, 14*(3), 233-240.
Urbani, L., & Simoni, R. D. (1990). Cholesterol and vesicular stomatitis virus G protein take separate routes from the endoplasmic reticulum to the plasma membrane. *Journal of Biological Chemistry, 265*(4), 1919-1923.
Vakkilainen, J., Steiner, G., Ansquer, J.-C., Aubin, F., Rattier, S., Foucher, C., . . . Taskinen, M.-R. (2003). Relationships Between Low-Density Lipoprotein Particle Size, Plasma Lipoproteins, and Progression of Coronary Artery Disease The Diabetes Atherosclerosis Intervention Study (DAIS). *Circulation, 107*(13), 1733-1737.
Van der Kraan, P. M., & van den Berg, W. B. (2000). Anabolic and destructive mediators in osteoarthritis. *Current Opinion in Clinical Nutrition & Metabolic Care, 3*(3), 205-211.
Van Eck, M., Twisk, J., Hoekstra, M., Van Rij, B. T., Van der Lans, C. A., Bos, I. S., . . . Van Berkel, T. J. (2003). Differential effects of scavenger receptor BI deficiency on lipid metabolism in cells of the arterial wall and in the liver. *J Biol Chem, 278*(26), 23699-23705. doi: 10.1074/jbc.M211233200
M211233200 [pii]
Vance, D., & Vance, J. (2002). Cholesterol biosynthesis. *Biochemistry of lipids, lipoproteins and membranes*, 399-421.
Velasquez, M. T., & Katz, J. D. (2010). Osteoarthritis: another component of metabolic syndrome? *METABOLIC SYNDROME AND RELATED DISORDERS, 8*(4), 295-305.
Vikram, N. K., Pandey, R. M., Misra, A., Sharma, R., Devi, J. R., & Khanna, N. (2003). Non-obese (body mass index< 25 kg/m 2) Asian Indians with normal waist circumference have high cardiovascular risk. *Nutrition, 19*(6), 503-509.
Vingård, E. (1991). Overweight predisposes to coxarthrosis: body-mass index studied in 239 males with hip arthroplasty. *Acta Orthopaedica, 62*(2), 106-109.
Vingård, E., Alfredsson, L., & Malchau, H. (1997). Lifestyle factors and hip arthrosis: a case referent study of body mass index, smoking and hormone therapy in 503 Swedish women. *Acta Orthopaedica, 68*(3), 216-220.
Vlad, S. C., Neogi, T., Aliabadi, P., Fontes, J. D., & Felson, D. T. (2011). No association between markers of inflammation and osteoarthritis of the hands and knees. *J Rheumatol, 38*(8), 1665-1670.
von der Mark, K., Gauss, V., von der Mark, H., & Müller, P. (1977). Relationship between cell shape and type of collagen synthesised as chondrocytes lose their cartilage phenotype in culture.
von Eckardstein, A., Huang, Y., Wu, S., Funke, H., Noseda, G., & Assmann, G. (1995). Reverse cholesterol transport in plasma of patients with different forms of familial HDL deficiency. *Arteriosclerosis, Thrombosis, and Vascular Biology, 15*(5), 691-703.
Wang, L., Park, P., La Marca, F., Than, K., Rahman, S., & Lin, C. Y. (2013). Bone formation induced by BMP-2 in human osteosarcoma cells. *Int J Oncol, 43*(4), 1095-1102. doi: 10.3892/ijo.2013.2030
Wang, N., Silver, D. L., Costet, P., & Tall, A. R. (2000). Specific binding of ApoA-I, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. *J Biol Chem, 275*(42), 33053-33058. doi: 10.1074/jbc.M005438200
M005438200 [pii]

Wang, W., Yang, L., & Huang, H. W. (2007). Evidence of cholesterol accumulated in high curvature regions: implication to the curvature elastic energy for lipid mixtures. *Biophysical journal, 92*(8), 2819-2830.

Weisberg, S. P., Hunter, D., Huber, R., Lemieux, J., Slaymaker, S., Vaddi, K., . . . Ferrante Jr, A. W. (2006). CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *Journal of Clinical Investigation, 116*(1), 115.

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante Jr, A. W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation, 112*(12), 1796.

Wellsandt, E., Gardinier, E.S., Manal, K., Axe, M.J., Buchanan, T.S., Snyder-Mackler, L., 2016. Decreased Knee Joint Loading Associated With Early Knee Osteoarthritis After Anterior Cruciate Ligament Injury. The American journal of sports medicine 44, 143-151. Wojtanik, K. M., & Liscum, L. (2003). The transport of low density lipoprotein-derived cholesterol to the plasma membrane is defective in NPC1 cells. *Journal of Biological Chemistry, 278*(17), 14850-14856.

Wolff, K.J., Ramakrishnan, P.S., Brouillette, M.J., Journot, B., McKinley, T.O., Buckwalter, J.A., Martin, J.A., 2013. Mechanical Stress and ATP Synthesis are coupled by Mitochondrial Oxidants in Articular Cartilage. Journal of Orthopaedic Research 31, 191-196.

Woolf, A., Breedveld, F., & Kvien, T. (2006). Controlling the obesity epidemic is important for maintaining musculoskeletal health. *Annals of the rheumatic diseases, 65*(11), 1401-1402.

Wu, J., Liu, W., Bemis, A., Wang, E., Qiu, Y., Morris, E. A., . . . Yang, Z. (2007). Comparative proteomic characterization of articular cartilage tissue from normal donors and patients with osteoarthritis. *Arthritis & Rheumatism, 56*(11), 3675-3684.

Wüstner, D., Mondal, M., Tabas, I., & Maxfield, F. R. (2005). Direct observation of rapid internalization and intracellular transport of sterol by macrophage foam cells. *Traffic, 6*(5), 396-412.

Xia, X., Jung, D., Webb, P., Zhang, A., Zhang, B., Li, L., . . . Lesage, G. D. (2012). Liver X receptor beta and peroxisome proliferator-activated receptor delta regulate cholesterol transport in murine cholangiocytes. *Hepatology, 56*(6), 2288-2296. doi: 10.1002/hep.25919

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., ... & Chen, H. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *The Journal of clinical investigation,, 112*(12), 1821–1830. doi: 1821–1830

Yamada, K., Healey, R., Amiel, D., Lotz, M., & Coutts, R. (2002). Subchondral bone of the human knee joint in aging and osteoarthritis. *Osteoarthritis and Cartilage, 10*(5), 360-369.

Yang, S., Kim, J., Ryu, J. H., Oh, H., Chun, C. H., Kim, B. J., . . . Chun, J. S. (2010). Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. *Nat Med, 16*(6), 687-693. doi: 10.1038/nm.2153

Yao, P. M., & Tabas, I. (2001). Free cholesterol loading of macrophages is associated with widespread mitochondrial dysfunction and activation of the mitochondrial apoptosis pathway. *Journal of Biological Chemistry, 276*(45), 42468-42476.

Yoshimura, N., Muraki, S., Oka, H., Tanaka, S., Kawaguchi, H., Nakamura, K., & Akune, T. (2012). Accumulation of metabolic risk factors such as overweight, hypertension, dyslipidaemia, and impaired glucose tolerance raises the risk of occurrence and progression of knee osteoarthritis: a 3-year follow-up of the ROAD study. *Osteoarthritis and Cartilage, 20*(11), 1217-1226. doi: http://dx.doi.org/10.1016/j.joca.2012.06.006

Yu, L., Bharadwaj, S., Brown, J. M., Ma, Y., Du, W., Davis, M. A., . . . Rudel, L. L. (2006). Cholesterol-regulated translocation of NPC1L1 to the cell surface facilitates free cholesterol uptake. *Journal of Biological Chemistry, 281*(10), 6616-6624.

Yu, W., Gong, J.-S., Ko, M., Garver, W. S., Yanagisawa, K., & Michikawa, M. (2005). Altered cholesterol metabolism in Niemann-Pick type C1 mouse brains affects mitochondrial function. *Journal of Biological Chemistry, 280*(12), 11731-11739.

Yudoh, K., & Karasawa, R. (2010). Statin prevents chondrocyte aging and degeneration of articular cartilage in osteoarthritis (OA). *Aging (Albany NY), 2*(12), 990.

Zamzami, N., Marchetti, P., Castedo, M., Zanin, C., Vayssiere, J.-L., Petit, P. X., & Kroemer, G. (1995). Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death in vivo. *The Journal of experimental medicine, 181*(5), 1661-1672.

Zerega, B., Pagano, A., Pianezzi, A., Ulivi, V., Camardella, L., Cancedda, R., & Cancedda, F. D. (2004). Expression of serum amyloid A in chondrocytes and myoblasts differentiation and inflammation: possible role in cholesterol homeostasis. *Matrix Biol, 23*(1), 35-46. doi: 10.1016/j.matbio.2004.02.002 S0945053X04000150 [pii]

Zhang, Y., Yu, C., Liu, J., Spencer, T. A., Chang, C. C., & Chang, T.-Y. (2003). Cholesterol is superior to 7-ketocholesterol or 7α-hydroxycholesterol as an allosteric activator for acyl-coenzyme A: cholesterol acyltransferase 1. *Journal of Biological Chemistry, 278*(13), 11642-11647.

Zhao, X., Liu, Y., Zhong, Y., Liu, B., Yu, K., Shi, H., . . . Zeng, Q. (2015). Atorvastatin Improves Inflammatory Response in Atherosclerosis by Upregulating the Expression of GARP. *Mediators Inflamm, 2015*, 841472. doi: 10.1155/2015/841472

Zhuo, Q., Yang, W., Chen, J., & Wang, Y. (2012). Metabolic syndrome meets osteoarthritis. *Nat Rev Rheumatol, 8*(12), 729-737.

Zhuo, Q., Yang, W., Chen, J., & Wang, Y. (2012). Metabolic syndrome meets osteoarthritis. *Nat Rev Rheumatol, 8*(12), 729-737. doi: 10.1038/nrrheum.2012.135