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The effects of inflammation and anti-inflammatory treatment on lipid metabolism and liver morphology in a rheumatoid arthritis rat model

Tshimangadzo Moreblessings Denga

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree

of

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Declaration

I, Tshimangadzo Moreblessings Denga, declare that this dissertation is my own, except to the extent indicated in the contribution and acknowledgments sections. It is being submitted for the degree of Master of Science in Medicine in the School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. The work contained in this dissertation has not been submitted for any degree or examination in this or any other University.

I hereby certify that the studies contained in this dissertation have been approved by the Animal Ethics Research Committee, University of the Witwatersrand, Johannesburg. The animal ethics approval numbers are 2017/03/21/C and 2019/02/10C.



Tshimangadzo Moreblessings Denga

Signed on Wednesday of 20th of January 2021.



Dr Lebogang Mokotedi



Assoc Prof Aletta Millen

Abstract

High-grade inflammation plays a key role in the development of dyslipidaemia in rheumatoid arthritis (RA) patients. Biologic disease-modifying antirheumatic drugs (DMARDs), for example TNF-α inhibitors and IL-6 receptor blockers, are used to mitigate systemic inflammation. However, the effect of these DMARDs on lipid metabolism is inconsistent. Additionally, these drugs have been associated with liver dysfunction in RA, however, these mechanisms are unclear. This study aimed to determine the effect of inflammation and biologic DMARD treatments on lipid metabolism and liver morphology in the collagen-induced arthritis (CIA) rat model.

Three-month-old male (n=38) and female (n=30) Sprague-Dawley rats were randomly divided into the control (n=20), inflammation (n=18), TNF-α inhibitor (n=15) and IL-6 blocker (n=15) groups. To induce arthritis, bovine type-II collagen emulsified in incomplete Freund's adjuvant was injected at the base of the tail, in the inflammation, TNF-α inhibitor and IL-6 blocker groups. At the first signs of arthritis, rats in the TNF-α inhibitor group received 10mg/kg intraperitoneal injections of Etanercept (a TNF-α inhibitor), every third day for six weeks and rats in the IL-6 blocker group received 8mg/kg intraperitoneal injections of Tocilizumab (an IL-6 receptor blocker), once a week for six weeks. At termination, body weight, tail-cuff blood pressure, arthritis scores and paw thickness were assessed. Serum concentrations of glucose, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured. The TNF-α, IL-6, C-reactive protein (CRP), and ATP-binding cassette transporter A1 (ABCA1) serum concentrations were measured using enzyme-linked immunosorbent assays (ELISA). Liver lipid content was determined using the Soxhlet technique and liver steatosis was determined by histological haematoxylin and eosin (H&E) staining. Liver fibrosis was determined by histological Masson’s trichrome and picrosirius red staining. Liver enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured using the IDEXX colorimetric chemistry analyser. Group differences in serum lipid profile,

liver morphology and inflammatory markers were assessed using a two-way analysis of variance (ANOVA) with a Tukey *post-hoc* test. Associations between inflammatory markers with markers of liver fibrosis and liver toxicity were determined by Pearson's correlations.

There were no significant differences in body weight or tail-cuff blood pressure between the groups at termination (all $p>0.05$). The HDL-C concentrations were lower in the inflammation (6.16 ± 0.34μM; $p<0.0001$) and TNF-α inhibitor groups (7.07 ± 0.37μM; $p=0.0004$) compared to the control group (9.17 ± 0.32μM). The HDL-C concentrations were higher in the IL-6 blocker group (8.10 ± 0.43μM; $p=0.004$) compared to the inflammation group (6.16 ± 0.34μM). The ABCA1 concentrations were lower in the inflammation group (1.53 ± 0.26 ng/ml; $p=0.04$) compared to the control group (2.44 ± 0.22ng/ml). The ABCA1 concentrations were higher in the TNF-α inhibitor (3.00 ± 0.25 ng/ml; $p=0.0007$) and IL-6 blocker groups (3.28 ± 0.26 ng/ml; $p<0.0001$) compared to the inflammation group (1.53 ± 0.26 ng/ml; $p=0.04$). There were no differences in serum concentrations of glucose, triglycerides, LDL-C or markers of liver steatosis between the groups (all $p>0.05$). Liver lipid content was significantly lower in the inflammation (6.79 ± 0.40%; $p<0.0001$), TNF-α inhibitor (6.90 ± 0.40%; $p<0.0001$) and IL-6 blocker (8.03 ± 0.35%; $p=0.01$) groups compared to the control group (9.68 ± 0.36%). There were no significant differences in liver fibrosis or liver toxicity enzyme markers between the control and inflammation groups (all $p>0.05$). Markers of liver fibrosis were significantly higher in the TNF-α inhibitor (Masson's trichrome collagen area fraction: 2.81 ± 0.31%; <0.0001, picrosirius red collagen area fraction: 2.37 ± 0.23%; $p=0.03$) and IL-6 blocker (Masson's trichrome collagen area fraction: 3.04 ± 0.25%; $p<0.0001$, picrosirius red collagen area fraction: 2.91 ± 0.21%; $p<0.0001$) groups compared to the control group (Masson's trichrome collagen area fraction: 1.19 ± 0.12%, picrosirius red collagen area fraction: 1.51 ± 0.18%; $p=0.03$ and $p<0.0001$ respectively) and the inflammation group (Masson's trichrome collagen area fraction: 1.82 ± 0.24%, picrosirius red collagen area fraction: 1.95 ± 0.16%, $p=0.03$ and $p=0.004$ respectively). The serum concentrations of ALP were significantly higher in the TNF-α inhibitor (116 ± 5.81μl; $p=0.02$)

and IL-6 blocker (141.85 ± 9.38μL; p<0.0001) groups compared to the control group (87.16 ± 4.96μl). The serum concentrations of ALP were significantly higher in the IL-6 blocker group (141.85 ± 9.38μl) compared to the inflammation group (103.16 ± 6.97μl; p=0.001).

In conclusion, chronic inflammation impaired the serum concentrations of HDL-C, ABCA1 and liver lipid content in the CIA rat model. Although TNF-α inhibitor therapy improved the inflammation-induced changes in ABCA1, TNF-α inhibitors induced liver fibrosis and increased the ALP concentration. Despite improving the overall lipid profiles of rats exposed to CIA, IL-6 receptor blockers also induced liver fibrosis and liver toxicity. Although biologic anti-inflammatory drugs may ameliorate inflammation-induced alterations in lipid metabolism, the potential adverse effect on the liver highlights the need for further investigation.

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Statement of contribution to data collection and analysis

I declare that I designed this study in conjunction with my supervisors Professor AME Millen and Dr L Mokotedi. I was part of a team of researchers (including Prof Millen, Prof Michel, Dr Mokotedi, Dr Gunter, Ms Manilall) and postgraduate students responsible for collecting data for a larger study, of which this dissertation forms a part. I was responsible for collecting data for the main outcome variables in this dissertation, namely lipid profiles, ABCA1 measures, liver steatosis, liver fibrosis and liver enzymes. I performed the data analysis for this dissertation with the help of Dr Mokotedi and Prof Millen. I wrote this dissertation which was reviewed by Dr Mokotedi and Prof Millen.

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Abbreviations

ABCA1	ATP-binding cassette transporter A1
AIA	adjuvant-induced arthritis
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BP	blood pressure
BW	body weight
CAS	Central Animal Services
CIA	collagen-induced arthritis
CRP	C-reactive protein
CVD	cardiovascular disease
DBP	diastolic blood pressure
DMARDs	disease modifying anti-rheumatic drugs
eNOS	endothelial nitric oxide synthase
iNOS	inducible nitric oxide synthase
EDHF	endothelium-derived hyperpolarising factor
EDCF	endothelium-derived contracting factor
ELISA	enzyme-linked immunosorbent assay

ESR	erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
gp130	glycoprotein 130
HDL	high-density lipoprotein
H&E	haematoxylin and eosin
ICAM-1	intercellular adhesion molecule 1
IL-1β	interleukin 1 beta
IL-6	interleukin-6
IQR	interquartile range
Jak-STAT	Janus-activated kinase—signal transducer and activator of transcription
LDL	low-density lipoprotein
MTX	methotrexate
MMP	matrix metalloproteinase
mRNA	messenger RNA
NF-κB	nuclear factor kappa B
NO	nitric oxide
NIBP	non-invasive blood pressure
NSAID	non-steroidal anti-inflammatory drugs

RA	rheumatoid arthritis
ROS	reactive oxygen species
SBP	systolic blood pressure
SEM	standard error of the mean
TIMP	tissue-inhibitor of metalloproteinase
TC	total cholesterol
TG	triglycerides
TNF-α	tumour necrosis factor-alpha
TNFR1	tumor necrosis factor receptor 1
TNFR2	tumor necrosis factor receptor 2
USA	United States of America
VCAM-1	vascular cell adhesion molecule 1
VLDL	very low-density lipoprotein

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Chapter 1

Introduction

Cardiovascular disease (CVD) is a major health burden and a leading cause of death worldwide (Bansilal et al., 2015; Liu et al., 2019). CVD represents more than 50% of the non-communicable disease burden and contributes to a substantial portion of the global mortality rates (The Lancet Global Health, 2018). In 2015, an estimated 17.5 million CVD related deaths were reported, constituting approximately 31% of global deaths (Kapwata and Manda, 2018). In South Africa, deaths attributed to non-communicable diseases are equivalent to deaths as a consequence of human immunodeficiency virus and tuberculosis combined (Nojilana et al., 2016). In this regard, stroke and ischaemic heart disease account for approximately 19% of the total mortality rates in South Africa (Peer et al., 2018).

It is well known that traditional risk factors such as age, obesity, smoking, hypertension and dyslipidaemia contribute to CVD risk (Liao and Solomon, 2013). However, traditional risk factors do not fully account for all cardiovascular events (Zhang et al., 2014). Emerging evidence suggests that high-grade systemic inflammation plays a key role in the pathogenesis of CVD (García-Gómez et al., 2014; Mehta and Li, 1999). Irrespective of traditional risk factors, elevated circulating inflammatory cytokines have been associated with an increased risk for CVD (Bartunek and Vanderheyden, 2012; Kaptoge et al., 2010; Testa et al., 1996). Several circulating cytokines, including C-reactive protein (CRP), tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) have been associated with increased risk of myocardial infarction, stroke, heart failure, and cardiovascular mortality (Schumacher and Naga Prasad, 2018). Recently, it has been proposed that microvascular inflammation induced by comorbidities such as hypertension, diabetes mellitus and obesity provide the pathophysiological link between these disease states and subclinical CVD (Paulus and Tschöpe, 2013; Tschöpe and Van Linthout, 2014). Moreover, the increased CVD risk in chronic high-grade inflammatory conditions is well established (Choy and Sattar, 2009). In this regard, CVD is a major cause of morbidity and mortality in high-grade chronic inflammatory disorders such as systemic lupus erythematosus and rheumatoid arthritis (RA)

(Choy et al., 2014). Although the pathophysiological mechanisms leading to the increased CVD risk in these conditions are not fully understood, the heightened immune response is known to play a key role (Arida et al., 2018; Choy and Sattar, 2009). Consequently, the mechanisms whereby chronic inflammation contributes to increased CVD risk in RA is currently under investigation.

The complex interaction between traditional risk factors and chronic systemic inflammation in RA patients play a significant role in the increased CVD risk (Choy et al., 2014; Hurt-Camejo et al., 2001; Liao and Solomon, 2013; McFarlane et al., 2019; Popa et al., 2007b). In this regard, systemic inflammation has been linked to altered traditional CVD risk profiles in RA patients (Choy et al., 2014). Systemic inflammation causes changes in the body composition and metabolic profiles of RA patients compared to the general population (Urman et al., 2018). Furthermore, the association between traditional risk factors and CVD risk in RA is impacted by systemic inflammation (Choy et al., 2014). RA patients have an altered lipid profile, a phenomenon known as the “lipid paradox” where decreased total cholesterol (TC) and low-density lipoprotein (LDL) concentrations and increased high-density lipoprotein (HDL) concentrations are not necessarily associated with reduced CVD risk (McGrath and Young, 2015). Nevertheless, the presence of confounding factors, including pharmacological treatment, in clinical studies makes it difficult to ascertain the independent effect of inflammation on lipid metabolism in RA.

Traditionally, synthetic disease-modifying anti-rheumatic drugs (DMARDs), including methotrexate (MTX), have been used to manage RA (Gaffo et al., 2006; Sizova, 2008). However, synthetic DMARDs have failed to fully control disease activity in patients which has led to the development of biologic DMARDs. Biologic DMARDs have occasionally shown considerable benefits over synthetic DMARDs in controlling joint damage and disease progression (Ginosyan, 2019; Taylor, 2003). However, there are contradictory

findings on the effects of biologic DMARDs on CVD risk markers (Curtis et al., 2012; Daïen et al., 2012; McGrath and Young, 2015; Nurmohamed, 2007; Schimmel and Yazici, 2009; Souto et al., 2015; van den Oever et al., 2013). Recent studies showed improvements in the lipid profiles and cardiovascular outcomes of RA patients treated with biologic DMARDs (Bortoluzzi et al., 2018; Cacciapaglia et al., 2018; Gabay et al., 2016; Rein and Mueller, 2017). Despite the improvement in lipid profiles, some studies have reported liver injuries in patients treated with biologic DMARDs (Leise et al., 2014; Rigby et al., 2017; Weiler-Normann and Schramm, 2011). Because of several confounding factors in these clinical studies, it is uncertain whether the liver injuries in RA patients are a direct result of the systemic inflammation or the biologic DMARDs (Brass, 1993; Cacciapaglia et al., 2014; Chen et al., 2015; Pandit, 2012; Popa et al., 2007b; Rigby et al., 2017). Therefore, the independent effects of inflammation and biologic DMARDs on liver injuries warrant further investigation.

This dissertation begins with a literature review in chapter 2, which summarises the current knowledge in the literature on the effects of inflammation on CVD, specifically the risk for dyslipidaemia in RA. Chapter 2 will further highlight the effects of anti-inflammatory treatment on lipid metabolism in RA. Chapter 3 outlines the methodological procedures used and Chapter 4 presents the results obtained in the current study. Chapter 5 will discuss the findings in the context of the current literature and will highlight the conclusion and limitations of the current study.

Chapter 2

Literature review

2.1 Rheumatoid arthritis

Rheumatoid arthritis (RA) is the most common chronic, inflammatory arthritic disorder (Firestein and McInnes, 2017), affecting approximately 0.5-1% of people globally (García-Gómez et al., 2014; Srivastava et al., 2018). Data on the prevalence of RA in Africa is limited, largely because the healthcare systems in African countries are rudimentary (Usenbo et al., 2015). In South Africa, the prevalence of RA ranges from 0.07 to 2.5% (Usenbo et al., 2015). This large range in prevalence is largely ascribed to urbanisation and the resultant epidemiological transition in a developing nation (Elshafie et al., 2016).

RA is characterised by persistent inflammation of the synovium, leading to joint destruction, which can significantly affect the quality of life (Daffner and Watkins, 2014; Souto et al., 2015; Urman et al., 2018). Although RA is a synovial-joint inflammatory disease, it also causes chronic systemic inflammation that can affect other organ systems (Hasan et al., 2018; McFarlane et al., 2019; Ridgley et al., 2018). Extra-articular manifestations in RA are associated with severe disease activity and premature mortality (Cojocaru et al., 2010; McFarlane et al., 2019). In RA, the chronic inflammatory state is associated with several diseases, including CVD (Kitas and Gabriel, 2011), which may partly account for the increased risk of premature mortality in this population (Dessein et al., 2016; Pérez-Baos et al., 2017; Urman et al., 2018).

2.2 Cardiovascular disease in rheumatoid arthritis

Compared to the general population, RA patients experience double the risk of developing CVD (Ormseth and Stein, 2016; Zhang et al., 2014). Although traditional cardiovascular risk factors including age, obesity, hypertension, smoking and dyslipidaemia contribute to the increased risk of developing CVD in RA patients, they do not fully account for all cardiovascular events in RA (Crowson et al., 2018; Hameed and Al-Barzinjy, 2017; Liao and

Solomon, 2013; van den Oever et al., 2013). Chronic high-grade systemic inflammation contributes to the increased CVD risk (González-Gay and González-Juanatey, 2014; Schumacher and Naga Prasad, 2018). Indeed, markers of systemic inflammation, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) have been associated with a high incidence of cardiovascular events, independent of traditional risk factors in patients with RA (López-Mejías et al., 2016; McFarlane et al., 2019; Zhang et al., 2014a). Although both traditional risk factors and inflammation play a role in the increased risk of CVD in RA, the high burden of CVD in RA is largely attributable to the interaction between traditional CVD risk factors and disease characteristics specific to RA (Crowson et al., 2018). Hence it is not surprising that the control of systemic inflammation is central to the guidelines for the treatment and management of not only disease progression and joint damage, but also comorbidities and risk of CVD in RA.

With regards to the pathogenesis of CVD, RA patients have a significant increased risk of mortality from ischaemic heart disease and stroke (Dessein et al., 2016; Jagpal and Navarro-Millán, 2018; Urman et al., 2018). Ischaemic pathologies, largely attributable to atherosclerosis, are common in RA patients and confer the greatest risk for increased cardiovascular related mortality (Avina-Zubieta et al., 2012). Compared to age and sex-matched control subjects, RA patients have more unstable carotid plaques that are more prone to rupture (Urman et al., 2018). Both traditional risk factors and RA disease-related systemic inflammation contributes to the accelerated atherosclerosis in this population (Gonzalez-Gay et al., 2005; McFarlane et al., 2019; González-Juanatey et al., 2011; Jagpal and Navarro-Millán, 2018; Youssef et al., 2018). Atherosclerosis often presents atypically in RA, which makes it difficult to diagnose (Crowson et al., 2018) and manage (González-Gay and González-Juanatey, 2014). Although a detailed discussion of the process of atherosclerosis is beyond the scope of this dissertation, the subsequent section will briefly highlight the role of dyslipidaemia in the pathogenesis of atherosclerosis.

2.3 The role of dyslipidaemia in the pathogenesis of atherosclerosis

Atherosclerosis is a chronic disease affecting sites on aortic, carotid and coronary vessel walls that are prone to turbulent or oscillatory shear stress (Marchio et al., 2019). In atherosclerosis, fatty streaks in the arterial walls gradually develop into atheroma and characteristic atherosclerotic plaques (Mahmoudi et al., 2017; Toms et al., 2011). Clinical events of atherosclerosis stem from the acute rupture of these plaques causing thrombosis, which ultimately leads to obstruction of blood vessels (Herrington et al., 2016; Mahmoudi et al., 2017). The development of atherosclerosis is complex and depends on the involvement of multiple interacting factors. In the general population, an atherogenic lipid profile, which is characterised by increased total cholesterol and low-density lipoprotein cholesterol (LDL-C) concentrations and a decreased high-density lipoprotein cholesterol (HDL-C) concentration, are well-known causes of atherosclerosis (Nurmohamed, 2007). The section below will briefly explain lipid metabolism and the contribution of altered lipid metabolism to the development of atherosclerosis.

Lipid metabolism involves the synthesis and degradation of plasma lipoproteins through exogenous and endogenous pathways (Remmerie and Scott, 2018). Lipoproteins, including HDL and LDL, contain apolipoproteins embedded in their phospholipid monolayer membrane, which transport poorly-soluble cholesterol and triglycerides in the body (Hussain, 2014; Remmerie and Scott, 2018; Stoffel, 1984). During lipid metabolism, chylomicrons are hydrolysed by lipoprotein lipase, losing some triglycerides into recipient cells as lipoprotein lipase anchors lipoproteins to vessel walls and facilitates fatty acid uptake (Wang and Eckel, 2009; Yang et al., 2012). The resulting remnant particles are then removed from the circulation via hepatic uptake (Nordestgaard, 2017; Remmerie and Scott, 2018). Very low-density lipoprotein (VLDL) is synthesised in the liver following the translation of apolipoproteins and undergoes a process of maturation, by acquiring triglycerides and

cholesteryl esters (Nordestgaard, 2017). Following the hydrolysis of the triglyceride-rich core by lipoprotein lipase, VLDL is secreted into the plasma and converted to LDL (Tall and Yvan-Charvet, 2015; Yang et al., 2012). Dyslipidaemia is a disorder of lipoprotein metabolism consisting of deficiencies or overproduction of lipoproteins and can be characterised by increases in total cholesterol, triglycerides, LDL-C and reductions in HDL-C (Hedayatnia et al., 2020). Dyslipidaemia is associated with almost all known risk factors for CVD and is an independent risk predictor of future cardiovascular events (Hameed and Al-Barzinjy, 2017; Jagpal and Navarro-Millán, 2018).

Dyslipidaemia is one of the main risk factors for the development of atherosclerotic CVD. In this regard, several lipid abnormalities have been associated with endothelial dysfunction (Pi et al., 2018), which is considered one of the earliest vascular changes in the process of atherosclerosis (Toms et al., 2011). Endothelial dysfunction is characterised by an imbalance between vasodilatory and vasoconstrictive responses of the vascular endothelium that results in damage to the endothelium (Ghosh et al., 2017). The endothelium is composed of a monolayer of cells lining the circulatory system. It plays an important role in the maintenance of vascular homeostasis by releasing endothelial-derived vasodilatory factors including nitric oxide (NO), prostacyclin, bradykinin and endothelium-derived hyperpolarising factor (EDHF) and endothelium-derived contracting factors (EDCFs), including angiotensin II, endothelin 1 and vasoconstrictor prostanoids (Godo and Shimokawa, 2017). Nitric oxide, bradykinin and prostacyclin have vasoprotective effects, that limit the process of atherosclerosis by inhibiting the oxidation of LDL (Ghosh et al., 2017; González-Gay and González-Juanatey, 2012). In contrast, an overproduction of endothelium-derived contracting factors has proatherogenic effects by promoting smooth muscle cell proliferation and pro-oxidant effects on LDL (Toms et al., 2011).

LDL is the major carrier of cholesterol in blood, from the liver to peripheral tissues, where the cholesterol is absorbed into target tissue (Remmerie and Scott, 2018). Under conditions of excess LDL in circulation, the LDL can undergo structural changes by attaching to proteins secreted by the liver that increase its ability to bind to the extracellular matrix and increase LDL deposition in vessels (Remmerie and Scott, 2018). Increased accumulation of LDL-C in cells poses a high risk for developing CVD (García-Gómez et al., 2014). Particularly, accumulation and oxidation of LDL-C in endothelial cells are the underlying causes of the formation of atherosclerotic plaque (Toms et al., 2011).

High levels of oxidised LDL directly inhibit the synthesis of endothelial nitric oxide synthase (eNOS) (Gliozzi et al., 2019), a key factor in the production of NO in the vascular wall (Norata and Catapano, 2005; Shi et al., 2014). The decreased production of eNOS facilitates the activation of its inducible isoform (iNOS) (Gliozzi et al., 2019). This altered eNOS/iNOS balance in endothelial cells reduces NO bioavailability and increases the production of free radicals. The reduced NO bioavailability and increased production of free radicals amplifies the oxidative damage, which enhances vascular wall inflammatory processes responsible for the development of atherosclerosis (Gliozzi et al., 2019). Furthermore, oxidised LDL also initiates the expression of inflammatory proteins associated with endothelial activation, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) (Salisbury and Bronas, 2014). Endothelial activation initiate monocyte-endothelial interaction, differentiation of monocytes into macrophages and stimulate biochemical processes where chemokines and foam cells (lipid-laden macrophages) accumulate in the intima of blood vessels (Salisbury and Bronas, 2014; Toms et al., 2011). In macrophages, digestion of excess lipids and oxidised lipoproteins in the lysosomes generates free cholesterol and free fatty acids. The free cholesterol is then re-esterified in the endoplasmic reticulum to cholesterol fatty acid esters and stored in lipid droplets in the cytosol, causing the development of foam cells (Remmerie and Scott, 2018). Taken together, the processes

associated with excess LDL results in the formation of a fatty streak in the vessels, which is one of the earliest signs of atherosclerotic plaque formation (Chistiakov et al., 2016; Jaipersad et al., 2014; Toms et al., 2011).

HDL is responsible for clearing cholesterol from the circulation via reverse cholesterol transport, which involves the removal and transportation of excess cholesterol from macrophages to the liver (Tran-Dinh et al., 2013). HDL plays an important role in counteracting the effects of oxidised LDL, and hence have atheroprotective properties (Cuchel and Rader, 2006; García-Gómez et al., 2014; Howes et al., 1997; März et al., 2017; Navab et al., 2009; Norata and Catapano, 2005; Yvan-Charvet et al., 2010). During lipid metabolism, nascent HDL particles synthesized in the liver are transported into the bloodstream where they can cross the vascular endothelium to collect cholesterol from vascular intima cells. Nascent HDL can accept free cholesterol in vascular cells using the ATP-binding cassette transporter (ABCA1) (Bag-Ozbek and Giles, 2015; Ben-Aicha et al., 2020; Sorci-Thomas and Thomas, 2013; Zhou et al., 2015). The free cholesterol bound to nascent HDL is then esterified with a fatty acid from lecithin by lecithin-cholesterol acyltransferase forming cholesteryl esters and generating mature HDL particles (Hernáez et al., 2019; Lewis and Rader, 2005; Sorci-Thomas and Thomas, 2013). The newly formed mature HDL particle is responsible for the systemic removal of excess cholesterol, for excretion in the liver (García-Gómez et al., 2014; Sorci-Thomas and Thomas, 2013). Considering the essential role of ABCA1 in lipid metabolism, dysfunction of this transporter and lipid transport impairments may result in the accumulation of cholesteryl-esters in various reticuloendothelial cells and significantly reduce the serum HDL concentration (Schumacher and Benndorf, 2017). In this regard, reduced HDL levels decrease the clearance of systemic LDL particles, resulting in a greater accumulation and oxidation of LDL (März et al., 2017; Yu et al., 2018).

Furthermore, HDL promotes eNOS activation and vessel relaxation by increasing cholesterol efflux (Gong et al., 2003; Norata and Catapano, 2005; Tran-Dinh et al., 2013). HDL also induces the release of prostacyclin which is vasoprotective and atheroprotective (Norata and Catapano, 2005). Ultimately, HDL regulates vascular tone, promotes endothelial protection, and is closely associated with a greater endothelial release of NO (Hernáez et al., 2019). Hence, reduced HDL concentrations contribute to the pathogenesis of atherosclerosis. The imbalance of HDL and oxidized LDL may lead to increased concentrations of total cholesterol. Increased total cholesterol: HDL-C ratio (total cholesterol/HDL-C) is considered an atherogenic lipid profile and an important prognostic marker for CVD risk (Parveen et al., 2017). Although the association between dyslipidaemia and atherosclerotic CVD is well characterised in the general population, this relationship between dyslipidaemia and atherosclerosis in RA is complex and atypical. The section below highlights the impact of RA on the relationship between dyslipidaemia and atherosclerosis.

2.4 Altered lipid metabolism and its association with atherosclerosis in rheumatoid arthritis

Compared to the general population, RA patients have a higher prevalence of dyslipidaemia (Hameed and Al-Barzinjy, 2017). Nevertheless, decreased concentrations of total cholesterol, LDL-C and HDL-C have been reported in RA compared to the general population. Despite the reductions in lipid concentrations, RA patients are at an increased risk of developing atherosclerotic CVD (Cacciapaglia et al., 2018; Charles-Schoeman et al., 2015; Pérez-Baos et al., 2017). In this regard, RA patients with lower total cholesterol, HDL-C and LDL-C concentrations have an increased risk of atherosclerosis (Choy et al., 2014; Choy and Sattar, 2009; McGrath and Young, 2015; Myasoedova et al., 2011; Nassir et al., 2007). This increased atherosclerotic risk is largely attributed to the unbalanced ratio between total cholesterol and HDL-C concentrations (total cholesterol/HDL-C), and hence an

increased atherogenic index (Cacciapaglia et al., 2018; Curtis et al., 2012; Pérez-Baos et al., 2017; Peters et al., 2010). Consistent evidence suggests that systemic inflammation is central to the altered lipid metabolism and its relation to atherosclerosis in RA (Charles-Schoeman et al., 2015; Gabay et al., 2016; García-Gómez et al., 2014; Jukema et al., 2019; van Halm et al., 2006). High circulating inflammatory marker concentrations, including IL-6, CRP and ESR have been associated with low total cholesterol, HDL-C and triglyceride concentrations and an increased atherogenic index ratio (Bag-Ozbek and Giles, 2015; Chung et al., 2010; Dabeer et al., 2019; Georgiadis et al., 2006; Nassir et al., 2007; Nurmohamed, 2007; Park et al., 1999; Peters et al., 2010; Steiner and Urowitz, 2009; van Halm et al., 2006; Zhang et al., 2014b). Reducing inflammatory cytokines coincide with increases in serum lipid concentrations and reduces the risk of CVD in RA patients (Robertson et al., 2013; Steiner and Urowitz, 2009). This inverse relationship between inflammation, lipid concentrations and atherosclerotic CVD in RA is known as the lipid paradox. The bi-directional interaction between dyslipidaemia and chronic inflammation in RA is fundamental to all stages of atherosclerotic plaque formation (Toms et al., 2011). Hence inflammation plays a central role in accelerating atherosclerosis (Naerr et al., 2016).

The suppression of HDL is the most consistently reported lipid metabolism abnormality in RA (Chung et al., 2010; Georgiadis et al., 2006; Guo et al., 2017; Hameed and Al-Barzinjy, 2017; Kim et al., 2016; Lazarevic et al., 1992; Nassir et al., 2007; Park et al., 1999; Roy and Das, 2016). The degree of HDL reduction is associated with RA disease activity and severity (Chung et al., 2010; Georgiadis et al., 2006). Although several studies have reported suppressed total cholesterol concentrations (Guo et al., 2017; Lazarevic et al., 1992), it is not as substantial as that seen in HDL concentrations (Georgiadis et al., 2006). Consequently, this produces an increased atherogenic index (total cholesterol/HDL-C ratio) in RA patients (Guo et al., 2017). Moreover, RA patients have reduced apolipoprotein A-I levels, reduced levels of HDL-associated enzymes and their HDL particles have oxidative

structural changes compared to the general population (Dessein et al., 2016; González-Gay and González-Juanatey, 2014; Ronsein and Vaisar, 2017). These changes in HDL metabolism associated with RA hinders its ability to facilitate cholesterol efflux in reverse cholesterol transport (Dessein et al., 2016) and ultimately impairs the antioxidant, anti-inflammatory and cardioprotective effects of HDL (Dessein et al., 2016; González-Gay and González-Juanatey, 2014).

Contradictory findings on the associations between LDL-C and disease activity in RA patients have been reported. Although several studies reported a decrease in LDL-C (Chung et al., 2010; Lazarevic et al., 1992), some reported a significant elevation in LDL-C concentrations (Georgiadis et al., 2006; Guo et al., 2017; Hameed and Al-Barzinjy, 2017; Roy and Das, 2016). Although lower LDL-C concentrations may appear to produce a less atherogenic lipid profile, chronic inflammation causes structural and oxidative changes in the LDL particle which may promote atherogenesis (Khovidhunkit, et al., 2000). LDL has a complex lipid-protein structure consisting of polyunsaturated fatty acyl chains, making it highly susceptible to oxidative modification by free radicals (Choi et al., 2017b; García-Gómez et al., 2014; Khovidhunkit, et al., 2000; Levitan et al., 2010; Parthasarathy, 2010), which also oxidize its major apolipoprotein B component (Steinberg, 1997; Yang et al., 2012). In chronic inflammatory conditions, a decrease in LDL-C concentrations is associated with a decrease in LDL particle size, resulting in small dense LDL particles that are more pro-atherogenic as they have a lower binding affinity for the LDL receptor (Hurt-Camejo et al., 2001; Jones, 2000). This leads to increased circulation time for LDL particles, increasing the potential for oxidation (Borén et al., 2020). Oxidation of these LDL particles takes place in the subendothelial space where antioxidants are much less compared to the circulation (Afonso and Spickett, 2019; Gianazza et al., 2019; Khovidhunkit et al., 2004; Levitan et al., 2010; Toms et al., 2011). Small, dense LDL particles can easily cross the endothelial barrier and bind to proteoglycans in the vascular wall intima, resulting in LDL retention (Chait et al.,

1993; Toms et al., 2011). These oxidised LDL particles are then no longer cleared from the subendothelial space during lipid metabolism (García-Gómez et al., 2014; Pignatelli et al., 2018).

Similar to LDL-C concentrations, the precise relationship between triglycerides and disease activity in RA remains controversial. While most studies have focused on the role of lipoproteins in CVD risk in RA, the role of triglycerides and its clinical relevance have not been clearly defined in RA (Rodríguez-Carrio et al., 2017). Some studies have reported high triglyceride concentrations (Hameed and Al-Barzinjy, 2017; Kim et al., 2016; Roy and Das, 2016), while others report no changes in triglyceride concentrations in untreated RA patients (Hurt-Camejo et al., 2001). While triglycerides are generally not directly atherogenic, its association with atherogenic remnant particles necessitates its consideration as a biomarker of CVD risk (Bag-Ozbek and Giles, 2015; Talayero and Sacks, 2011). The relationship between hypertriglyceridemia and increased CVD risk is possibly explained by the altered HDL functionality. Elevated triglyceride concentrations induce a greater cholesteryl ester exchange between triacylglycerol-rich lipoproteins and nascent HDL leading to smaller and more dense HDL particles that have a reduced functionality (Rodríguez-Carrio et al., 2017). The reduced HDL functionality reduce cholesterol efflux capacity (Rodríguez-Carrio et al., 2017), and hence reduce LDL-C clearance.

Taken together, inflammation plays a central role in the altered lipid metabolism and its association with atherosclerosis in RA. RA patients develop a pro-atherogenic lipid profile compared to healthy individuals without RA and remain at higher risk for CVD regardless of the reduced lipid levels (Crowson et al., 2018). Inflammation induces deleterious consequences on lipid metabolism which ultimately produces this unique pro-atherogenic lipid profile. The pro-atherogenic lipid profile accelerates atherosclerosis progression and increases the risk of CVD in this population. However, the true effect of inflammation on lipid

metabolism in RA is still controversial as findings are inconsistent and the mechanisms responsible for these changes remain largely unknown. The discrepancies in the reported findings may be attributed to small sample sizes, the cross-sectional nature of studies where cause and effect cannot be inferred and the large number of confounding factors in human RA studies (Bag-Ozbek and Giles, 2015; Choy and Sattar, 2009; Hassen Zrour et al., 2011; Nurmohamed, 2007; Toms et al., 2011). In this regard, animal studies have been done to gain a better understanding of the effects of inflammation on lipid metabolism, independent of confounding factors. The section below briefly describes findings of inflammation-induced changes in lipid metabolism in pre-clinical models of high-grade inflammation.

2.5 Lipid profiles in animal models of RA

Table 2.1 shows a summary of animal studies that have investigated the effects of inflammation on lipids. In the adjuvant-induced arthritis (AIA) model, studies have reported contradictory findings on the effects of inflammation on lipid metabolism (Abdin et al., 2012; El-Ashmawy et al., 2019; El-Gaphar et al., 2018; Kunitomo et al., 1987, 1986; Patel and Pundarikakshudu, 2016; Wendt et al., 2019; Yamaguchi et al., 2007). Some (Abdin et al., 2012; El-Ashmawy et al., 2019; El-Gaphar et al., 2018; Patel and Pundarikakshudu, 2016; Wendt et al., 2019; Yamaguchi et al., 1989) but not others (El-Ashmawy et al., 2019; He et al., 2013; Kunitomo et al., 1986) have reported reduced serum concentrations of HDL, LDL and total cholesterol. These discrepancies could possibly be explained by differences in rat strain and the onset, severity and duration of the different arthritic protocols used. Although the adjuvant-induced arthritis model is an established animal model of RA, the animal model generally shows less severe symptoms and is transient (Bendele, 2001). The collagen-induced arthritis (CIA) rat model on the other hand, is a model that closely resembles the disease activity in RA (Bendele, 2001). The CIA model shares many pathological features with RA including mononuclear cell infiltration, synovial hyperplasia, cartilage degradation,

and collagen type II-specific antibodies and rheumatoid factor production (Bendele, 2001). However, to the best of our knowledge, there is currently no direct evidence to support the role of chronic exposure to inflammation in the development of dyslipidaemia in the CIA model. CIA studies have only reported decreased plasma triglycerides and total cholesterol concentrations (Srivastava et al., 2018, 2014). Currently, no studies have investigated the effects of inflammation on HDL-C or LDL-C concentrations. *Hence in the present study, I aimed to determine whether exposure to chronic inflammation alters serum lipid concentrations in the CIA rat model.*

Considering the evidence that circulating inflammatory markers are associated with abnormal lipid metabolism (Cacciapaglia et al., 2018; Hashizume and Mihara, 2011; Nurmohamed, 2007; Seriolo, 2006), the question remains what are the possible mechanisms explaining the relationship between inflammation and altered lipid metabolism? The section below briefly highlights the mechanisms whereby inflammation may mediate abnormal lipid metabolism.

Table 2.1 Summary of animal studies showing the effects of inflammation on lipid profiles.

Authors	Animal model	Duration	Sample size	Findings
Abdin et al., (2012)	AIA	35 days	Control=10, AIA=10	Serum levels of LDL-C, TC and triglycerides increased, and HDL-C levels decreased in AIA group.
El-Ashmawy et al., (2019)	AIA	31 days	Control=21, AIA=21	Serum levels of LDL-C, TC and triglycerides increased, and HDL-C levels decreased in AIA group.
El-Gaphar et al., (2018)	AIA	28 days	Control=10, AIA=10	Serum levels of TC, LDL-C and triglycerides increased, and HDL-C levels decreased in AIA group.
Kunitomo et al., (1986)	AIA	8 weeks	Control=6, AIA=9	Serum levels of TC and HDL-C increased, and serum triglyceride levels decreased in AIA group.
Kunitomo et al., (1987)	AIA	4 weeks	Control=6, AIA=9	Serum levels of TC increased, and serum triglyceride levels decreased in AIA group.
Patel and Pundarikakshudu, (2016)	AIA	4 weeks	Control=6, AIA=6	Serum levels of HDL-C decreased, and LDL-C, TG and triglycerides increased in the AIA group despite unchanged body weight
Wendt et al., (2019)	AIA	18 days	Control=5, AIA=5	Serum levels of HDL-C, LDL-C, VLDL, TC and triglycerides decreased in AIA group.
Yamaguchi et al., (1989)	AIA	6 weeks	Control=6, AIA=6	Serum levels of HDL-C, TC and triglycerides decreased in the first 4 weeks but increased after the last 2 weeks in AIA group.
He et al., (2013)	CIA	8 weeks	Control=16, CIA=16	No change in body weight, blood glucose and serum levels of TC and triglycerides in CIA group.
Srivastava et al., (2014)	CIA	4 weeks	Control=10, CIA=10	Plasma levels of TC and triglycerides decreased in CIA group. HDL-C and LDL-C were not reported.
Srivastava et al., (2018)	CIA	4 weeks	Control=5, CIA=5	Plasma levels of TC and triglycerides decreased in CIA group. HDL-C and LDL-C were not reported.

HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TC: total cholesterol, VLDL: very low-density lipoprotein; AIA: adjuvant-induced arthritis, CIA: collagen-induced arthritis.

2.6 Mechanisms of inflammation-induced changes in lipid metabolism

2.6.1 The role of inflammation on impaired HDL metabolism

Although chronic inflammation is associated with reduced concentrations of HDL-C in RA patients, the mechanisms underlying this relationship remain controversial. Evidence suggests that chronic exposure to inflammation alters the HDL structure and functional capacity. Inflammation-induced structural changes in the HDL particle may impair its ability to mediate reverse cholesterol transport (Ronsein and Vaisar, 2017). In this regard, one possible mechanism that could explain the reduced HDL-C in RA is the effect of inflammation on apolipoprotein A-I. Apolipoprotein A-I is a lipid-poor protein, synthesized in the liver and intestines (Hui et al., 2019). It is the main constituent of HDL, that actively removes cholesterol and phospholipids from cells (Hui et al., 2019; Oram et al., 2000; Wang et al., 2017). Indeed, inflammatory cytokines decrease the mRNA expression of apolipoprotein A-I in pre-clinical and *in vitro* studies (Beers et al., 2006; Ettinger et al., 1994; Haas et al., 2003; Navarro et al., 2005; Parseghian et al., 2014). Several cohort studies in RA have reported that apolipoprotein A-I is reduced in the presence of increased CRP concentration and decreased HDL-C (Liao et al., 2015; Oliviero et al., 2009; Tanimoto et al., 2003). However, not all studies reported a reduced apolipoprotein A-I in conjunction with increased CRP concentrations (Cigliano et al., 2005) or with reduced HDL-C concentrations (Charles-Schoeman et al., 2012). Hence this suggests that other mechanisms may be involved.

Another possible link between inflammation and HDL-C is serum amyloid A. Chronic inflammation increases the production of serum amyloid A, which displace apolipoprotein A-1 from HDL (Kisilevsky and Manley, 2012; Prüfer et al., 2015). HDL particles containing serum amyloid A have a reduced ability to carry out reverse cholesterol transport (Kisilevsky and Manley, 2012; Prüfer et al., 2015). Although one study has shown an increased serum

amyloid A in RA as a potential cause of the reduced HDL-C (Tanimoto et al., 2003), this has not been confirmed by others. Others showed increased serum amyloid A in the presence of increased CRP concentrations, but HDL-C was not significantly reduced (Oliviero et al., 2009; Ormseth et al., 2016).

Another possible mechanism that may explain the reduced functionality of HDL-C in chronic inflammation is the conversion of nascent HDL to mature HDL. In this regard, lecithin-cholesterol acyltransferase is an essential enzyme involved in the synthesis of mature HDL particles (Zhou et al., 2015). Reduced lecithin-cholesterol acyltransferase impairs the ability to esterify cholesterol leading to decreased synthesis of mature HDL particles (Popa et al., 2007a; Zannis et al., 2017). In pre-clinical models, the administration of inflammatory cytokines reduced lecithin-cholesterol acyltransferase activity (De La et al., 2012; Ettinger et al., 1994, 1992; Ly et al., 1995). In RA patients, lecithin-cholesterol acyltransferase is also reduced (Charles-Schoeman et al., 2015, 2009; Tanimoto et al., 2003). However, there is still uncertainty about the contribution of reduced lecithin-cholesterol acyltransferase activity in decreasing HDL-C concentrations in RA. In the presence of increased CRP and ESR, lecithin-cholesterol acyltransferase activity remained unchanged while HDL-C insignificantly increased in RA patients (Cigliano et al., 2005).

Another mechanism involved in HDL maturation that may explain the reduced HDL-C concentration in inflammation is the expression and activity of ABCA1. ABCA1 is involved in the conversion of nascent HDL to mature HDL (Bag-Ozbek and Giles, 2015; Ben-Aicha et al., 2020; Sorci-Thomas and Thomas, 2013; Zhou et al., 2015). Several *in vitro* and pre-clinical studies have shown that inflammation downregulates the expression of ABCA1 in a variety of cells (Chen et al., 2009; Dragoljevic et al., 2018; Field et al., 2010; Ma et al., 2013). Nevertheless, the evidence of a reduced ABCA1 in RA patients and its relations to inflammation and HDL-C concentrations are controversial. Ronda et al. (2014) showed no

differences in ABCA1 concentrations between RA and non-RA patients. Furthermore, these authors showed no associations between inflammatory cytokine concentrations and ABCA1 concentrations, or any change in ABCA1 concentration after anti-inflammatory treatment (Ronda et al., 2014; Ronda et al., 2015). Moreover, the serum HDL-C concentrations were not correlated with ABCA1 concentrations in RA patients (Ronda et al., 2014; Ronda et al., 2015). Despite the potential of ABCA1 as a mediator of the functionality of HDL-C in high-grade inflammation shown in basic studies, RA studies in humans have not confirmed these results. The discrepancies in these studies may be explained by the confounding factors in cross-sectional studies. Hence, the role of ABCA1 in inflammation-induced lipid metabolism requires elucidation in pre-clinical models.

2.6.2 The role of inflammation on impaired LDL metabolism

While reductions in HDL-C are mostly consistent in studies, LDL-C levels remain controversial in RA. One possible mechanism to explain the relationship between high-grade inflammation and reduced LDL-C is apolipoprotein B. Apolipoprotein B is the major protein found on the surface of LDL particles and is responsible for LDL-C clearance via the LDL receptor pathway. Apolipoprotein B is used for VLDL formation in hepatocytes and plays a central role in maintaining the lipoprotein structure (Ismaili et al., 2019; Morita, 2016). Altering the normal structure and composition of LDL decreases their uptake by hepatocytes and increases their uptake by macrophages (Dabeer et al., 2019). *In vitro*, some studies reported that cytokines decreased the concentrations of apolipoprotein B by reducing secretion rates of apolipoproteins, while other studies reported that apolipoprotein B reduction was by marginally increased LDL reuptake in cells (Ettinger et al., 1994; Grove et al., 1991; Popa et al., 2007a). Administration of cytokines in healthy subjects decreased LDL-C and apolipoprotein B levels by reducing the apolipoprotein B production rate (Schectman et al., 1992). While inflammation mostly reduced LDL-C and apolipoprotein B in

basic studies, the effect of apolipoprotein B on LDL-C in RA patients is controversial. Some cohort studies have reported significant reductions in apolipoprotein B and LDL-C in the presence of increased CRP in RA patients (Kremer et al., 2017). Others showed increased apolipoprotein B when CRP was decreased (Ismaili et al., 2019). However, others found increased (van Halm et al., 2006) or unchanged apolipoprotein B in the presence of increased CRP (Peters et al., 2007).

Other mechanisms possibly altered in LDL lipid metabolism are the activity and expression of lipoprotein lipase and endothelial lipase in chronic inflammation. Lipoprotein lipase hydrolyses triacylglycerol-rich lipoproteins and releases the free fatty acids for usage or storage in cells (Alves-Bezerra and Cohen, 2017; Olivecrona, 2016), thus reducing circulatory concentrations. Reduced lipoprotein lipase activity in chronic inflammation causes a rise in systemic triglycerides and LDL-C (Dabeer et al., 2019). Pre-clinical and *in vitro* studies demonstrated that cytokines suppressed the mRNA expression, activity or secretion of lipoprotein lipase (Albalat et al., 2005; Chen et al., 2009; Greenberg et al., 1992; Lubrano et al., 2015; Zhang et al., 2002). Data on the effects of inflammation on lipoprotein lipase in RA are limited. An inverse relationship has been reported between CRP concentrations and lipoprotein lipase mass in RA patients (Wallberg-Jonsson et al., 1996). This demonstrates a possible relationship between inflammation and lipoprotein lipase activity in these patients. However, these authors failed to show an association between lipoprotein lipase and LDL-C, therefore the direct relationship remains uncertain.

Lastly, endothelial lipase is an enzyme involved in phospholipase activity and triglyceride hydrolysis to a lesser extent during lipid metabolism (Huang et al., 2010; Robert et al., 2013; Yu et al., 2018). Increased endothelial lipase results in increased uptake of cholesteryl esters into vasculature cells and mediates the binding and uptake of LDL (Kojma et al., 2004; Yue et al., 2016), which lowers the levels in circulation. In this regard, endothelial

lipase contributes to VLDL and LDL metabolism and reduces their serum concentrations during inflammation (Yu et al., 2018). In *in vitro* studies, cytokines upregulated the expression of endothelial lipase mRNA and the endothelial lipase plasma concentrations were positively correlated with increased inflammatory marker concentrations (Paradis et al., 2006; Yue et al., 2016). In humans, one study showed a correlation between high inflammatory cytokine concentrations and increased endothelial lipase concentrations in plasma of 858 human subjects with endotoxin-induced inflammation (Badellino et al., 2008). To the best of our knowledge, studies have not reported on endothelial lipase in RA patients, therefore the relationship between endothelial lipase and LDL-C remains undefined in this population. However, increased endothelial lipase activity may possibly contribute to atherogenesis in RA. Endothelial lipase expression has been detected in atheromatous plaque macrophages in human coronary arteries (Kojma et al., 2004). Taken together, several possible mechanisms may explain the altered lipid metabolism in RA. However, the cross-sectional nature of the human RA studies precludes the inference of cause and effect. Hence, the mechanisms of inflammation-induced alterations in lipid metabolism required further investigation.

2.7 Effects of anti-inflammatory treatment on lipid profiles in high-grade inflammation

Inflammation is at the centre of driving disease activity, joint damage, clinical symptoms and comorbidities in RA therefore reducing inflammation is the major therapeutic target in RA disease management (Smolen et al., 2016). Disease-modifying antirheumatic drugs (DMARDs) are used in the management of RA, as they reduced inflammation and reduce structural joint damage progression (Smolen et al., 2016). There are two major classes of DMARDs, namely synthetic and biologic. According to the current European League Against Rheumatism (EULAR) recommendations, treatment in RA patients should be initiated with conventional synthetic DMARDs (Smolen et al., 2017). Synthetic DMARDs such as

methotrexate, sulfasalazine, leflunomide and hydroxychloroquine are prescribed to reduce disease activity and severity in RA patients (Sizova, 2008; Smolen et al., 2016). However, not all patients tolerate synthetic DMARDs well, and some patients do not achieve a decrease in disease activity and severity when receiving synthetic DMARDs (Rigby et al., 2017). In patients with an intolerance to synthetic DMARDs, in those who have severe disease, presence of autoantibodies or early joint damage despite synthetic DMARD treatment, it is recommended that a biologic DMARD be given as monotherapy or that it be added to the synthetic DMARD treatment regimen (Smolen et al., 2017). Biologic DMARDs have been introduced as an alternative monotherapy, but mostly additive therapy to synthetic DMARDs over the past decade (Harmse and Reuter, 2016). Biologic DMARDs have markedly improved RA treatment strategies for patients with severe disease and have provided benefits in those who do not tolerate synthetic DMARDs (Rein and Mueller, 2017; Rigby et al., 2017). Several classes of biologic DMARDs are available, each with a different mode of action. The different classes of biologic DMARDs include TNF-α inhibitors (etanercept, certolizumab pegol, adalimumab, golimumab and infliximab), IL-6 receptor blockers (tocilizumab), IL-1 receptor blockers (anakinra), T-cell stimulation blockers (abatacept), and B-cell depletion (rituximab) (Monti et al., 2017; Smolen et al., 2016). Although these different classes of drugs are mechanistically discrete, the overall effects of the drugs are mediated by interfering with pro-inflammatory cytokine effects (Smolen et al., 2016).

Although synthetic DMARDs improve disease activity by suppression of the immune system, their effects on lipid metabolism have been controversial (Naerr et al., 2016). Some studies have shown improved lipid metabolism and reduced CVD risk (Bălănescu et al., 2018; Charles-Schoeman et al., 2017, 2016; Karimifar et al., 2018; Naerr et al., 2016; Rigby et al., 2017) but not all studies support this (Cacciapaglia et al., 2018; Chen et al., 2015; Ghosh et al., 2009; Singh et al., 2020). Synthetic DMARDs are often used as first-line therapy and as

a result, their effects on lipid metabolism and liver toxicity are well-studied. However, because biologic DMARDs are mostly prescribed in conjunction with synthetic DMARDs, their independent effects on lipid metabolism are not as well categorised.

Some clinical trials that have determined the effects of biologic DMARDs on the lipid profiles of RA patients are summarised in Table 2.2. Majority of the studies reported that TNF-α inhibitors improved lipid metabolism by increasing HDL-C concentrations in RA patients (Allanore et al., 2006; Chen et al., 2015; Gabay et al., 2016; Nishida et al., 2008; Peters et al., 2007; Popa et al., 2005; Seriolo, 2006; Spanakis et al., 2006; Vis et al., 2005; Wijbrandts et al., 2009; Yeh et al., 2014). Other studies reported worsened LDL-C (Allanore et al., 2006; Gabay et al., 2016; Popa et al., 2007b; Wijbrandts et al., 2009; Yeh et al., 2014), total cholesterol (Allanore et al., 2006; Gabay et al., 2016; Nishida et al., 2008; Peters et al., 2007; Popa et al., 2007b, 2005; Saiki et al., 2007; Seriolo, 2006; Soubrier et al., 2008; Vis et al., 2005; Wijbrandts et al., 2009; Yeh et al., 2014) and triglycerides (Allanore et al., 2006; Gabay et al., 2016; Saiki et al., 2007; Wijbrandts et al., 2009) compared to baseline values or control groups. A single study reported improved total cholesterol and triglycerides, although HDL-C remained unchanged (De Sanctis et al., 2013). A meta-analysis reported that the administration of TNF-α inhibitors increased total cholesterol and HDL-C concentrations and may be associated with an increased apolipoprotein A-I level without changes in LDL-C levels (Daïen et al., 2012). In contrast, others have reported no changes in lipid metabolism with the administration of TNF-α inhibitors, despite an effective reduction in disease activity (Soubrier et al., 2008). However, some authors reported that TNF-α inhibitors reduce CVD risk and improve cardiovascular outcomes in RA patients despite some elevations in lipids (de La Forest Divonne et al., 2017; Tocci et al., 2016).

Similar to the effects of TNF-α inhibitors, studies that have investigated the effects of IL-6 blockers (tocilizumab) on lipid metabolism in RA have reported conflicting results. Majority of

the studies have reported that IL-6 blockers moderately improved the atherogenic profiles of RA patients by increasing HDL-C concentrations (Genovese et al., 2008; Jones et al., 2010; Kawashiri et al., 2011; Maini et al., 2006; Schultz et al., 2010; Tournadre et al., 2017). However, some studies have reported that IL-6 blockers worsen the atherogenic profile by increasing total cholesterol, LDL-C concentrations and triglycerides without improvements in HDL-C concentrations (Cacciapaglia et al., 2018; Chen et al., 2015; McInnes et al., 2015). In other studies, administrations of IL-6 blockers in RA patients resulted in unchanged lipid profiles (Farah et al., 2016; Provan et al., 2015).

Taken together, the effects of biologic DMARDs on lipid metabolism are contradictory. These discrepancies may be explained by the differences in disease activity and severity in different study cohorts and the heterogeneity in the dosage, duration and follow-up after DMARD treatment. Furthermore, the relatively small sample sizes, the lack of control groups and the heterogeneity in the study population may also impact the outcomes. Lastly, the confounding effect of other cardiovascular drugs in these studies limit the interpretability of the independent effects of the DMARDs on lipid profiles. Therefore, animal models of chronic inflammation remain the prime setting to assess the direct effects of biologic DMARDs on lipid metabolism, independent of confounding factors. Currently, studies investigating the effects of biologic DMARDs on lipid metabolism in animal models of RA are limited. To the best of our knowledge, only one study has evaluated the effects of TNF-α inhibitors on lipid metabolism, which demonstrated etanercept did not affect the lipid profiles in an AIA rat model (El-Ashmawy et al., 2019). There are currently no studies that have investigated the effects of IL-6 blockers on lipid metabolism in the AIA or CIA rat models. *Hence, in the present dissertation, I aimed to determine the effects of a TNF-α inhibitor and an IL-6 receptor blocker on inflammation-induced lipid abnormalities in the CIA rat model.*

Table 2.2 Summary of clinical studies showing the effects of biologic DMARDs on lipid metabolism in RA

Authors	Study design	Dosage and duration	Findings
Chen et al., (2015)	Control=20 (RA patients receiving synthetic DMARDs Without biologic),Tx=16 (sDMARD+ oral corticosteroids+ etanercept)	Twice weekly for 24 weeks	Etanercept increased HDL-C but LDL-C and TC remained unchanged compared to baseline.
De Sanctis et al., (2013)	Tx=30 (sDMARD + NSAID + etanercept)	Once weekly for 48 weeks	Etanercept decreased TC and triglycerides but HDL-C remained unchanged compared to baseline. LDL-C was not reported.
Seriolo, (2006)	Tx=16 (sDMARD + NSAID + prednisone + etanercept)	Twice weekly for 24 weeks	Etanercept increased HDL-C and TC but triglycerides remained unchanged compared to baseline. LDL-C was not reported.
Soubrier et al., (2008)	Tx=6 (sDMARD + NSAID + etanercept)	Twice weekly for 14 weeks	Etanercept did not change HDL-C, LDL-C, TC and triglycerides compared to baseline.
Yeh et al., (2014)	Tx=23 (sDMARD + prednisolone + etanercept)	Twice weekly for 48 weeks	Etanercept increased HDL-C, LDL-C and TC but triglycerides decreased compared to baseline.
Gabay et al., (2016)	Tx=163	Once every 2 weeks for 24 weeks	Adalimumab increased HDL-C, LDL-C, TC and triglycerides compared to baseline.
Popa et al., (2005)	Tx=33 (NSAID + prednisone+ adalimumab)	Once-off for 2 weeks	Adalimumab increased HDL-C and TC but LDL-C and triglycerides remained unchanged compared to baseline.
Wijbrandts et al., (2009)	Tx=50 (NSAID + prednisone + adalimumab)	Once every 2 weeks for 52 weeks	Adalimumab increased the HDL-C, LDL-C, TC and triglycerides compared to baseline.
Allanore et al., (2006)	Control= 112 (RA patients not receiving anti-TNF-a therapy and healthy controls not on treatments), Tx=59 (sDMARD + infliximab)	Once every 8 weeks for 30 weeks	Infliximab increased HDL-C, LDL-C, TC and triglycerides compared to control groups.
Popa et al., (2007b)	Control=55 (Healthy controls not on treatments), Tx=55 (sDMARD + oral	Once every 8 weeks for 48 weeks	Infliximab increased HDL-C, LDL-C, and TC compared to control groups.

	corticosteroids + infliximab)		
Nishida et al., (2008)	Tx=97 (sDMARD + oral corticosteroids + infliximab)	Once every 8 weeks for 58 weeks	Infliximab increased HDL-C and TC but triglycerides remained unchanged compared to baseline. LDL-C was not reported.
Peters et al., (2007)	Tx=80 (sDMARD + prednisone + infliximab)	Once every 8 weeks for 48 weeks	Infliximab increased HDL-C and TC but triglycerides remained unchanged compared to baseline. LDL-C was not reported.
Saiki et al., (2007)	control= 64 (RA patients with successful outcome after methotrexate and healthy controls not on treatments), Tx=32 (sDMARD + prednisolone + infliximab)	Once every 8 weeks for 24 weeks	Infliximab increased TC and triglycerides compared to control groups. LDL-C was not reported.
Spanakis et al., (2006)	Tx=24 (sDMARD + prednisolone + infliximab)	Once at week 0, 2, 6 and every 8 weeks for 24 weeks	Infliximab increased HDL-C but TC, LDL-C and triglycerides remained unchanged compared to baseline.
Vis et al., (2005)	Tx=69 (sDMARD + corticosteroids + infliximab)	Once at week 0, 2 and 6 week	Infliximab increased HDL-C and TC but triglycerides remained unchanged compared to baseline. LDL-C was not reported.
Chen et al., (2015)	Control=20 (RA patients receiving synthetic DMARDs Without biologic), Tx=24 (sDMARD + oral corticosteroids + tocilizumab)	Once weekly for 24 weeks	Tocilizumab increased LDL-C, TC and triglycerides but HDL-C remained unchanged compared to baseline.
Cacciapaglia et al., (2018)	Control=20 (RA patients treated with methotrexate for at least 52 weeks), Tx=40 (sDMARD + tocilizumab)	Once every 28 days for 52 weeks	Tocilizumab increased TC and triglycerides but HDL-C and LDL-C remained unchanged compared to baseline.TC, LDL-C and triglycerides increased but HDL-C remained unchanged compared to control group.
Genovese et al., (2008)	Control=370 (RA patients on placebo and DMARDs), Tx=751(sDMARD + oral corticosteroids + NSAID + tocilizumab)	Once every 4 weeks for 24 weeks	Tocilizumab increased HDL-C, LDL-C and TC compared to baseline.
Farah et al., (2016)	Tx=15 (lipid lowering agents +	144 weeks, frequency of	Tocilizumab resulted in a trend towards

	tocilizumab)	treatment administration not defined	increased LDL-C but HDL-C, TC and triglycerides remained unchanged compared to baseline.
Jones et al., (2010)	Tx=268 (NSAID + oral corticosteroids + tocilizumab)	Once every 4 weeks for 24 weeks	Tocilizumab increased HDL-C, LDL-C and TC compared to baseline.
Kawashiri et al., (2011)	Control=10 (RA patients on conventional DMARDs), Tx=9 (prednisolone + tocilizumab)	Once every 4 weeks for 12 weeks	Tocilizumab increased HDL-C, LDL-C, TC but not triglycerides compared to baseline.
Maini et al., (2006)	Control=49 (RA patients receiving placebo infusions and methotrexate), Tx= 52 (sDMARD + tocilizumab)	Once every 4 week for 20 weeks	Tocilizumab increased HDL-C, TC and triglycerides compared to control groups. LDL-C was not reported.
McInnes et al., (2015)	Control=63 (RA patients receiving placebo and methotrexate), Tx=69 (sDMARD + glucocorticoids + tocilizumab)	Once every 4 weeks for 24 weeks	Tocilizumab increased TC, LDL-C and triglycerides but HDL-C remained unchanged compared to control groups.
Provan et al., (2015)	Tx=7	12 weeks, frequency of treatment administration not defined	Tocilizumab did not change HDL-C, LDL-C, TC and triglycerides compared to baseline.
Schultz et al., (2010)	Tx=11(sDMARD + prednisone+ tocilizumab)	12 weeks, frequency of treatment administration not defined	Tocilizumab increased HDL-C, LDL-C and triglycerides but not significantly compared to baseline.
Tournadre et al., (2017)	Control=21 (Healthy subjects not on any treatments), Tx=21(sDMARD + lipid lowering agents + hypertensive medication + oral corticosteroids + tocilizumab)	24 weeks, frequency of treatment administration not defined	Tocilizumab increased HDL-C and TC but LDL-C and triglycerides remained unchanged compared to baseline.

Tx: treatment groups, sDMARD: synthetic DMARD, NSAID: non-steroidal anti-inflammatory drugs, HDL-C: high-density lipoprotein cholesterol,

LDL-C: low-density lipoprotein cholesterol, TC: total cholesterol.

2.8 Liver damage associated with DMARD treatment

Liver injuries are not a common extra-articular manifestation in rheumatic diseases (Carlo et al., 2011; Wagan et al., 2020). In treatment naïve RA patients, liver fibrosis is present in a small number of patients (4-8%) (Kremer et al., 1994; Ruderman et al., 1997). However, the risk of liver injury with DMARD treatment increases substantially (Osuga et al., 2015). Long-term use of methotrexate monotherapy has been associated with aminotransferase elevations more than three times the upper limit of normal in 32% of RA patients (Akhlaghi et al., 2018; Curtis et al., 2010; Dehestani et al., 2015; Jones et al., 2010; Oan et al., 2000; Schiff et al., 2011; Sotoudehmanesh et al., 2010). Synthetic DMARD combination therapy has been associated with elevated liver enzymes in up to 43% of RA patients (Akhlaghi et al., 2018; Curtis et al., 2010; Dubey et al., 2016).

Despite the benefits of biologic DMARDs on disease progression and some positive reports on lipid metabolism, liver damage has also been reported as a result of biologic DMARD treatment (Fisher et al., 2015; Lopetuso et al., 2018). Since RA patients mostly receive biologic DMARDs in conjunction with synthetic DMARDs and concomitant treatments, such as non-steroidal anti-inflammatory drugs, oral glucocorticoids and anti-hypertensive drugs, the independent effects of biologic DMARDs on hepatotoxicity in RA are not well established. The administration of TNF-α inhibitors have been associated with significantly elevated hepatic transaminase levels in RA patients (Oan et al., 2000; Rubbert-Roth, 2012). Aminotransferase elevations from TNF-α inhibitors are usually mild to moderate, and severe elevations have been reported in ≤1% of patients (Rubbert-Roth, 2012). However, there seems to be a difference in the extent of hepatotoxicity for different types of TNF-α inhibitors. In 6861 RA patients, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations were elevated in 5.9% of patients with the administration of infliximab or

adalimumab compared to etanercept, which had a lower hepatotoxicity risk (<1%) (Nanau and Neuman, 2014; Sokolove et al., 2010).

Moderate increases in liver transaminase were also observed with the administration of IL-6 blockers in RA patients (Baek et al., 2019; Emery et al., 2008; Genovese et al., 2017, 2008; Jones et al., 2010; Maini et al., 2006; Schiff et al., 2011). However, severe transaminase elevations are rare with tocilizumab (Genovese et al., 2017) and occur primarily in the first year of treatment (Ogata et al., 2019). In 4171 RA patients receiving tocilizumab with concomitant DMARDs, ALT and AST were moderately elevated in 24% and 13% of patients respectively and severely elevated in 0.2% and 0.3% of patients respectively (Genovese et al., 2017). Following the cessation of tocilizumab therapy, abnormal transaminase concentrations are likely to return to normal (Genovese et al., 2017).

In addition to increasing liver transaminases, biologic DMARDs have been associated with the development of liver fibrosis. Cirrhosis has been reported in patients receiving TNF-α inhibitors (Tang et al., 2020). However, there is limited evidence of cirrhosis associated with IL-6 receptor blocker use (Genovese et al., 2017). In most cases of reported liver fibrosis, patients were receiving concomitant treatments including synthetic DMARDs (Ogdie et al., 2018; Tang et al., 2020). However, patients receiving oral corticosteroids and non-steroidal anti-inflammatory drugs were at a higher risk of developing cirrhosis compared to those taking concomitant synthetic DMARDs (Ogdie et al., 2018) and RA patients had the least cirrhosis incidences compared to patients with other immune-related diseases (Ogdie et al., 2018; Tang et al., 2020). These findings indicate that multiple interacting factors may contribute to the development and progression of fibrosis in patients with immune-related diseases.

Taken together, some evidence suggests that biologic DMARDs may induce liver injury in chronic inflammatory disorders. However, the direct contribution of biologic DMARDs on

underlying hepatic injury in RA is uncertain as a result of the confounding hepatotoxic effects of other drugs used in the management of RA. Therefore, the independent effect of biologic DMARDs on liver morphology, injury and toxicity needs investigation. *Hence, in the present study, I aimed to determine the effects of etanercept and tocilizumab on liver morphology in the CIA rat model.*

2.9 Problem statement

RA patients are at greater risk of developing atherosclerotic CVD compared to the general population. Traditional CVD risk factors and systemic inflammation comprise the majority of the increased CVD risk in RA. In RA patients, the bi-directional relationship between inflammation and abnormal lipid metabolism is believed to increase the risk of atherosclerosis. Nevertheless, confounding factors in human studies limit the ability to determine the independent role of inflammation on the altered lipid metabolism in RA. In animal studies of high-grade inflammation, the contribution of inflammation to altered lipid metabolism is controversial and requires elucidation.

The management of systemic inflammation constitutes the cornerstone of RA therapy. Current DMARDs are aimed at controlling inflammation and slowing disease progression and joint damage. The effects of DMARDs on CVD in RA patients are inconsistent. Biologic DMARDs are mostly given as adjunct therapy to synthetic DMARDs, hence their independent role in the prevention of CVD are uncertain. Clinical studies have reported conflicting results on the effects of biologic DMARDs on lipid profiles in RA. Moreover, biologic DMARDs have been associated with hepatotoxicity and liver morphological changes in clinical studies. However, due to confounding factors in clinical studies, the direct impact of TNF-α inhibitors and IL-6 receptor blockers on the liver morphology remains largely unknown. Therefore, a better understanding of the role of inflammation and biologic

DMARDs on lipid metabolism and liver morphology may contribute to the prevention and management of atherosclerosis and CVD risk stratification in RA patients.

2.10 Aims

The aim of this study was to determine the effect of high-grade inflammation on lipid metabolism in Sprague-Dawley rats exposed to collagen-induced arthritis (CIA). In addition, this study aimed to assess whether biologic DMARDs alter the outcomes of inflammation-induced changes in lipid metabolism and whether they are associated with drug-induced liver morphological changes in Sprague-Dawley rats exposed to CIA.

The specific objectives of the current study were to determine

1. the lipid concentrations in Sprague-Dawley rats exposed to CIA.

2. whether treatment with Etanercept (a TNF-α inhibitor) and Tocilizumab (an IL-6 receptor blocker) alters inflammation-induced lipid concentration in Sprague Dawley rats exposed to CIA.

3. whether the administration of Etanercept (a TNF-α inhibitor) and Tocilizumab (an IL-6 receptor blocker) are associated with liver injuries in Sprague Dawley rats exposed to CIA.

Chapter 3

Methods

3.1 Animals and ethical clearance

Sixty-eight, male (n =38) and female (n=30), three-month-old Sprague Dawley rats were obtained from the Central Animal Services at the University of the Witwatersrand. The rats were housed individually, in temperature-controlled rooms with a 12-hour light-dark cycle and allowed free access to food and water. Male and female rats were kept in separate rooms. All experimental procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC approval number: 2017/03/21C and 2019/02/10C). This study was a part of a larger, ongoing study aimed at elucidating the mechanisms of inflammation-induced cardiometabolic changes.

3.2 Experimental design

The rats underwent a two-week habituation period, where body weight and arthritis scores were measured once a week and blood pressure was measured twice a week. After the two-week habituation period, rats were randomly divided into the control (n=20, 9 females and 11 males), inflammation (n=18, 7 females and 11 males), tumour necrosis factor-alpha inhibitor (TNF-α inhibitor; n=15, 7 females and 8 males) or interleukin-6 blocker (IL-6 blocker; n=15, 7 females and 8 males) groups. The control group received intraperitoneal injections of saline solution. The inflammation, TNF-α inhibitor, and IL-6 blocker groups were injected with an arthritis inducing emulsion. Following the onset of arthritis, rats in the TNF-α inhibitor group received a TNF-α inhibitor (etanercept; Enbrel) for six weeks and the rats in the IL-6 blocker group received an IL-6 receptor blocker (tocilizumab; Actemra), for six weeks. Body weight, blood pressure, and arthritis scores were measured once every two weeks. After six weeks of treatment, the rats were anaesthetised by intramuscular injections of ketamine (100 $mg.kg^{-1}$) and xylazine (5 $mg.kg^{-1}$) and were terminated by thoracotomy. Blood samples were obtained and the liver and adipose tissue were removed and weighed before being frozen (-80°C) until further analysis. A summary of the experimental design is outlined in Figure 3.1.

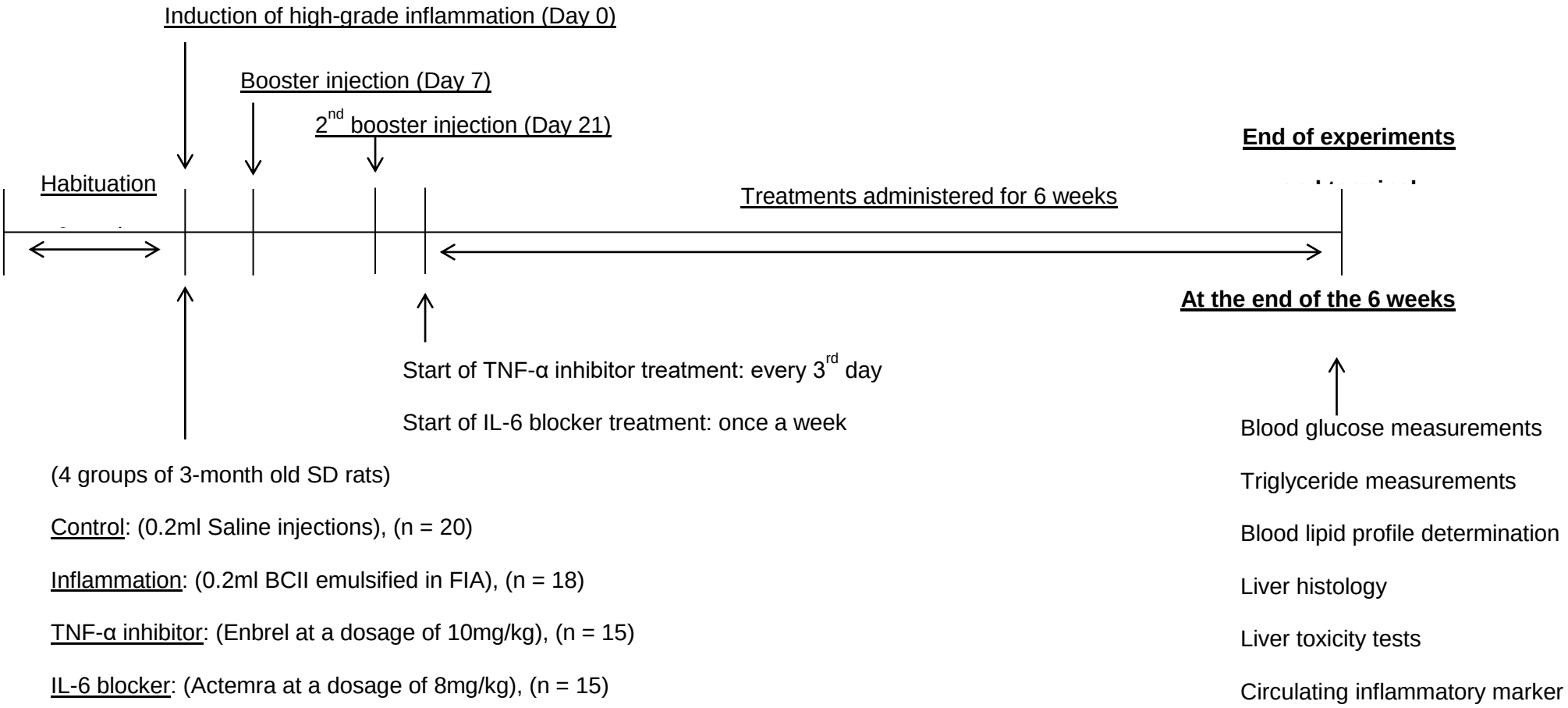

Figure 3.1 Experimental design

3.2.1 Induction and monitoring of arthritis

After the two-week habituation period, systemic inflammation was induced in rats in the inflammation, TNF-α inhibitor, and IL-6 inhibitor groups, as previously described (Jasemiam et al., 2011). Briefly, bovine type II collagen (BCII) (Chondrex cat. #20021, Redmond, WA, USA) was dissolved in 0.05M acetic acid by gently stirring overnight at 4°C. Equal amounts of dissolved bovine type II collagen (2mg/ml) and incomplete Freund's adjuvant (FIA) (2mg/ml) (Chondrex cat. #7002, Redmond, WA, USA) were mixed using an electric homogenizer. The arthritis-inducing emulsion was prepared immediately before immunisation. Under general anaesthesia (2.5-3.5% isoflurane), the rats were immunised with 0.2ml (200µg) of the arthritis inducing emulsion by subcutaneous injection at the base of the tail. To ensure a high incidence and severity of arthritis, 0.1ml (100µg) booster injections were administered seven and 21 days after the first immunisation. Control rats received a subcutaneous injection of 0.1ml (100µg) of saline at the base of the tail.

3.2.2 Drug intervention

Following the onset of arthritis, the rats in the TNF-α inhibitor group received a subcutaneous injection of etanercept, a TNF-α inhibitor (Enbrel) at a dosage of 10 mg/kg every third day for six weeks. The rats in the IL-6 blocker group received a subcutaneous injection of tocilizumab, an IL-6 receptor blocker (Actemra) at a dosage of 8 mg/kg once a week for six weeks. Following the onset of arthritis, rats in the inflammation, TNF-α inhibitor and IL-6 blocker groups received 2mg/kg Tramazec injected subcutaneously daily for pain relief.

3.3 Measurement of arthritis scores and paw thickness

To quantitatively evaluate the severity of arthritis, rat paws were scored once a week using a previously described five-point scoring system (Kim et al., 2010). In the scoring system, a score of 0 indicates no swelling or focal redness (normal); 1 indicates slight swelling and/or focal redness; 2 indicates low-to-moderate oedema, 3 indicates pronounced oedema with reduced paw function and 4 indicates excessive oedema with deformity and joint rigidity. The cumulative score for the two hind paws of each rat (a maximum score of eight) was used to represent the overall disease severity. To include a more objective measure of swelling, the paw thickness at the ankle joints and the tarsometatarsal joints of the hind paws were measured using a digital calliper (GS5071523 Vernier Caliper Mastercraft, Cape Town, South Africa).

3.4 Determination of blood pressure

Blood pressure was measured every two weeks by a tail-cuff technique employing non-evasive blood pressure (NIBP) amplifiers (Biopac Systems, Santa Barbara, CA, USA). Three measurements were taken for blood pressure and the average of the last two measures were used. The rats were placed in restrainers and tails were heated for ten minutes in order to obtain the blood pressure. To avoid diurnal variation, blood pressure measurements were taken at midday. The rats were habituated to the restrainers during the two-week habituation period prior to the first measurement being obtained.

3.5 Measurement of blood glucose and triglyceride concentrations

To ensure that differences in lipoproteins between the groups were not as a result of metabolic changes, blood glucose and triglyceride concentrations were measured in a sub-set of the control (n=15) and inflammation (n=15) group rats. Three days before termination, rats were fasted overnight, and fasting blood glucose and triglyceride concentrations were

determined from a drop of blood obtained from the tail vein via a pinprick. Glucose concentrations were determined using a calibrated glucometer (Ascensia EliteTM Blood glucose meter, Bayer Corporation, Mishawaka, USA) and triglyceride concentrations were determined using a calibrated Accutrend triglyceride analyzer (Roche, Mannheim, Germany).

3.6 Determination of circulating inflammatory marker concentrations

After thoracotomy, blood was sampled and allowed to clot for 2 hours at room temperature. Blood samples were centrifuged and serum was collected and stored at -80°C until assayed. Enzyme-linked immunosorbent assay (ELISA) kits (Elabscience Biotechnology Co. Ltd, Wuhan, China) were used to measure serum levels of TNF-α, IL-6, and CRP according to the instructions of the manufacturer (Elabscience Biotechnology Co. Ltd, Wuhan, China). The lower detection limit for TNF-α, IL-6, and CRP were 78.13 pg/ml, 62.50 pg/ml and 0.31 ng/ml respectively, all with coefficients of variation of <10%.

3.7 Determination of blood lipid profile

The HDL and LDL/VLDL Cholesterol Assay Kit (Cell Biolabs Inc., San Diego, CA, USA) was used to measure serum concentrations of HDL-C and LDL-C according to the manufacturer instructions. Absorbance was read at 570 nm with a microplate spectrophotometer and sample cholesterol concentrations were determined by interpolation from a standard curve.

3.8 Determination of ATP-binding cassette transporter A1

Serum concentration of ATP-binding cassette transporter A1 (ABCA1), a regulator of cholesterol metabolism, was measured by enzyme-linked immunosorbent assay (ELISA) (Elabscience Biotechnology Co. Ltd, Wuhan, China), according to the manufacturer

instructions). The lower detection limit for the ABCA1 kit was 0.16 ng/ml with a coefficient of variation <10%.

3.9 Determination of hepatic lipid content

3.9.1 Liver sample collection and storage

At termination, the liver was removed and weighed. The left lateral and medial lobes of the liver were stored at -80°C. A section of the left lateral lobe was used for liver lipid extractions. A section of the right median lobe was stored in 10% formalin and used for histology.

3.9.2 Liver lipid extraction

Liver lipid extractions were performed using the Tecator Soxtec method (Official Methods of Analysis of Analytical Chemists, 2005). Briefly, 0.5g of freeze-dried ground liver was packed into paper extraction thimbles (Whatman™, Buckinghamshire, United Kingdom) and placed into the extraction chamber of the Soxhlet apparatus. Laboratory distillation flasks (250ml) were weighed when empty and then filled with 200ml petroleum ether solvent and positioned onto the heating pads. The extraction procedure ran for 2 hours at the boiling point of the solvent (50 ± 10°C), while cool water was continuously supplied to the condensers. Thereafter, petroleum ether was evaporated with a rotary vacuum evaporator (Labex®, Krugersdorp, South Africa) and the oil remained in the flask. Flasks were dried in an oven at 60°C (Salvis Lab, Rotkreuz, Switzerland) for 20 minutes and then weighed. To obtain the lipid mass, the flask weight prior to the experiment was subtracted from the weight after the experiment. The lipid mass was expressed as a function of the total liver mass (% of liver mass).

3.10 Hepatic histological analysis

3.10.1 Tissue processing and sectioning

Liver tissue fixed in 10% formalin underwent a series of dehydration steps followed by the infiltration of Paraplast using a tissue processor (Thermo Fischer Scientific™, Citadel 2000 Tissue Processor). Once embedded onto cassettes, tissues were sectioned into 5µm thick sections with a microtome and were placed onto glass microscope slides. The slides were allowed to air-dry and adhere onto a 20°C heating block.

3.10.2 Haematoxylin and Eosin stain

The Haematoxylin and Eosin (H&E) stain was used to quantify the degree of steatosis (the accumulation of fat droplets) in liver tissue samples. Briefly, samples were deparaffinised with xylene, rehydrated through a graded series of ethanol and stained with Harris' haematoxylin and alcoholic eosin solution. The slides were then washed (dehydrated) through ethanol and xylene and a coverslip was placed with DPX mountant. The slides were viewed using a Zeiss Axioskop 2 Plus microscope equipped with a Zeiss AxioCam (Zeiss, Peabody, MA, USA) to examine the degree of steatosis.

Steatosis was scored by an experienced observer using a grid method (Biesterfeld et al., 2012; Franzén et al., 2005) whereby a grid consisting of several squares was superimposed on the images using ImageJ software (ImageJ version 1.4.3.67, NIH, Bethesda, USA). The steatosis touching the grid lines and the steatosis adjacent to the grid intersections were considered. The numbers of micro and macro steatosis per image were counted on a 400× magnification on a computer screen. Microsteatosis was characterised by the cytoplasmic appearance of small foamy fat droplets that do not displace the nucleus whereas macrosteatosis was characterised by the cytoplasmic appearance of enlarged vacuole sized fat droplets that displace the nucleus to the edge of the cell (Brown and Kleiner, 2016).

3.10.3 Masson's trichrome stain

To quantify the degree of liver fibrosis (the accumulation of collagen fibers), liver tissue samples were deparaffinised, rehydrated and fixed in Bouins solution for one hour and rinsed under running tap water. Samples were immersed in Weigert's iron hematoxylin working solution for 10 minutes and washed in distilled water. Thereafter, samples were immersed in Biebrich scarlet-acid fuchsin solution for 10 minutes, washed in distilled water and then differentiated in phosphomolybdic-phosphotungstic acid for 10 minutes. Samples were transferred to an aniline blue solution for 5-10 minutes, dehydrated in a 1% acetic acid solution for five minutes and washed in distilled water. Slides were dehydrated, cleared with xylene and mounted with DPX mounting medium. The samples were analysed using a Zeiss Axioskop 2 Plus microscope equipped with a Zeiss AxioCam (Zeiss, Peabody, MA, USA). Tissue sections were viewed with a 10× objective lens (100× magnification) under bright-field. The collagen area fraction was calculated for each tissue section by dividing the collagen area by the total tissue area using ImageJ software (ImageJ version 1.4.3.67, NIH, Bethesda, USA).

3.10.4 Picrosirius-Red stain

The Picrosirius red stain was used in the present study as an additional measure to quantify the degree of liver fibrosis. The Picrosirius red stain is highly specific and differentiates between different collagen fibre types when viewed under polarised light. Briefly, liver samples were deparaffinised, rehydrated and stained with a 0.1% Sirius Red solution dissolved in aqueous saturated picric acid for 60 minutes at room temperature. After washing in two changes of acidified water, slides were dehydrated, cleared with xylene and mounted with DPX mounting medium. The samples were analysed using a Zeiss Axioskop 2 Plus microscope equipped with a Zeiss AxioCam (Zeiss, Peabody, MA, USA). Tissue sections were viewed with a 10× objective lens (100× magnification) under bright-field and

polarized light. The collagen area fraction was calculated for each tissue section by dividing the collagen area by the total tissue area using ImageJ software (ImageJ version 1.4.3.67, NIH, Bethesda, USA).

3.11 Determination of surrogate markers of liver function

To evaluate potential drug-induced toxic effects on the liver, the serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), surrogate markers of liver function, were determined as previously described (Arhoghro *et al.*, 2009). Briefly, 150 µL of plasma was placed on the IDEXX panels and inserted in the colorimetric chemistry analyser (IDEXX, VetTest® Chemistry Analyzer, Netherlands) to determine the concentration of the liver enzymes.

3.12 Statistical Analysis

Statistical analysis was performed using the SAS software, version 9.4 (SAS Institute Inc., USA). Parametric data are expressed as means ± standard error of the mean (SEM) and nonparametric data are expressed as median (interquartile range, IQR). Differences in body weight, arthritis scores and blood pressure at baseline and termination were determined by repeated measures analysis of variance (ANOVA) followed by a Tukey *post-hoc* test. Differences in circulating inflammatory markers, lipid profile measurements (HDL-C and LDL-C, ABCA1, hepatic lipid content), liver morphology (degree of fibrosis), and liver toxicity enzymes between the groups were determined using a two-way ANOVA, followed by a Tukey *post-hoc* test. Differences in fasting glucose and triglyceride concentrations were determined using a student's unpaired t-test. The Kruskal-Wallis test followed by Dunn's *post-hoc* test was used to determine the differences in the degree of steatosis between the groups. Except for body weight, there were no sex differences in lipid profile measurements, hence the male and female data were pooled to increase statistical power. Associations

between circulating inflammatory marker concentrations and markers of fibrosis and liver toxicity enzymes were determined by Pearson's correlation coefficients in all the groups and in the control and CIA groups only. Because of the differences in body mass between the male and female rats, correlations were adjusted for body mass and sex. Differences were considered statistically significant if $p<0.05$.

Chapter 4

Results

4.1 Body weight, liver and adipose tissue weight, blood pressure, arthritis scores and circulating inflammatory marker concentrations

Table 4.1 shows the body weight, liver and adipose tissue weight, tail-cuff blood pressure and arthritis scores at baseline and termination as well as the circulating inflammatory marker concentrations at termination in the control, inflammation, TNF-α inhibitor, and IL-6 blocker groups. The body weight, systolic and diastolic blood pressures were similar between the groups at baseline and termination (all $p>0.05$). At termination the liver weight was similar between the groups (all $p>0.05$), however the adipose tissue weight was significantly lower in the inflammation (9.3 ± 0.7; $p=0.048$), TNF-α inhibitor (6.8 ±0.8; $p=0.001$) and IL-6 blocker (6.7 ±0.8; $p<0.0001$) groups compared to the control group (11.8 ± 0.7). When adjusting the adipose tissue weight for body weight, there were no longer differences between the control and inflammation groups ($p=0.23$) however differences between the control and TNF-α inhibitor ($p=0.0004$) and IL-6 blocker ($p=0.0008$) were materially unaltered. At termination, the mean (± SEM) arthritis scores were significantly higher in the inflammation (5.04 ± 0.32), TNF-α inhibitor (3.62 ± 0.46) and IL-6 blocker (4.87 ± 0.43) groups compared to the control group (0.00 ± 0.34; all $p<0.0001$). There were no significant differences in the arthritis scores between the inflammation, TNF-α inhibitor, and IL-6 blocker groups at termination (all $p>0.05$). The paw thickness at the ankle joints and tarsometatarsal joints were similar between the groups at baseline ($p>0.05$). At termination, the paw thickness at the ankle joint was significantly increased in the inflammation group compared to the control group ($p=0.01$). There were no differences in the paw thickness at the ankle joint between the control, TNF-inhibitor and IL-6 blocker groups ($p>0.05$). At termination, the paw thickness at the tarsometatarsal joint was significantly increased in the inflammation group compared to the control group ($p=0.03$). There were no differences in the paw thickness at the tarsometatarsal joint between the control, TNF inhibitor and IL-6 blocker groups ($p>0.05$).

The mean (±SEM) serum concentrations of CRP were significantly higher in the inflammation (0.64 ± 0.04 pg/ml; $p<0.0001$), TNF-α inhibitor (0.43 ± 0.05 pg/ml; $p<0.0001$) and IL-6 blocker (0.66 ± 0.06 pg/ml; $p<0.0001$) groups compared to the control group (0.14 ± 0.04 pg/ml). The mean (±SEM) serum concentrations of TNF-α were significantly higher in the inflammation (179.06 ± 9.48 pg/ml; $p<0.0001$), TNF-α inhibitor (145.58 ± 11.07pg/ml; $p=0.006$) and IL-6 blocker (162.08 ± 11.55pg/ml; $p=0.0001$) groups compared to the control group (96.21 ± 9.43 pg/ml). The mean (±SEM) serum concentrations of IL-6 were significantly higher in the inflammation (33.17 ± 2.17 pg/ml; $p<0.0001$), TNF-α inhibitor (27.79 ± 2.42 pg/ml; $p=0.02$) and IL-6 blocker (31.28 ± 2.53 pg/ml; $p=0.0008$) groups compared to the control group (18.19 ± 2.03 pg/ml). There were no significant differences in the serum concentrations of CRP, TNF-α and IL-6 between the inflammation, TNF-α inhibitor, and IL-6 blocker groups at termination (all $p>0.05$).

Table 4.1 Body weight, tail-cuff blood pressure, arthritis scores and circulating inflammatory marker concentrations in the control, inflammation, TNF-α inhibitor, and IL-6 blocker groups.

	Control	Inflammation	TNF-α inhibitor	IL-6 blocker
n	20	18	15	15
Male n (%)	17 (65)	14 (70)	8 (53)	8 (53)
Baseline				
Body weight (g)	403 ± 8	391 ± 9	391± 10	399 ± 11
Systolic blood pressure (mm Hg)	129 ± 2	127 ± 2	129 ± 2	131 ± 2
Diastolic blood pressure (mm Hg)	89 ± 2	89± 2	86 ± 2	88 ± 2
Arthritis score	0	0	0	0
Paw thickness				
Ankle joint (mm)	8.08 ± 0.20	8.30 ± 0.18	8.09 ± 0.24	7.93 ± 0.24
Tarsometatarsal joint (mm)	4.98 ± 0.15	4.71 ± 0.13	4.86 ± 0.18	4.73 ± 0.18
Termination				
Body weight (g)	478 ± 25	451± 24	393 ± 33	386± 31
Adipose tissue weight (g)	11.8 ± 0.7	9.3 ± 0.7*	6.8 ±0.8*	6.7 ±0.8*
Liver weight (g)	17.0 ±0.9	14.9 ±1.1	14.1 ±1.1	13.9 ±1.1
Systolic blood pressure (mm Hg)	127 ± 2	128 ± 2	130 ± 2	129 ± 2
Diastolic blood pressure (mm Hg)	87 ± 1	88 ± 2	86 ± 2	90 ± 2
Arthritis score	0.00 ± 0.34	5.04 ± 0.33*	3.62 ± 0.46*	4.87 ± 0.43*
Paw thickness				
Ankle joint (mm)	8.58 ± 0.24	9.59 ± 0.22*	9.14 ± 0.29	9.23 ± 0.29
Tarsometatarsal joint (mm)	5.04 ± 0.25	6.00 ± 0.23*	5.75 ± 0.30	5.68 ± 0.30
Inflammatory cytokines				
C-reactive protein (ng/ml)	0.14 ± 0.04	0.64 ± 0.04*	0.43 ± 0.05*	0.66 ± 0.06*
Tumour necrosis factor-alpha (pg/ml)	96.2 ± 9.4	179.1 ± 9.4*	145.6 ± 11.1*	164.1 ± 11.6*
Interleukin 6 (pg/ml)	18.2 ± 1.9	33.2 ± 2.2*	27.8 ± 2.4*	31.3 ± 2.5*

Data presented as mean ± SEM. * $p<0.05$ compared to control.

4.2 Fasting glucose and triglyceride concentrations

Table 4.2 shows fasting glucose and triglyceride concentrations measured prior to termination in a subset of the rats of the control and inflammation groups. There were no significant differences in the mean (±SEM) fasting glucose concentrations between the control (3.93 ± 0.17 mmol/l) and inflammation (3.55 ± 0.25 mmol/l; p=0.21) groups. There were no differences in the mean (±SEM) triglyceride concentrations between the control (3.35 ± 0.34 mmol/l) and inflammation (2.67 ± 0.38 mmol/l; p=0.18) groups.

Table 4.2 Fasting glucose and triglyceride concentrations in a subset of the control and inflammation group rats

	Control	**Inflammation**	**p-value**
n	15	15	
Glucose (mmol/l)	3.93 ± 0.17	3.55 ± 0.25	0.21
Triglycerides (mmol/l)	3.35 ± 0.34	2.67 ± 0.38	0.18

Data presented as mean ± SEM.

4.3 Blood lipids and ATP-binding cassette transporter A1 (ABCA1) concentrations

Serum HDL-C concentrations (mean ± SEM) were significantly lower in the inflammation (6.16 ± 0.34 μM; $p<0.0001$) and TNF-α inhibitor groups (7.07 ± 0.37 μM; $p=0.0004$) compared to the control group (9.17 ± 0.32 μM; Figure 4.1A). Serum HDL-C concentrations were significantly higher in the IL-6 blocker group (8.10 ± 0.43 μM) compared to the inflammation group ($p=0.004$; Figure 4.1A). There were no significant differences in the serum HDL-C concentrations of the control and IL-6 blocker groups ($p>0.05$; Figure 4.1A).

Serum ABCA1 concentrations (mean ± SEM) were significantly lower in the inflammation group (1.53 ± 0.26 ng/ml) compared to the control group (2.44 ± 0.22 ng/ml; $p=0.0448$; Figure 4.1 B). Serum ABCA1 concentrations were significantly higher in the TNF-α inhibitor group (3.00 ± 0.25 ng/ml; $p=0.0007$) and IL-6 blocker group (3.28 ± 0.26 ng/ml; $p<0.0001$) compared to the inflammation group (Figure 4.1B). There were no significant differences in the serum ABCA1 concentrations between the control, TNF-α inhibitor and IL-6 blocker groups ($p>0.05$).

There were no significant differences in the serum LDL-C concentrations between the control (4.22 ± 0.22 μM), inflammation (5.02 ± 0.25 μM), TNF-α inhibitor (4.53 ± 0.26 μM) or IL-6 blocker (4.33 ± 0.3 μM) groups ($p>0.05$; Figure 4.1C) groups. However, there was a trend towards an increased LDL-C concentration in the inflammation group compared to the control group ($p= 0.06$).

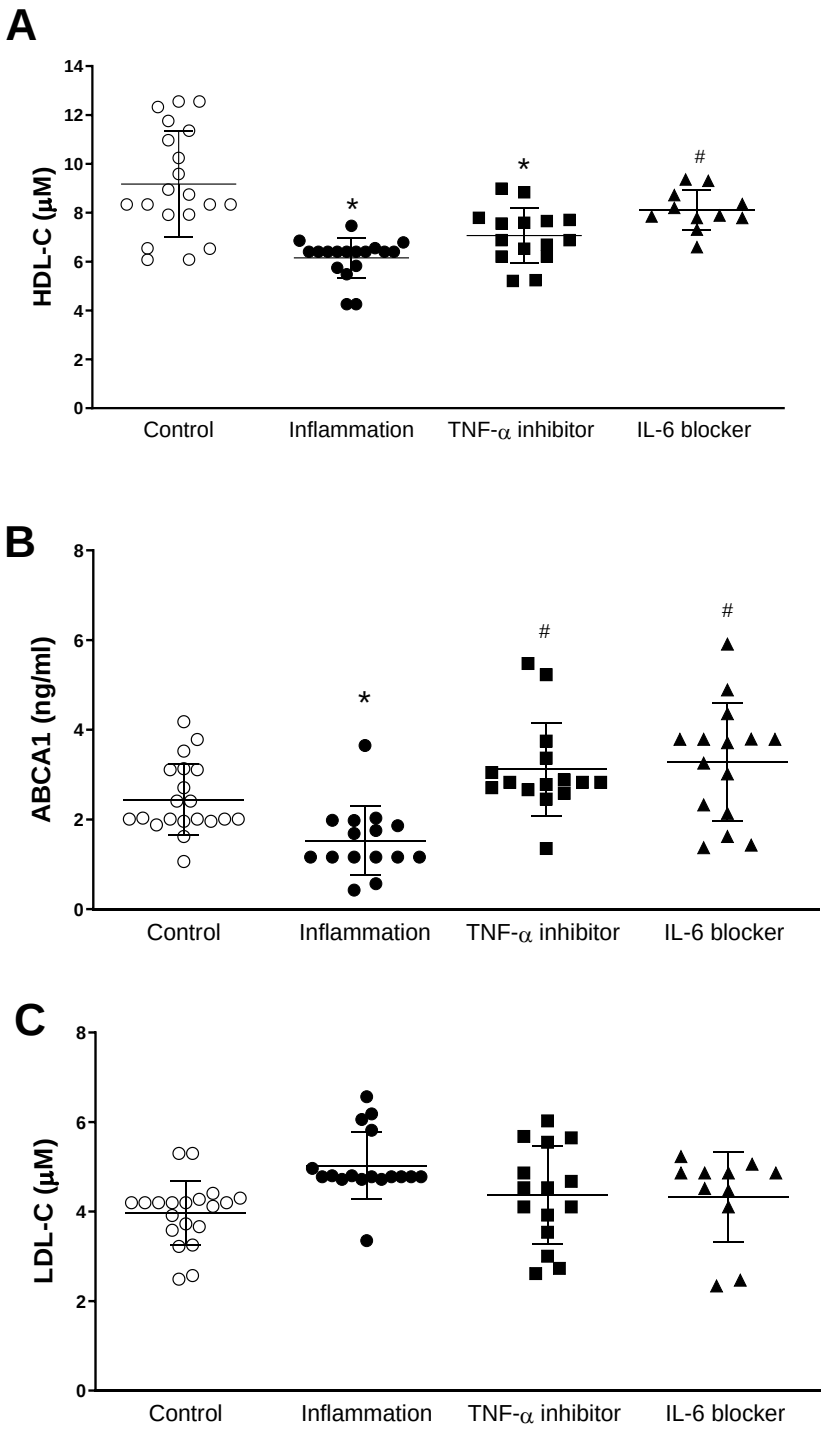

Figure 4.1 The serum concentrations of **(A)** HDL-C, **(B)** ABCA1 and **(C)** LDL-C in the control, inflammation, TNF-α inhibitor and IL-6 blocker groups. Data presented as mean ± SEM. HDL-C, high-density lipoprotein cholesterol; ABCA1, ATP-binding cassette transporter A1; LDL-C, low-density lipoprotein cholesterol. * $p<0.05$ compared to control. # $p<0.05$ compared to Inflammation

4.4 Liver lipid content

Compared to the control group (9.68 ± 0.36 %), the liver lipid content, expressed as a percentage of liver mass (mean ± SEM) was significantly lower in the inflammation (6.79 ± 0.40 %; p<0.0001), TNF-α inhibitor (6.90 ± 0.40 %; p<0.0001) and IL-6 blocker (8.03 ± 0.35 %; p=0.01) groups (Figure 4.2). There were no significant differences in the liver lipid content between the inflammation, TNF-α inhibitor, and IL-6 blocker groups (p>0.05; Figure 4.2).

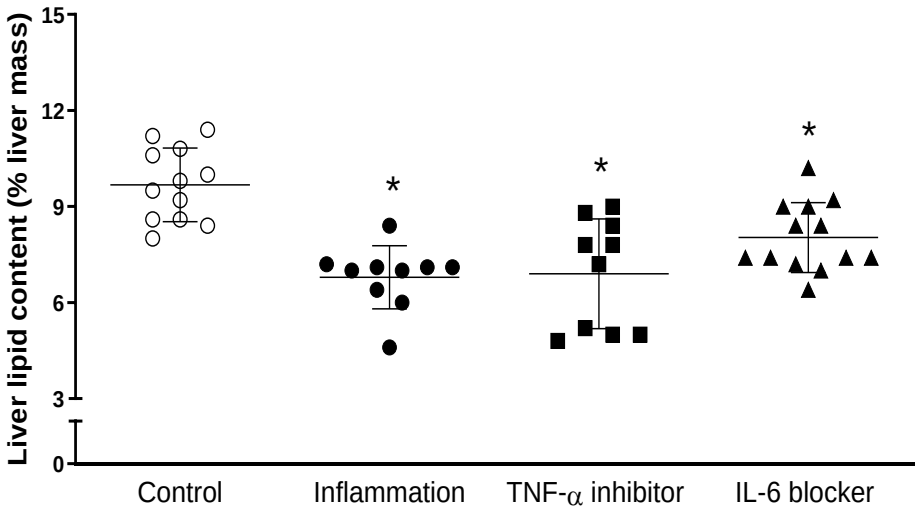

Figure 4.2 The lipid liver lipid content in the control (n=12), inflammation (n=10), TNF-α inhibitor (n=10) and IL-6 blocker (n=13) groups.* p<0.05 compared to control.

4.5 Degree of steatosis

There were no significant differences in the degree of steatosis, as indicated by micro or macro steatosis, between the control, inflammation, TNF-α inhibitor and the IL-6 blocker groups ($p>0.05$) (Table 4.3). Figure 4.3 shows that there were no differences in the representative liver histology sections stained with hematoxylin and eosin (400x magnification) between the groups.

Table 4.3 The degree of steatosis in liver samples of the control, inflammation, TNF-α inhibitor, and IL-6 blocker groups.

	Control	**Inflammation**	**TNF-α inhibitor**	**IL-6 blocker**
n	20	18	15	11
Micro steatosis	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)
Macro steatosis	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)

Data presented as median (interquartile range)

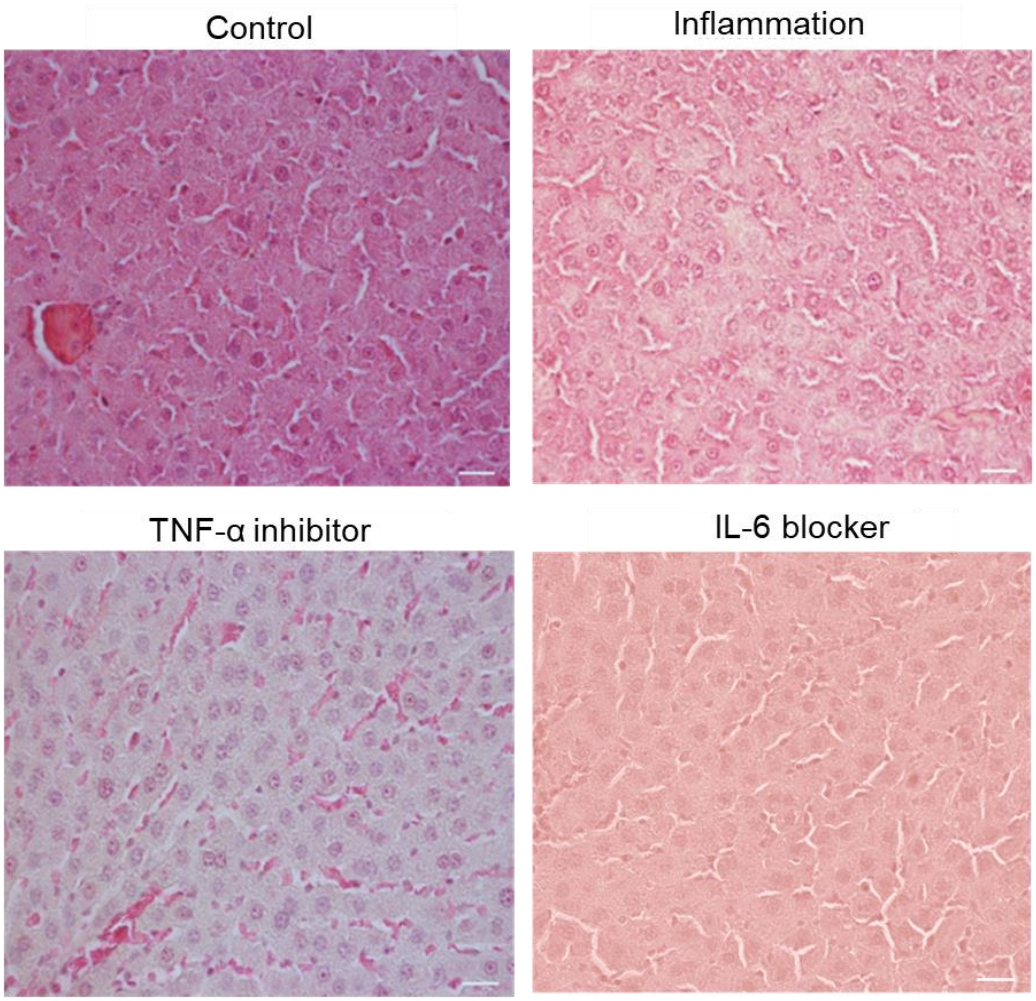

Figure 4.3 Representative liver histology stained with hematoxylin and eosin (400× magnification) from the control, inflammation, TNF-α inhibitor and IL-6 inhibitor groups. Scale bar = 20 μm

4.6 Degree of fibrosis

4.6.1. Masson's trichrome

There was greater collagen accumulation (more fibrosis) in the TNF-α inhibitor and IL-6 blocker groups compared to the control and inflammation groups, as evidenced by the increased blue staining of liver tissue sections visualised under bright-field microscopy (Figure 4.4A). Figure 4.4B shows that the collagen area fraction (mean ± SEM) was significantly greater in the TNF-α inhibitor (2.81 ± 0.31 %; $p<0.0001$) and IL-6 blocker (3.04 ± 0.25 %; $p<0.0001$) groups compared to the control group (1.19 ± 0.12 %; both $p<0.0001$) and the inflammation group (1.83 ± 0.27; $p=0.03$ and $p=0.006$ respectively).

4.6.2. Picrosirius red

There was greater collagen accumulation (more fibrosis) in the TNF-α inhibitor and IL-6 blocker groups compared to the control and inflammation groups, as evidenced by the increased red staining of liver tissue sections visualised under bright-filed microscopy (Figure 4.5A). Under polarized light, hepatic tissue sections of the TNF-α inhibitor and IL-6 blocker groups showed increased presence of large and thin collagen fibres, where large collagen fibres appear orange or yellow and thin fibres appear green (Figure 4.5B). The collagen area fraction (mean ± SEM) was significantly greater in the TNF-α inhibitor (2.37 ± 0.23 %; $p=0.03$) and IL-6 blocker (2.91 ± 0.21 %; $p<0.0001$) groups compared to the control group (1.51 ± 0.18 %; Figure 4.5C). There were no significant differences between the collagen area fraction of the control and inflammation groups (1.95 ± 0.16 %; $p=0.30$; Figure 4.5C). The collagen area fraction was significantly greater in the IL-6 blocker group compared to the inflammation group ($p=0.004$; Figure 4.5C). There were no significant differences in the collagen area fraction between the inflammation and TNF-α inhibitor groups ($p=0.47$; Figure 4.5C).

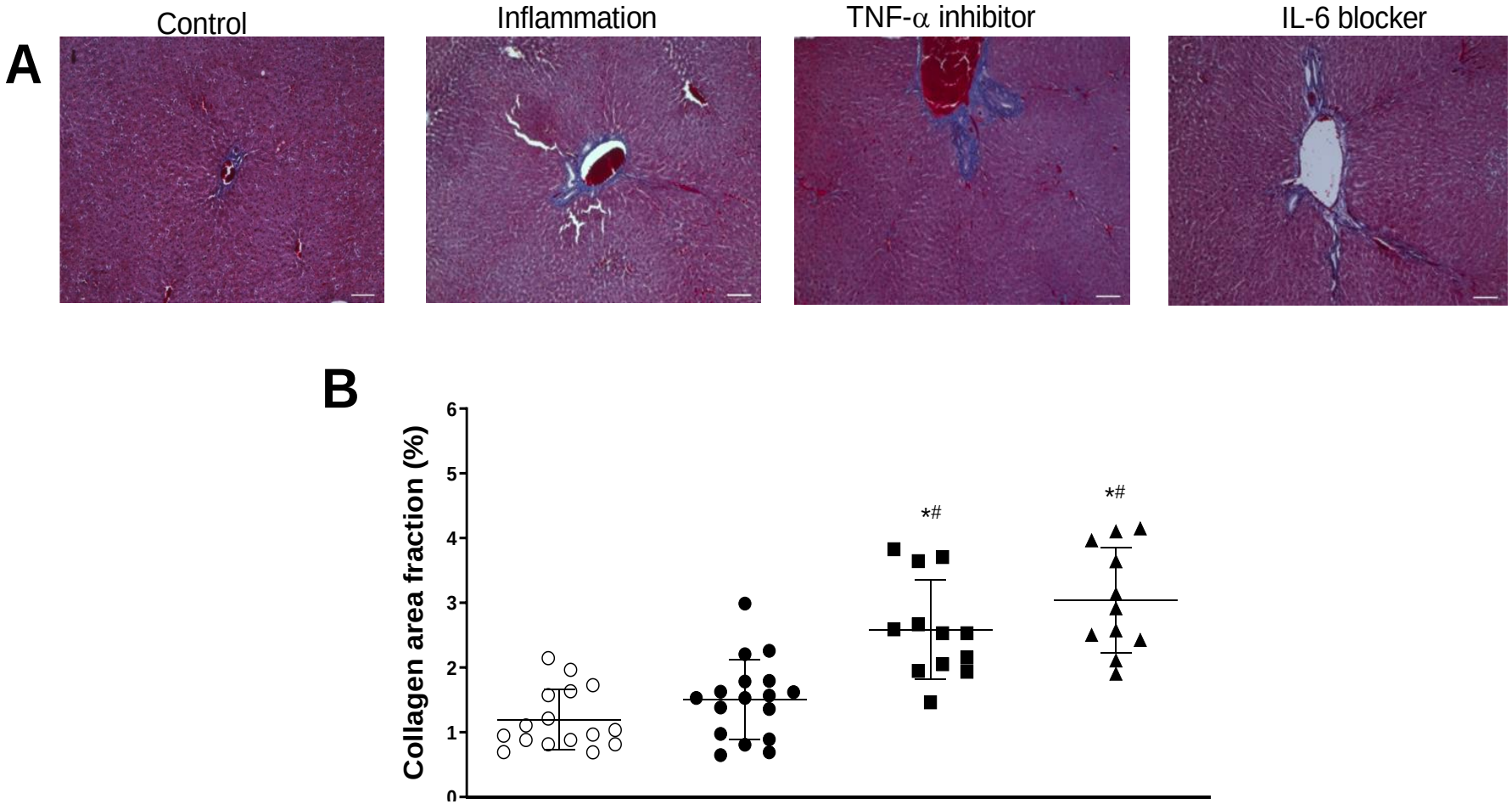

Figure 4.4 (A) Representative liver histology sections stained with Masson's trichrome (100x magnification) viewed in bright field. **(B)** Collagen area fraction (degree of fibrosis) calculated from the stained liver sections of the control (n=16), inflammation (n=17), TNF-α inhibitor (n=12) and IL-6 blocker (n=11) groups. * $p<0.05$ compared to control. # $p<0.05$ compared to inflammation. Scale bar = 200μm

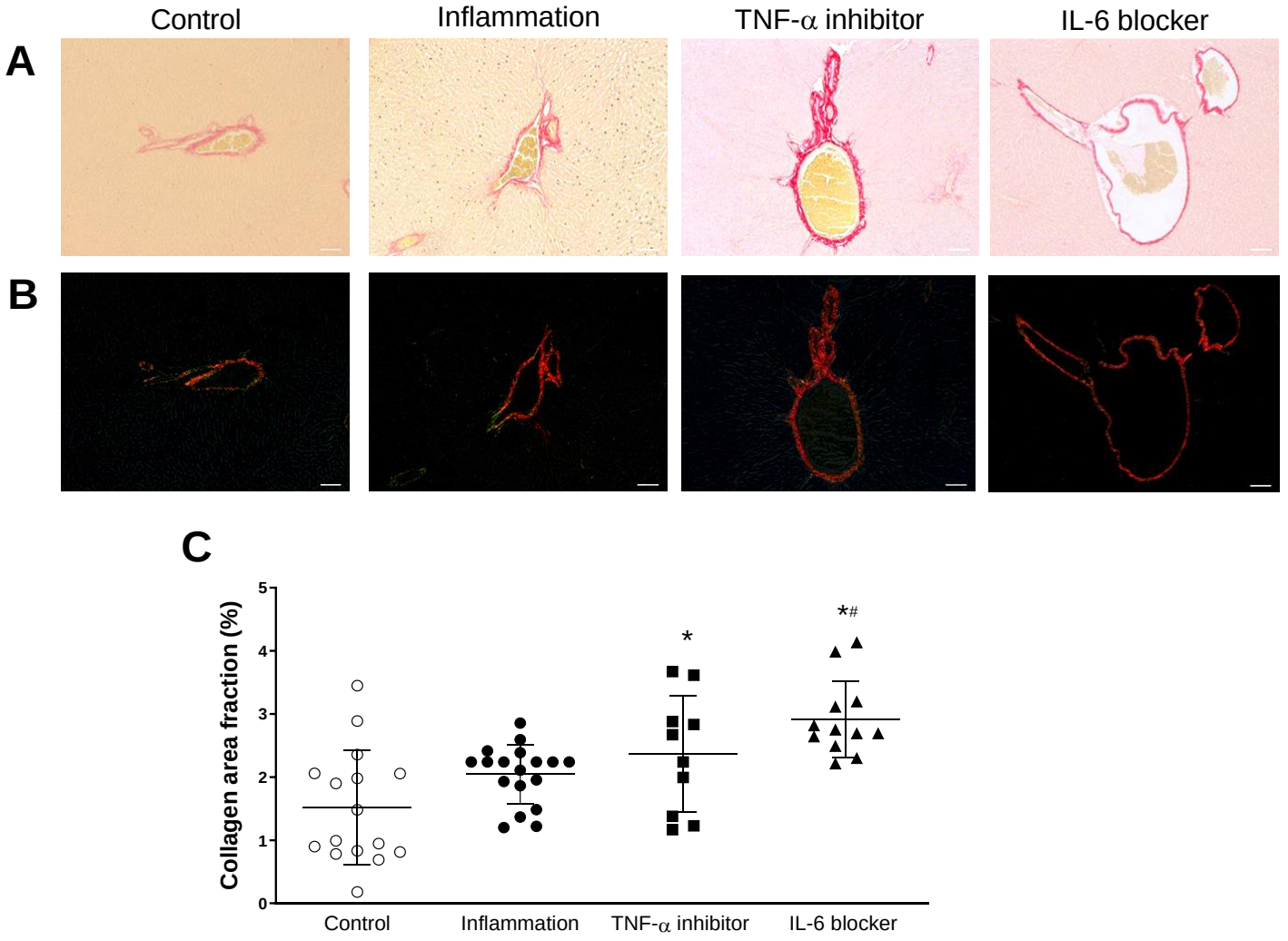

Figure 4.5 Representative liver histology sections stained with picrosirius red (100x magnification) viewed in **(A)** bright field and **(B)** under polarized light. **(C)** Collagen area fraction (degree of fibrosis) calculated from the stained liver sections of the control (n=16), inflammation (n=20), TNF-α inhibitor (n=10) and IL-6 blocker (n=12) groups. * $p<0.05$ compared to control. $^{\#}$ $p<0.05$ compared to inflammation. Scale bar = 200µm

4.7 Associations between circulating inflammatory markers and liver fibrosis

4.7.1 Masson's trichrome collagen area fraction

When including all the groups in the analysis, no significant associations were observed between the serum concentrations of CRP (r=0.21, p=0.12), TNF-α (r=0.11, p=0.49) or IL-6 (r=0.27, p=0.05) with the degree of fibrosis (Table 4.4). These associations were materially unaltered when adjusting for body weight and sex (Table 4.4).

When including only the control and inflammation groups in the analysis, no significant associations were observed between the circulating inflammatory markers and the degree of fibrosis (Table 4.4).

4.7.2 Picrosirius red collagen area fraction

When including all the groups in the analysis, higher serum concentrations of CRP were associated with a greater degree of fibrosis (r=0.31, p=0.02; Table 4.4). This association remained unaltered when adjusting for body weight and sex (CRP: partial r=0.29, p=0.04; Table 4.4). There were no significant associations between the serum concentrations of TNF-α (r=0.08, p=0.56) or IL-6 (r=0.11, p=0.47) and the degree of fibrosis (Table 4.4). These associations were materially unaltered when adjusting for body weight and sex (Table 4.4).

When including only the control and inflammation groups in the analysis, no significant associations were observed between the circulating inflammatory markers and the degree of fibrosis (Table 4.4).

Table 4.4 Associations between circulating inflammatory markers with the degree of liver fibrosis in all groups and in the control and inflammation groups only

	Unadjusted				**Body weight and sex adjusted**			
	Masson's trichrome collagen area fraction		**Picrosirius red collagen area fraction**		**Masson's trichrome collagen area fraction**		**Picrosirius red collagen area fraction**	
	r(95% CI)	p-value	r(95% CI)	p-value	partial r (95%CI)	p-value	partial r (95%CI)	p-value
All groups								
CRP	0.21(-0.06-0.46)	0.12	**0.31(0.12 - 0.51)**	**0.02**	0.25(-0.05-0.51)	0.11	**0.29(0.04 - 0.54)**	**0.04**
TNF-α	0.11(-0.18-0.37)	0.49	0.08(-0.19 - 0.34)	0.56	0.14(-0.17-0.42)	0.38	0.05(-0.25 - 0.34)	0.74
IL-6	0.27(-0.001-0.50)	0.05	0.11(-0.18 - 0.37)	0.47	0.22(-0.08-0.49)	0.15	-0.03(-0.33 - 0.27)	0.84
Control and inflammation groups only								
CRP	0.05(-0.31-0.39)	0.81	0.25(-0.10 - 0.54)	0.17	0.14(-0.27-0.50)	0.52	0.21(-0.20 - 0.56)	0.32
TNF-α	0.04(-0.34-0.42)	0.83	0.11(-0.26 - 0.46)	0.56	0.12(-0.29-0.49)	0.59	0.08(-0.32 - 0.47)	0.68
IL6	0.33(-0.04-0.61)	0.08	0.09(-0.25 - 0.45)	0.61	0.24(-0.17-0.58)	0.25	0.003(-0.41 - 0.39)	0.99

Significant values are represented in bold. r(95% CI), r-value (95% confidence interval). CRP: C- reactive protein, TNF-α: tumor necrosis factor alpha, IL-6: interleukin 6

4.8 Liver toxicity

The serum concentrations (mean ± SEM) of alkaline phosphatase (ALP) were significantly higher in the TNF-α inhibitor (116 ± 5.81 µl; p=0.02) and IL-6 blocker (141.85 ± 9.38 µl; p<0.0001) groups compared to the control group (87.16 ± 4.96 µl; Table 4.5). There were no significant differences between the serum concentrations (mean ± SEM) of ALP of the inflammation group (103.16 ± 6.97 µl) and the control group (p=0.26; Table 4.5). The serum concentrations of ALP were significantly higher in the IL-6 blocker group compared to the inflammation group (p=0.001; Table 4.5). There were no significant differences between the serum concentrations of ALP in the inflammation and TNF-α inhibitor groups (p>0.05; Table 4.5).

There were no significant differences in the serum concentrations (mean ± SEM) of alanine aminotransferase (ALT) in the control (61.93 ± 3.41 µl), inflammation (64.73 ± 3.45 µl), TNF-α inhibitor (65.85 ± 5.77 µl) or IL-6 blocker (54.75 ± 1.55 µl) groups (p>0.05; Table 4.5).

There were no significant differences in the serum concentrations of aspartate transaminase (AST) in the control (182.12 ± 9.75 µl), inflammation (182.12 ± 10.66 µl), TNF-α inhibitor (162.23 ± 9.54 µl) or IL-6 blocker (155.57 ± 6.63 µl) groups (p>0.05 Table 4.5).

Table 4.5 Serum concentrations of liver toxicity enzymes in the control, inflammation, TNF-α inhibitor, and IL-6 blocker groups

	Control	**Inflammation**	**TNF-α inhibitor**	**IL-6 blocker**
n	20	18	13	14
ALP (μl)	87.16 ± 4.96	103.16 ± 6.97	116 ± 5.81*	141.85 ± 9.38*#
ALT (μl)	61.93 ± 3.41	64.73 ± 3.45	65.85 ± 5.77	54.75 ± 1.55
AST (μl)	182.12 ± 9.75	182.18 ± 10.66	162.23 ± 9.54	155.57 ± 6.63

Data presented as mean ± SEM. * $p<0.05$ compared to control, # $p<0.05$ compared to inflammation. ALP, alkaline phosphatase; ALT, Alanine aminotransferase; AST, Aspartate transaminase.

4.9 Associations between circulating inflammatory markers and liver toxicity

When including all the groups in the analysis, higher serum concentrations of CRP was associated with higher serum concentrations of ALP (r=0.31, p=0.03; Table 4.6). This association remained unaltered when adjusting for body weight and sex (partial r=0.28; p=0.04; Table 4.6). There were no significant associations between the serum concentrations of TNF-α (r=0.14, p=0.28) or IL-6 (r=0.11, p=0.46) with serum concentrations of ALP (Table 4.6). These associations were materially unaltered when adjusting for body weight and sex (Table 4.7). There were no associations between circulating inflammatory markers and serum concentrations of AST or ALT (all p>0.05; Table 4.6).

When including only the control and inflammation groups in the analysis, no significant associations were observed between circulating inflammatory markers and serum concentrations of ALP, AST or ALT (all p>0.05; Table 4.6).

Table 4.6 Associations between circulating inflammatory markers with markers of liver toxicity in all groups and in the control and inflammation groups only

	ALP versus		**ALT versus**		**AST versus**	
	r(95% CI)	p-value	r(95% CI)	p-value	r(95% CI)	p-value
In all groups						
CRP	**0.31(0.05-0.52)**	**0.03**	-0.11(-0.33-0.15)	0.45	0.01(-0.23-0.25)	0.94
TNF-α	0.14(-0.12-0.38)	0.28	-0.07(-0.32-0.19)	0.59	-0.13(-0.38-0.12)	0.31
IL-6	0.11(-0.17-0.35)	0.46	-0.09(-0.33-0.16)	0.48	-0.05(-0.29-0.21)	0.73
In control and inflammation groups only						
CRP	0.07(-0.28-0.40)	0.71	0.03(-0.29-0.34)	0.85	0.03(-0.29-0.34)	0.85
TNF-α	0.07(-0.28-0.40)	0.71	-0.02(-0.35-0.32)	0.91	-0.23(-0.54-0.11)	0.18
IL-6	0.12(-0.22-0.43)	0.48	0.001(-0.32-0.33)	0.99	-0.09(-0.41-0.24)	0.58

Significant values are represented in bold. r(95% CI), r-value (95% confidence interval). ALP, alkaline phosphatase; ALT, Alanine aminotransferase; AST, Aspartate transaminase; CRP. C- reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin 6.

Table 4.7 Body weight and sex adjusted associations between circulating inflammatory markers with markers of liver toxicity in all groups and in the control and inflammation groups only

	ALP (µl)		**ALT (µl)**		**AST (µl)**	
	Partial r(95% CI)	p-value	Partial r(95% CI)	p-value	Partial r(95% CI)	p-value
In all groups						
CRP	**0.28(0.02 - 0.48)**	**0.04**	-0.08(-0.33 - 0.19)	0.56	-0.01(-0.27 - 0.25)	0.94
TNF-α	0.03(-0.24 - 0.31)	0.83	-0.22(-0.46 - 0.04)	0.10	-0.12(-0.37 - 0.15)	0.39
IL-6	0.08(-0.19 - 0.34)	0.57	-0.11(-0.36 - 0.16)	0.42	-0.01(-0.27 - 0.26)	0.95
In control and inflammation groups only						
CRP	0.19(-0.18 - 0.51)	0.32	-0.13(-0.46 - 0.24)	0.50	-0.33(-0.61 - 0.02)	0.07
TNF-α	0.09(-0.27 - 0.44)	0.62	-0.01(-0.36 - 0.35)	0.96	-0.14(-0.47 - 0.22)	0.44
IL-6	0.16(-0.22 - 0.49)	0.41	-0.13(-0.47 - 0.23)	0.47	-0.16(-0.49 - 0.21)	0.39

Significant values are represented in bold. r(95% CI), r-value (95% confidence interval). ALP, alkaline phosphatase; ALT, Alanine aminotransferase; AST, Aspartate transaminase; CRP. C- reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin 6.

Chapter 5

Discussion

The present study investigated the effects of inflammation and anti-inflammatory drug treatment on lipid metabolism and liver morphology in rats exposed to CIA. The main findings of the current study are that exposure to high-grade inflammation impaired lipid metabolism by reducing the serum concentrations of HDL-C and ABCA1 and the total liver lipid content. These findings indicate that lipid metabolism is adversely affected in rats exposed to high-grade inflammation. Treatment with TNF-α inhibitors marginally improved circulating ABCA1 concentrations, but that did not translate into increased HDL-C concentrations. TNF-α inhibitors induced liver injury in rats exposed to CIA as evidenced by the increased degree of fibrosis and ALP concentrations. Treatment with IL-6 blockers improved the overall lipid profiles of rats exposed to CIA as evidenced by increased concentrations of ABCA1 and HDL-C. Despite the improved lipid profiles, IL-6 blockers resulted in increased liver fibrosis and liver toxicity. These findings suggest that treatment with TNF-α inhibitors and IL-6 blockers have beneficial effects on inflammation-induced lipid profiles but may induce hepatotoxicity.

5.1 The effects of high-grade inflammation on lipid profiles

In the present study, exposing Sprague-Dawley rats to CIA, resulted in changes in lipid profiles as evidenced by reduced serum concentrations of HDL-C, ABCA1 and total hepatic lipid content. Despite the changes in HDL-C, findings from the current study showed no changes in the LDL-C concentrations after exposure to inflammation. Similarly, previous animal models of high-grade inflammation also reported no changes in LDL-C concentrations following exposure to high-grade inflammation (Carbajo-Pescador et al., 2019). In contrast, others have shown reduced LDL-C concentrations in rats exposed to inflammation (Pérez-Baos et al., 2017; Wendt et al., 2019). Available data on the effects of inflammation on the LDL-C concentrations in clinical RA studies are controversial. Some RA studies have reported reduced LDL-C concentrations (Charles-Schoeman et al., 2015;

Chung et al., 2010; Lazarevic et al., 1992; Wendt et al., 2019), while others reported no changes in LDL-C concentrations (Cacciapaglia et al., 2018; Chen et al., 2015; Kim et al., 2016; Nassir et al., 2007; Popa et al., 2005; Serhan et al., 2015; Spanakis et al., 2006; Tournadre et al., 2017). Regardless of the LDL-C changes in clinical and experimental studies of RA, there is an agreement that chronic high-grade inflammation in RA leads to a more atherogenic lipid profile (total cholesterol/HDL-C), largely as a result of reduced HDL-C concentrations (Guo et al., 2017; Nurmohamed, 2007; Yeh et al., 2014).

The reduced HDL-C, ABCA1 and liver lipid content in the inflammation group in the current study is consistent with previous clinical studies that reported reduced serum concentrations of HDL-C, ABCA1, total lipids and total cholesterol in patients with RA compared to healthy controls (Field et al., 2010; Guo et al., 2017; Hameed and Al-Barzinjy, 2017; Kim et al., 2016; Lazarevic et al., 1992; Machado et al., 2012; Nassir et al., 2007; Nurmohamed, 2007; Ronda et al., 2015, 2014; Sha Ma et al., 2013; Voloshyna et al., 2013; Yin et al., 2010). Similar to the current findings, previous animal studies of high-grade inflammation also reported reduced HDL-C of rats exposed to AIA despite unchanged body weight, glucose or triglyceride concentrations (El-Gaphar et al., 2018; He et al., 2013; Patel and Pundarikakshudu, 2016; Wendt et al., 2019).

Besides the change in HDL-C concentrations, it has been shown that the HDL lipidome and proteome, and hence its function, are significantly altered under conditions of inflammation (Kajani et al., 2018). The inflammation-induced changes in the HDL lipidome and proteome reduces its cholesterol efflux capacity (Kajani et al., 2018). Although several functional features contribute to the cholesterol efflux capacity of HDL particles (Genest and Choi, 2017; Kajani et al., 2018) one of the factors that determine HDL-C concentration and functionality is ABCA1 expression.

ABCA1 is predominantly expressed in tissues that maintain lipid homeostasis or in tissues involved in lipid turnover including the liver, adipose tissue, small intestine and macrophages (Kang et al., 2013; Yin et al., 2010). ABCA1 functions by forming an extracellular hydrophobic tunnels or pathways that facilitate the transfer of phospholipids (Qian et al., 2017). Apolipoproteins such as apolipoprotein A-I, the backbone of HDL, then bind to the ABCA1 to form complexes between the apolipoprotein A-I and lipids, which form the nascent HDL particles (Segrest et al., 2015). These nascent HDL particles are then able to interact with other lipid transporters to take up more cholesterol (Rosenson et al., 2012). Hence the binding of apolipoprotein to ABCA1 is considered a key step in the functionality of HDL cholesterol efflux capacity (Segrest et al., 2015). Therefore, the expression of ABCA1 is considered a major determinant of the functionality of HDL particles.

Inflammatory cytokines have previously been shown to change ABCA1 expression. In this regard, TNF-α downregulates ABCA1 gene expression in ABCA1 expressing cells by decreasing promoter activity through activation of the nuclear factor-kappa B (NF-κB) dependant pathways (Edgel et al., 2010; Field et al., 2010; Gerbod-Giannone et al., 2006; Yin et al., 2010; Zhao and Dong, 2008). Furthermore, TNF-α has also been shown to enhance the rate of ABCA1 protein degradation (Field et al., 2010). Indeed studies have shown that incubating ABCA1 expressing cells with exogenous TNF-α downregulated ABCA1 mRNA and protein expression in some human (Field et al., 2010; Jang et al., 2008; Mei et al., 2007; Sha Ma et al., 2013; Zhang et al., 2010) and animal (Edgel et al., 2010; Ma et al., 2008; Sato et al., 2018; Tang, 2012; Zhang et al., 2010; Zhao and Dong, 2008) cell lines in a time and dose-dependent manner. Pre-clinical studies that have reported on the administration of exogenous IL-6 to ABCA1 expressing cells have been contradictory (Frisdal et al., 2011; Mao et al., 2014; Stamatikos et al., 2019). While some have shown that IL-6 downregulates the mRNA and protein expression of ABCA1 via the Janus-activated kinase-signal transducer and activator of transcription (Jak-STAT) signalling pathways

(Frisdal et al., 2011, Mao et al., 2014), a different study failed to support these results (Stamatikos et al., 2019). A study identified signal transducers and activators of the transcription-3 element in the first intron of the human ABCA1 gene (Frisdal et al., 2011), which may be involved in the regulation of ABCA1 gene expression by IL-6.

Nevertheless, the downregulation of ABCA1 results in decreased ABCA1-mediated cholesterol efflux which hinders the maturation process of nascent HDL to mature HDL (Babashamsi et al., 2019). Thus, decreased HDL maturation may have contributed to the reduced circulatory HDL-C in the current study. Furthermore, a reduction in HDL-C as a result of reduced ABCA1 activity may result in decreased clearance of excess cholesterol via reverse cholesterol transport, which may explain the trend towards an increased LDL-C in the inflammation group in the current study.

With regards to the total liver lipid content, the evidence of hepatic lipid accumulation in RA is controversial. In arthritis, hyperlipidaemia is not common, as both HDL and total cholesterol are decreased (Choy et al., 2014; Robertson et al., 2013). However, in arthritis lipolysis in adipose tissue is increased, which results in increased plasma free fatty acids (Wendt et al., 2019). Even in the fed state, adipose tissue lipolysis is increased in arthritis (Wendt et al., 2019). Similar to previous reports (Wendt et al., 2019), in the current study, we showed significantly reduced adipose tissue mass, independent of body mass in the rats exposed to inflammation. Despite the evidence of increased plasma free fatty acids, there is controversy regarding the uptake of free fatty acids in the liver and muscle (Frayn et al., 2006; Wendt et al., 2019). Some suggest that free fatty acid uptake in the liver is decreased (Frayn et al., 2006), while others reported increased uptake (Wendt et al., 2019; Delano et al., 2006). Furthermore, it has been reported that inflammation induces hepatic *de novo* lipid synthesis and suppresses fatty acid β-oxidation, which would further result in hepatic lipid accumulation (Chen et al., 2009; Khovidhunkit et al., 2004).

Nevertheless, it is well accepted that in RA, hepatic lipid metabolism is altered as a result of the increased circulating inflammatory cytokines that increase oxidative stress in the liver (Robertson et al., 2017; Strang et al., 2013). The current study showed that total liver lipid content was reduced in the group exposed to inflammation compared to the control group. These findings are in agreement with a previous study in AIA rats that reported that chronic inflammation reduced the liver lipid content, despite an increased free fatty acid plasma concentration and uptake in the liver (Wendt et al., 2019). Nevertheless, these authors reported that total cholesterol, HDL-C and LDL-C, as well as triglycerides, were reduced in rats exposed to inflammation (Wendt et al., 2019). Hence, the authors suggested that despite increased free fatty acid uptake in the liver, fatty acid synthesis in the liver may also be reduced, as the acetyl-CoA carboxylase enzyme activity, an important enzyme in fatty acid synthesis, were downregulated (Wendt et al., 2019). Furthermore, oxidation of endogenous and exogenous fatty acids were increased in the livers of rats exposed to inflammation in the previous study, suggesting that the hepatic metabolism of fatty acids are catabolic rather than anabolic, mainly as a result of increased oxidative stress (Wendt et al., 2019). The current study supports previous findings, as there was no evidence of either increased liver weight or micro or macro steatosis in any of the groups exposed to inflammation in the current study. Although some studies have reported steatosis in RA patients (Tang et al., 2020) steatosis is likely induced by methotrexate therapy rather than the inflammation (Akhlaghi et al., 2018; Curtis et al., 2010; Dehestani et al., 2015; Jones et al., 2010; Oan et al., 2000; Schiff et al., 2011; Sotoudehmanesh et al., 2010).

Taken together the current results show, in an animal model which closely resembles the pathogenesis of RA in humans, that exposure to high-grade systemic inflammation independently impairs lipid metabolism. In this regard, I showed a decreased production of HDL, which may be attributed to a decreased ABCA1 expression. The current study further confirms that liver lipid metabolism was altered, as liver lipid content was decreased in rats

exposed to inflammation. However, since plasma free fatty acid concentrations or molecular markers of liver metabolism were not measured in this study, the direct cause of lipid metabolism impairments in chronic inflammation cannot be confirmed. Future studies should investigate the mechanisms of inflammation-induced changes in lipid metabolism.

5.2 The effects of TNF-α inhibitor therapy on the lipid profiles

In the present study, treatment with TNF-α resulted in a significant increase in ABCA1 concentrations. Although there have been no previous reports on the effect of TNF-α inhibitor treatments on circulating ABCA1 levels, previous pre-clinical studies have reported that exogenous TNF-α administration directly reduced the expression of ABCA1 (Field et al., 2010; Gerbod-Giannone et al., 2006; Voloshyna et al., 2014). Furthermore, TNF-α neutralizing antibodies improved the gene expression of ABCA1 (Voloshyna et al., 2014). Despite the improvement in ABCA1 levels following TNF-α inhibitor treatment, this improvement did not translate to increased HDL-C or LDL-C concentrations or liver lipid content in the current study. Consistent with our findings, treatment with anti-TNF-α drugs did not affect inflammation-induced lipid alterations in the adjuvant-induced rat model (El-Ashmawy et al., 2019; Totoson et al., 2016).

Despite the well-known cardioprotective effects of HDL, it has been suggested that it may not be the quantity of HDL that is predictive of atherosclerosis, but rather the quality of the HDL particles (Genest and Choi, 2017). Indeed, it has been shown that increasing HDL-C concentrations does not necessarily translate into reduced atherosclerotic CVD risk (Boekholdt et al., 2013; Kingwell et al., 2014; Madsen et al., 2017). The mass of HDL is only weakly correlated to the anti-inflammatory and anti-oxidative effects of HDL (Boekholdt et al., 2013; Kingwell et al., 2014; Kontush et al., 2013; Madsen et al., 2017; Vaisar et al., 2015). In contrast, the lipidome and proteome quality of HDL particles are more strongly related to these cardioprotective effects of HDL (Boekholdt et al., 2013; Kingwell et al., 2014; Kontush

et al., 2013; Madsen et al., 2017; Vaisar et al., 2015). Increasing HDL-C concentration as a therapeutic target does not reduce the risk of atherosclerotic CVD, as an improved HDL-C concentration does not guarantee improved functionality (Choi et al., 2017a; Lüscher et al., 2014). Indeed, it has been shown the functionality and cholesterol efflux capacity of HDL particles are more strongly linked to atherosclerosis and cardiovascular events (Khera et al., 2017, 2011; Rohatgi et al., 2014). Therefore, in disease states, it has been suggested that HDL-C is not a reliable predictor of CV outcomes, but rather the HDL functional properties (Genest and Choi, 2017). Hence, in the current study, even though HDL-C concentrations did not increase after TNF-α inhibitor treatment, the increased ABCA1 concentrations suggest that there may be greater amounts of mature HDL particles with greater cholesterol efflux capacity. Indeed, studies have shown that a reduced breakdown of ABCA1 improves HDL cholesterol efflux capacity and reduced the risk of atherosclerosis (Arakawa et al., 2009; Huang et al., 2015). Nevertheless, the aforementioned studies showed an increased HDL concentration when ABCA1 breakdown was reduced. This raises the question, whether the increased functionality of HDL particles, as evidenced by increased ABCA1 concentrations, independent of the HDL-C concentrations, translates into long term protection against atherosclerosis. In this regard, in a murine model of atherosclerosis (apolipoprotein E deficient mice), it has been shown that increased ABCA1 concentrations increases the cholesterol efflux capacity of HDL particles and reduced the risk of atherosclerosis, despite very small changes in HDL-C concentrations (Singaraja et al., 2002). Similarly, Wang et al (2017) reported that treatment with Tofacitinib, a JAK inhibitor, increased ABCA1 expression without a change in HDL-C concentrations. These authors showed reduced atherosclerosis in apolipoprotein E deficient mice, which was ascribed to the change in ABCA1, independent of lipid concentrations (Wang et al., 2017b). Furthermore, a clinical study using anti-inflammatory treatments (IL-1β blockers), showed that reducing systemic inflammation decreased the incidence of cardiovascular events

independent of a change in HDL or LDL cholesterol levels (Ridker et al., 2017). Nevertheless, the clinical study did not report any markers of HDL functionality, hence further studies need to elucidate the effect of anti-inflammatory treatment on the functionality of HDL particles and specifically on ABCA1 concentrations.

There are several possible reasons why inhibiting TNF-α may have ameliorated the inflammation-induced changes in lipid profiles in the current study. In this regard, TNF-α is a pro-inflammatory cytokine that exerts its effects on lipid metabolism at varying cells, tissues and organs (Chen et al., 2009). It exists biologically in the soluble and transmembrane TNF-α form (which is the precursor form of soluble TNF-α) (Deora et al., 2017; Wullaert et al., 2007). TNF-α mediates biological processes by acting on plasma membrane receptors: tumor necrosis factor-alpha receptor 1 (TNFR1; also known as p55 or p60) and receptor 2 (TNFR2; p75 or p80) to initiate intracellular signalling transduction which regulate gene transcription (Parameswaran and Patial, 2010; Schumacher and Naga Prasad, 2018). TNFR1 and TNFR2 can be proteolytically cleaved to release the soluble receptor form (sTNFR) (Cawthorn and Sethi, 2008). TNF-α is deactivated when binding to the sTNFR which dampens the inflammatory response (Narayanan et al., 2011), therefore this sTNFR is possibly involved in the neutralisation and excretion of TNF-α (Cawthorn and Sethi, 2008). Soluble TNF-α acts primarily through TNFR1, which is constitutively expressed in most body cells. Transmembrane TNF-α mediates its biological activities on both TNF-α receptors but mainly through the highly regulated TNFR2 that is mostly expressed in certain neurons, immune and endothelial cells (Deora et al., 2017; Parameswaran and Patial, 2010; Pegoretti et al., 2018; Ruder et al., 2019; Wullaert et al., 2007).

Etanercept is a genetically engineered human dimetric fusion protein consisting of the extracellular portion of the human p75 TNF receptor linked to the Fc portion of human immunoglobulin G1 (IgG1) (Garrison and McDonnell, 1999). It functions as a TNF-α inhibitor

and competitively binds to circulating TNF-α as it has a higher affinity for TNF-α than the naturally occurring soluble TNF receptor. On this basis, etanercept blocks the interaction between soluble TNF-α and the membrane-bound receptors, inhibiting TNF-α induced pro-inflammatory activity (Garrison and McDonnell, 1999). Etanercept has a prolonged half-life in the bloodstream and induces a greater biologic effect than the soluble TNF receptor, showing more effectiveness in preventing activation of the inflammatory cascade (Madhusudan et al., 2005). For this reason, TNF-α inhibitors have been used as a treatment strategy to alleviate inflammation in RA. However, etanercept has a lower binding affinity for the transmembrane TNF-α and shows 2-fold less activity on transmembrane TNF-α compared to competitor TNF inhibitors (Deora et al., 2017; Horiuchi et al., 2010). While etanercept may successfully inhibit the effects of soluble TNF-α in cells predominantly expressing TNFR1, it is less effective in inhibiting biological activity mediated by transmembrane TNF-α acting on TNFR2 expressing cells which include macrophages. In this regard, etanercept will not effectively inhibit the downregulation of ABCA1 expression in these particular cells as compared to other ABCA1 cells predominately expressing TNFR1. Therefore, since macrophages are one of the sites for ABCA1-mediated HDL-C efflux activity, this mechanism of etanercept possibly explains the significant improvement in ABCA1 while HDL-C concentrations were unaffected. The continued downregulation of ABCA1 expression in hepatocytes due to transmembrane TNF-α binding on hepatocyte TNFR1 may explain why total liver lipid content was not improved.

Taken together, TNF-α inhibitors ameliorated the inflammation-induced reductions in ABCA1 concentration but not HDL-C, LDL-C concentrations or liver lipid content. Hence it may be possible that the functionality of the HDL particles may have been improved. Nevertheless, the functionality of the HDL particles in this study can only be implied. A direct measure of HDL functionality should be included in future investigations. Furthermore, the lack of TNF-α inhibitor effects on HDL-C concentrations and overall lipid metabolism may be as a result of

the short duration of the intervention or as a result of the specific binding of etanercept to TNF-α receptors. Further investigations are required to determine the molecular effects of specific anti-TNF-α drugs on inflammation-induced changes in ABCA1 expression, HDL functionality and liver lipid metabolism.

5.3 The effects of IL-6 inhibitor therapy on the lipid profiles

In the present study, treatment with an IL-6 inhibitor improved the serum concentrations of HDL and ABCA1 compared to the inflammation rats. Pre-clinical studies have demonstrated that exogenous IL-6 reduces the concentrations of HDL and ABCA1 (Mao et al., 2014; Stamatikos et al., 2019). Furthermore, a previous animal study showed that downregulating inflammation via the NF-κB pathway, increased HDL-C concentrations (Hadi et al., 2013). Also, decreasing circulating levels of IL-6 by administering other anti-inflammatory substances, have been associated with increased expression of ABCA1, which led to decreased atherosclerotic plaques in apolipoprotein E deficient mice (Jia et al., 2019; Li et al., 2020; Zhang et al., 2018). However, to my knowledge, there has been no evidence on the direct effects of IL-6 receptor blockers on either HDL or ABCA1 concentrations in pre-clinical models of high-grade inflammation.

In clinical RA studies, while some studies have reported that tocilizumab treatment improved the atherogenic lipid profiles (Cacciapaglia et al., 2018; Genovese et al., 2008; Jones et al., 2010; Kawashiri et al., 2011; Maini et al., 2006; Schultz et al., 2010; Tournadre et al., 2017), others have not reported any changes (Chen et al., 2015; Farah et al., 2016; McInnes et al., 2015; Provan et al., 2015; Tournadre et al., 2017). With regards to the cholesterol efflux capacity, a recent study showed that despite unchanged HDL concentrations, cholesterol efflux capacity was increased in RA patients after 12 weeks of tocilizumab treatment (Greco et al., 2020). The authors reported that the cholesterol efflux capacity was mediated by an increase in the scavenger receptor class B type 1 transporter, and not ABCA1 expression

(Greco et al., 2020). Nevertheless, this study was confounded by a small sample size (n = 8). Similarly, others have shown that despite unchanged HDL concentrations, the composition of HDL particles are improved after tocilizumab treatment, which exhibits greater anti-inflammatory properties (McInnes et al., 2015). These positive results necessitate briefly discussing the effect of IL-6 and its inhibition on the signalling pathways involved in lipid metabolism.

IL-6 is a pleiotropic cytokine with various pathophysiologic functions in several target cells that regulate the inflammatory response (Naka et al., 2002; Papanicolaou, 2000). The IL-6 receptor exists in two forms namely the membrane-bound IL-6 receptors on cells or the soluble IL-6 receptors that circulates in the blood (Cronstein, 2007; Witte, 2015). IL-6 binds to either the membrane-bound IL-6 receptor or the soluble IL-6 receptor, forming a complex that associates with a signal-transducing glycoprotein 130 (gp130) subunit resulting in the dimerization of gp130 (Sebba, 2008). This gp130 dimerisation then triggers downstream trans-signalling via several members of the Jak-STAT pathways on target cells and initiating a pro-inflammatory response (Sebba, 2008). While gp130 is expressed in all cell types, the membrane-bound IL-6 receptor is expressed on fewer types of cells including hepatocytes, hepatic stellate cells, some immune cells and epithelial cells (e.g. biliary epithelial cells) (Mudter and Neurath, 2007; Schmidt-Arras and Rose-John, 2016). Therefore, gp130 enables IL-6 signaling in a broad range of cells that do not express the membrane-bound receptor on their surface.

Tocilizumab is a humanised monoclonal antibody that functions as an IL-6 receptor antagonist and prevents IL-6 from binding to the IL-6 receptor (Scott, 2017). In this regard, tocilizumab disrupts the Jak-STAT pathways in IL-6 signal transduction by competitively binding to soluble IL-6 and membrane-bound receptors which inhibits the classical (via membrane-bound receptors) and tans-signaling (gp130 dimerization) pathways and

subsequently suppresses the pro-inflammatory effects of IL-6 in cells (Hennigan and Kavanaugh, 2008; Sebba, 2008). However, tocilizumab bound to the IL-6 receptor hinders the clearance of IL-6 from the circulation (Sakkas, 2016). Therefore, tocilizumab does not necessarily reduce the circulating IL-6 concentrations but rather increases the concentrations of IL-6 through negative feedback even though its pro-inflammatory effects are reduced. In this regard, the results in the current study suggest that tocilizumab may induce a significant improvement in HDL-C by restoring ABCA1-mediated cholesterol efflux in cells expressing ABCA1, therefore enabling maturation of nascent HDL and increasing concentrations of mature HDL in circulation. Indeed, exogenous administration of IL-6 to ABCA1 expressing cells have shown altered ABCA1 expression via activation of Jak-STAT signalling pathways (Frisdal et al., 2011; Mao et al., 2014; Stamatikos et al., 2019). Hence, blocking IL-6 signalling may increase ABCA1 expression in these cells, which may have increased the HDL-C concentration. Despite improvements in ABCA1 and HDL-C concentrations, tocilizumab did not restore the liver lipid content to the pre-inflammatory state. Although the liver lipid content in the IL-6 blocker group was higher than the inflammation group, this result was not statistically significant. Considering the abundance of IL-6 receptors expressed on the liver, it is possible that blocking IL-6 may have influenced normal liver function. In this regard, IL-6 is important in the protection against liver damage as it stimulates regeneration of the liver (Streetz, 2000). Hence blocking IL-6 may have prevented the regeneration of the liver to restore normal function. These potential negative effects of blocking IL-6 on liver function will be further discussed in sections 5.4 and 5.5 below. Taken together, blocking IL-6 increased the concentration of HDL-C and possibly functionality of HDL particles, as evidenced by increased ABCA1 concentrations. Despite unchanged circulating IL-6 concentrations, IL-6 blockers effectively improved lipid profiles in rats exposed to arthritis. However, the improved lipid profiles were not reflected in the liver lipid content. Hence IL-6 blockers may interfere with normal liver function. Future studies

should elucidate the mechanisms whereby IL-6 inhibitors alter inflammation-induced lipid metabolism in animals and investigate the role of blocking IL-6 on liver lipid metabolism.

5.4 Effects of inflammation and anti-inflammatory treatment on liver fibrosis

In the present study, the total collagen area fraction, a marker of liver fibrosis, was significantly increased in the TNF-α inhibitor and IL-6 blocker rats compared to control and inflammation group rats. In addition, these results showed that increased concentrations of CRP were associated with increased total collagen area fraction as measured by Masson's trichrome and picrosirius red staining when including all the groups in the correlation analysis. However, the association between CRP and fibrosis were abrogated when including only the control and inflammation groups in the correlation analysis. These results suggest that it is the treatment rather than the exposure to inflammation that likely caused the fibrosis. Consistent with our results, others have reported that TNF-α inhibitors and IL-6 blockers caused liver fibrosis in RA patients (Ghabril et al., 2013; Leise et al., 2014; Weiler-Normann and Schramm, 2011). However, animal models have not reported liver fibrosis caused by TNF-α inhibitors and IL-6 receptor blockers.

Although the mechanisms of biologic DMARD-induced fibrosis have not been defined, studies have reported that sTNFR can reduce matrix metalloproteinases (MMPs) expression in synovial fluid and anti-TNF-α antibody may cause similar effects (Aggarwal, 2004; Catrina et al., 2002). MMPs are matrix-degrading enzymes with proteolytic abilities and are able to cause tissue destruction (Aggarwal, 2004). TNF-α inhibitors in RA patients significantly downregulated the serum levels of MMP-1 (collagenase), MMP-2, MMP-3 and MMP-9 (Brennan et al., 1997; Catrina et al., 2002; Klimiuk et al., 2015, 2004), while etanercept specifically has been shown to downregulate MMP-1 (Aggarwal, 2004; Catrina et al., 2002).

Tissue destruction occurs when there is an imbalance between MMPs and tissue inhibitor of MMP (TIMP) which regulate MMPs activity (Aggarwal, 2004; Klimiuk et al., 2004). In hepatocyte injury, fibrogenesis prevails over fibrolysis which is accompanied by a downregulation of MMPs (Zhang et al., 2016). Myofibroblasts are the primary collagen source in the liver and they secrete essential components required for tissue remodelling including extracellular matrix proteins for collagen deposition (Arthur, 2000; Zhang et al., 2016). The excess fibrillar matrix deposited by myofibroblasts during remodelling consists mainly of fibrillary collagens, such as collagen types I and III (Zhang et al., 2016). Collagen type-I fibres form a greater proportion of the total collagen compared to the collagen type-III fibres (Zhang et al., 2016). Collagen type-I fibres increase the tensile stiffness of the extracellular matrix in tissues, compared to the more flexible properties of the thinner type-III collagen fibres resulting in excess collagen deposition and tissue fibrosis (Zhang et al., 2016). Downregulation of MMPs causes a reduction in collagen degradation which results in the development of fibrosis (Zhang et al., 2016). Therefore, it is possible that etanercept may have downregulated the serum MMP levels and upregulated TIMP levels in CIA rats, resulting in increased liver tissue fibrosis. Indeed etanercept has shown to decrease serum levels of MMPs in animal models of arthritis (El-Ashmawy et al., 2019; Kaneko et al., 2016; Seki et al., 2019).

The effect of IL-6 inhibitors on fibrosis in RA has not been extensively documented, hence the mechanisms that are responsible for liver fibrosis are not yet defined. High IL-6 levels have been associated with high synovial MMP levels (Aoki et al., 2014) and tocilizumab decreased MMP levels in juvenile idiopathic arthritis and RA patients (Aoki et al., 2014; Avdeeva et al., 2013). Therefore, IL-6 inhibitors may induce similar effects on MMPs in CIA and subsequently increase collagen deposition.

The present study demonstrates that administration of biologic DMARDs, rather than the presence of inflammation induces liver injury in the CIA model. Future studies should elucidate the mechanisms whereby TNF-α inhibitors and IL-6 blockers induce liver fibrosis.

5.5 Effects of inflammation and anti-inflammatory treatment on hepatotoxicity

In the present study, treatment with TNF-α inhibitors and IL-6 inhibitors significantly increased the concentrations of ALP, but not AST or ALT. In addition, the associations between circulating CRP concentrations with ALP were abrogated when including only the control and inflammation groups in the correlation analysis. These results suggest that it is more likely the biologic DMARD treatment and not the inflammation that caused the hepatotoxicity. Several clinical studies have reported that TNF-α inhibitors and IL-6 blockers increase serum activity of ALT, AST and ALP in RA (Brazdilova et al., 2019; French et al., 2016; Frider et al., 2013; Genovese et al., 2017; Pandit, 2012; Yazici and Curtis, 2012). Currently, no studies have investigated the impact of TNF-α inhibitors and IL-6 blockers on liver transaminases in animal models of RA. To my knowledge, this is the first study to report on the effects of etanercept and tocilizumab on liver toxicity enzymes in the CIA rat model.

The mechanisms whereby biologic DMARDs induce hepatotoxicity are not well established (Weiler-Normann and Schramm, 2011). Drug-induced hepatotoxicity may occur via hepatocellular necrosis (Brass, 1993; Pandit, 2012). Anti-inflammatory drugs may potentially induce cell apoptosis or necrosis and this is possibly due to the drugs ability to induce harmful events such as mitochondrial toxicity, oxidative stress, metabolite instability, alterations of transporters involved in drug metabolism, protein composition abnormalities and immunoallergic reactions which ultimately contributes to liver cell injury (Weiler-Normann and Schramm, 2011). When hepatocytes are damaged, hepatic enzymes leak into the bloodstream and elevate serum liver enzyme activity (Sookoian et al., 2016). Anti-inflammatory drugs may induce idiosyncratic drug reactions that generate reactive

compounds, either as a derivative of the drug or ROS, which can lead to hepatocyte protein covalent modifications that induce hepatocellular necrosis and damages hepatocytes (Brass, 1993).

TNF-α inhibitors, containing the Fc portion of human IgG, display opsonizing capabilities and promote antibody-dependent cell-mediated cell death of cells that produce TNF-α (French et al., 2016). In the case of etanercept, immune complexes are cleared via Fc receptor-mediated interactions in a process involving hepatic sinusoids which may lead to the activation of kupffer cells that subsequently release ROS or induce local liver cell damage (Pandit, 2012). However, etanercept forms immune complexes less frequently (Pandit, 2012; Parekh and Kaur, 2014) and is least associated with liver transaminase elevations (Fisher et al., 2015; Ghabril et al., 2013) compared to other monoclonal antibody TNF-α inhibitors, hence its less harmful to the liver.

On the other hand, since hepatocytes express abundant IL-6 receptors, it would be expected that these receptors may reflect a potential route of liver damage for tocilizumab in these cells. However, tocilizumab does not induce cellular damage from antibody-dependant cellular cytotoxicity (Ogata et al., 2019). Serum liver enzyme elevation incidences are similar in sirukumab (an IL-6 ligand antibody) and tocilizumab, suggesting that liver damage may result from IL-6 signal interference as opposed to direct toxicity (Ogata et al., 2019; Takeuchi et al., 2017). Furthermore, tocilizumab may also interfere with the IL-6 mediated regeneration of the liver (Streetz, 2000).

Liver damage in RA has frequently been reported as a result of combination therapy (Xiang et al., 2015). However, the current study provides further evidence that biological DMARDs induce liver toxicity, independent of inflammation, confounding factors or concomitant treatments. Furthermore, this study confirmed that biologic DMARDs have varying effects on liver enzymes as only ALP activity was significantly raised while ALT and AST activity

remained unchanged after both therapies. Most previous studies with DMARDs have shown elevations in AST and ALT activity (Curtis et al., 2010; Genovese et al., 2017; Gupta et al., 2011; Sokolove et al., 2010). The differential effect on the different liver enzymes may be explained by the interpretation of these enzymes. Elevations in AST and ALT activity are associated with a hepatocellular pattern of liver damages, such as with viral hepatitis or cirrhosis (Lala et al., 2020). Elevations in ALP, without elevations in AST and ALT are associated with either hepatobiliary causes such as bile duct obstruction, infiltrating diseases of the liver such as sarcoidosis, cystic fibrosis and cholestasis (Lala et al., 2020). However, there may also be non-hepatic causes of elevated ALP, such as heart failure, renal failure, infection or inflammation (Lala et al., 2020). Hence, in the current study, the increased ALP may be as a result of inflammation. Nevertheless, this is unlikely as the inflammation group did not show an increased ALP concentration. Furthermore, this study showed that IL-6 inhibitor therapy increased the ALP concentrations more than the TNF-α inhibitor therapy. Therefore, it is likely that the increased ALP may be as a result of the liver damage caused by the medication, rather than non-hepatic causes. Future studies should investigate the mechanisms responsible for the biological DMARD induced changes in ALP.

5.6 Limitations

The present study had several limitations. Including both male and female rats may have impacted the results. However, there were no differences in any of the lipid markers or fibrosis makers between males and females. Furthermore two-way ANOVAs, with group and sex as main effects were used to account for the potential sex difference. In the data analysis, sex and body weight were included as potential confounders in all correlation analysis, which did not materially alter the results. In the current study glucose and triglyceride concentrations were measured in the control and inflammation rats, hence the effects of the biologic DMARDs on glucose and triglyceride concentrations are not known.

Furthermore, LDL-C, HDL-C and ABCA1 serum concentrations were measured, however, the functionality of the LDL and HDL particles can only be implied in the current study. The expression of ABCA1 on different tissues may have improved the interpretations of the current study. Furthermore, the cellular mechanisms responsible for the changes in liver metabolism were not measured and should be included in future studies. Moreover, whether the changes in the lipid profiles translated into a reduced risk of atherosclerosis were not measured and should be included in future studies.

The present study investigated the role of biologic DMARDs on lipid metabolism, liver morphology and liver toxicity using a humanised soluble TNF-α inhibitor and a humanised anti-IL-6 monoclonal antibody. Although these biologic DMARDs had significant effects on lipid profiles, ABCA1, liver fibrosis and liver enzymes, these humanised drugs may have been suboptimal as it is possible that these treatments did not bind effectively to the rat cytokines and the receptors. In the current study, I showed no decrease in circulating inflammatory markers in the drug-treated group. However, receptor inhibition is usually accompanied by increased serum activity of the cognate ligand, in this instance circulating concentrations of TNF-α and IL-6 (Gibiansky and Frey, 2012; Mann et al., 2008; Nishimoto et al., 2008). Nevertheless, CRP concentrations were not reduced, which was a surprising finding. The lack of white blood cell measures prohibited the confirmation of effective drug effects. Nevertheless, previous studies animal studies using etanercept (Totoson et al., 2016) and tocilizumab (Abdel-Maged et al., 2018) have shown significant effects of these drugs on inflammation-induced outcomes in rats. Future studies should nevertheless consider using rat specific TNF-α inhibitors and IL-6 blocker therapy.

5.7 Conclusion

The results presented in this work increase our understanding of the role of high-grade inflammation and biologic DMARDs on lipid metabolism, liver morphology and toxicity. In this

regard, the present study showed that collagen immunisation resulted in the development of high-grade systemic inflammation *in vivo* which impaired lipid metabolism. High-grade inflammation impaired lipid profiles, however, inflammation was not associated with fibrosis and toxicity markers in the CIA rat model. The use of TNF-α inhibitor therapy effectively reversed inflammation-induced changes ABCA1, but this improvement did not translate into an increase in HDL-C concentration and it resulted in significant liver fibrosis and increased ALP concentration. IL-6 receptor blocker therapy effectively reversed the inflammation-induced decreases in HDL-C and ABCA1 concentrations. This suggests that blocking the effects of circulating IL-6 may improve the functionality and the concentration of HDL particles. Despite the positive effects on lipid profiles, IL-6 receptor blockers induced significant liver fibrosis and increased ALP concentrations. IL-6 blockade appears to have more favourable outcomes on lipid metabolism compared to TNF-α inhibitors. However, it is uncertain whether their harmful effects on the liver outweigh their beneficial effect on lipid metabolism. The mechanism whereby biologic DMARDs improve lipid metabolism and induce liver injury in RA requires further investigation.

References

Abdel-Maged, A.E., Gad, A.M., Abdel-Aziz, A.K., Aboulwafa, M.M., Azab, S.S., 2018. Comparative study of anti-VEGF Ranibizumab and Interleukin-6 receptor antagonist Tocilizumab in Adjuvant-induced Arthritis. Toxicol. Appl. Pharmacol. 356, 65–75.

Abdin, A.A., Abd El-Halim, M.S., Hedeya, S.E., El-Saadany, A.A.E., 2012. Effect of atorvastatin with or without prednisolone on Freund's adjuvant induced-arthritis in rats. Eur. J. Pharmacol. 676, 34–40.

Afonso, C.B., Spickett, C.M., 2019. Lipoproteins as targets and markers of lipoxidation. Redox Biol. 23, 101066.

Aggarwal, A., 2004. Effect of etanercept on matrix metalloproteinases and angiogenic vascular endothelial growth factor: a time kinetic study. Ann. Rheum. Dis. 63, 891–892.

Akhlaghi, S., Sahebari, M., Mahmoodi, M., Yaseri, M., Mansournia, M.A., Rafatpanah, H., Zeraati, H., 2018. Additional effect of etanercept or infliximab on the liver function tests of patients with rheumatoid arthritis: a cohort study. Ther. Clin. Risk Manag. 14, 1943–1950.

Albalat, A., Liarte, C., MacKenzie, S., Tort, L., Planas, J. V., Navarro, I., 2005. Control of adipose tissue lipid metabolism by tumor necrosis factor-α in rainbow trout (Oncorhynchus mykiss). J. Endocrinol. 184, 527–534.

Allanore, Y., Kahan, A., Sellam, J., Ekindjian, O.G., Borderie, D., 2006. Effects of repeated infliximab therapy on serum lipid profile in patients with refractory rheumatoid arthritis. Clin. Chim. Acta 365, 143–148.

Alves-Bezerra, M., Cohen, D.E., 2017. Triglyceride Metabolism in the Liver. Compr. Physiol.

8, 1–8.

Aoki, C., Inaba, Y., Choe, H., Kaneko, U., Hara, R., Miyamae, T., Imagawa, T., Mori, M., Oba, M.S., Yokota, S., Saito, T., 2014. Discrepancy Between Clinical and Radiological Responses to Tocilizumab Treatment in Patients with Systemic-onset Juvenile Idiopathic Arthritis. J. Rheumatol. 41, 1171–1177.

Arakawa, R., Tsujita, M., Iwamoto, N., Ito-Ohsumi, C., Lu, R., Wu, C.-A., Shimizu, K., Aotsuka, T., Kanazawa, H., Abe-Dohmae, S., Yokoyama, S., 2009. Pharmacological inhibition of ABCA1 degradation increases HDL biogenesis and exhibits antiatherogenesis. J. Lipid Res. 50, 2299–2305.

Arida, A., Protogerou, A., Kitas, G., Sfikakis, P., 2018. Systemic Inflammatory Response and Atherosclerosis: The Paradigm of Chronic Inflammatory Rheumatic Diseases. Int. J. Mol. Sci. 19, 1–27.

Arthur, M.J.P., 2000. Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis. Am J Physiol Gastrointest Liver Physiol 279, G245–G249.

Avdeeva, A.S., Aleksandrova, E.N., Novikov, A.A., Cherkasova, M. V, Panasyuk, E.Y., Nasonov, E.L., 2013. Relationship of the clinical efficiency of tocilizumab therapy to the serum level of matrix metalloproteinase-3 in patients with rheumatoid arthritis. Ter. Arkh. 85, 24–9.

Avina-Zubieta, J.A., Thomas, J., Sadatsafavi, M., Lehman, A.J., Lacaille, D., 2012. Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies. Ann. Rheum. Dis. 71, 1524–1529.

Babashamsi, M.M., Koukhaloo, S.Z., Halalkhor, S., Salimi, A., Babashamsi, M., 2019. ABCA1 and metabolic syndrome; a review of the ABCA1 role in HDL-VLDL production,

insulin-glucose homeostasis, inflammation and obesity. Diabetes Metab. Syndr. Clin. Res. Rev. 13, 1529–1534.

Badellino, K.O., Wolfe, M.L., Reilly, M.P., Rader, D.J., 2008. Endothelial Lipase Is Increased In Vivo by Inflammation in Humans. Circulation 117, 678–685.

Baek, H.J., Lim, M.J., Park, W., Park, S.H., Shim, S.-C., Yoo, D.-H., Kim, H.A., Lee, S.K., Lee, Y.J., Park, Y.E., Cha, H.-S., Song, Y.-W., 2019. Efficacy and safety of tocilizumab in Korean patients with active rheumatoid arthritis. Korean J. Intern. Med. 34, 917–931.

Bag-Ozbek, A., Giles, J.T., 2015. Inflammation, Adiposity, and Atherogenic Dyslipidemia in Rheumatoid Arthritis: Is There a Paradoxical Relationship? Curr. Allergy Asthma Rep. 15, 497.

Bălănescu, A., Bojincă, V., Bojincă, M., Donisan, T., Bălănescu, S., 2018. Cardiovascular effects of methotrexate in immune‑mediated inflammatory diseases (Review). Exp. Ther. Med. 17, 1024–1029.

Bansilal, S., Castellano, J.M., Fuster, V., 2015. Global burden of CVD: focus on secondary prevention of cardiovascular disease. Int. J. Cardiol. 201, S1–S7.

Bartunek, J., Vanderheyden, M., 2012. Inflammation and related biomarkers in cardiovascular disease. Biomarkers Med 6, 1–3.

Beers, A., Haas, M.J., Wong, N.C.W., Mooradian, A.D., 2006. Inhibition of Apolipoprotein AI Gene Expression by Tumor Necrosis Factor α: Roles for MEK/ERK and JNK Signaling. Biochemistry 45, 2408–2413.

Ben-Aicha, S., Badimon, L., Vilahur, G., 2020. Advances in HDL: Much More than Lipid Transporters. Int. J. Mol. Sci. 21, 732.

Bendele, A.M., 2001. Animal models of rheumatoid arthritis. J Musculoskel Neuron Interact 1, 377–385.

Biesterfeld, S., Knapp, J., Bittinger, F., Götte, H., Schramm, M., Otto, G., 2012. Frozen section diagnosis in donor liver biopsies: observer variation of semiquantitative and quantitative steatosis assessment. Virchows Arch. 461, 177–183.

Boekholdt, S.M., Arsenault, B.J., Hovingh, G.K., Mora, S., Pedersen, T.R., LaRosa, J.C., Welch, K.M.A., Amarenco, P., DeMicco, D.A., Tonkin, A.M., Sullivan, D.R., Kirby, A., Colhoun, H.M., Hitman, G.A., Betteridge, D.J., Durrington, P.N., Clearfield, M.B., Downs, J.R., Gotto, A.M., Ridker, P.M., Kastelein, J.J.P., 2013. Levels and Changes of HDL Cholesterol and Apolipoprotein A-I in Relation to Risk of Cardiovascular Events Among Statin-Treated Patients. Circulation 128, 1504–1512.

Borén, J., Chapman, M.J., Krauss, R.M., Packard, C.J., Bentzon, J.F., Binder, C.J., Daemen, M.J., Demer, L.L., Hegele, R.A., Nicholls, S.J., Nordestgaard, B.G., Watts, G.F., Bruckert, E., Fazio, S., Ference, B.A., Graham, I., Horton, J.D., Landmesser, U., Laufs, U., Masana, L., Pasterkamp, G., Raal, F.J., Ray, K.K., Schunkert, H., Taskinen, M.-R., van de Sluis, B., Wiklund, O., Tokgozoglu, L., Catapano, A.L., Ginsberg, H.N., 2020. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. Eur. Heart J. 41, 2313–2330.

Bortoluzzi, A., Furini, F., Generali, E., Silvagni, E., Luciano, N., Scirè, C.A., 2018. One year in review 2018: novelties in the treatment of rheumatoid arthritis. Clin. Exp. Rheumatol. 36, 347–361.

Brass, E.P., 1993. Hepatic toxicity of antirheumatic drugs. Cleve. Clin. J. Med. 60, 466–472.

Brazdilova, K., Koller, T., Killinger, Z., Payer, J., 2019. Prevalence and Risk Factors for Drug-Induced Liver Injury Among Patients With Rheumatic Diseases Treated With Biological Therapy: A Single-Center Experience. Physiol. Res. 68, S157–S163.

Brennan, F.M., Browne, K.A., Green, P.A., Jaspar, J.M., Maini, R.N., Feldmann, M., 1997. Reduction of serum matrix metalloproteinase 1 and matrix metalloproteinase 3 in rheumatoid arthritis patients following anti- tumour necrosis factor-alpha (cA2) therapy. Rheumatology 36, 643–650.

Brown, G.T., Kleiner, D.E., 2016. Histopathology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Metabolism 65, 1080–1086.

Cacciapaglia, F., Anelli, M.G., Rinaldi, A., Fornaro, M., Lopalco, G., Scioscia, C., Lapadula, G., Iannone, F., 2018. Lipids and Atherogenic Indices Fluctuation in Rheumatoid Arthritis Patients on Long-Term Tocilizumab Treatment. Mediators Inflamm. 2018, 1–7.

Cacciapaglia, F., Anelli, M.G., Rinaldi, A., Serafino, L., Covelli, M., Scioscia, C., Iannone, F., Lapadula, G., 2014. Lipid Profile of Rheumatoid Arthritis Patients Treated with Anti-Tumor Necrosis Factor-Alpha Drugs Changes According to Disease Activity and Predicts Clinical Response. Drug Dev. Res. 75, S77–S80.

Carbajo-Pescador, S., Porras, D., García-Mediavilla, M.V., Martínez-Flórez, S., Juarez-Fernández, M., Cuevas, M.J., Mauriz, J.L., González-Gallego, J., Nistal, E., Sánchez-Campos, S., 2019. Beneficial effects of exercise on gut microbiota functionality and barrier integrity, and gut-liver crosstalk in an in vivo model of early obesity and non-alcoholic fatty liver disease. Dis. Model. Mech. 12, 1–14.

Carlo, S., Maria, D.S., Eric M, G., 2011. Liver involvement in subjects with rheumatic disease. Arthritis Res. Ther. 13, 226.

Catrina, A.I., Lampa, J., Ernestam, S., Af Klint, E., Bratt, J., Klareskog, L., Ulfgren, A.-K., 2002. Anti-tumour necrosis factor (TNF)-a therapy (etanercept) down-regulates serum matrix metalloproteinase (MMP)-3 and MMP-1 in rheumatoid arthritis. Rheumatology 41, 484–489.

Cawthorn, W.P., Sethi, J.K., 2008. TNF-α and adipocyte biology. FEBS Lett. 582, 117–131.

Chait, A., Brazg, R.L., Tribble, D.L., Krauss, R.M., 1993. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. Am. J. Med. 94, 350–356.

Charles-Schoeman, C., Fleischmann, R., Davignon, J., Schwartz, H., Turner, S.M., Beysen, C., Milad, M., Hellerstein, M.K., Luo, Z., Kaplan, I. V., Riese, R., Zuckerman, A., McInnes, I.B., 2015. Potential Mechanisms Leading to the Abnormal Lipid Profile in Patients With Rheumatoid Arthritis Versus Healthy Volunteers and Reversal by Tofacitinib. Arthritis Rheumatol. 67, 616–625.

Charles-Schoeman, C., Lee, Y.Y., Grijalva, V., Amjadi, S., FitzGerald, J., Ranganath, V.K., Taylor, M., McMahon, M., Paulus, H.E., Reddy, S.T., 2012. Cholesterol efflux by high density lipoproteins is impaired in patients with active rheumatoid arthritis. Ann. Rheum. Dis. 71, 1157–1162.

Charles-Schoeman, C., Wang, X., Lee, Y.Y., Shahbazian, A., Navarro-Millán, I., Yang, S., Chen, L., Cofield, S.S., Moreland, L.W., O'Dell, J., Bathon, J.M., Paulus, H., Louis Bridges, S., Curtis, J.R., 2016. Association of Triple Therapy With Improvement in Cholesterol Profiles Over Two-Year Followup in the Treatment of Early Aggressive Rheumatoid Arthritis Trial. Arthritis Rheumatol. 68, 577–586.

Charles-Schoeman, C., Watanabe, J., Lee, Y.Y., Furst, D.E., Amjadi, S., Elashoff, D., Park,

G., McMahon, M., Paulus, H.E., Fogelman, A.M., Reddy, S.T., 2009. Abnormal function of high-density lipoprotein is associated with poor disease control and an altered protein cargo in rheumatoid arthritis. Arthritis Rheum. 60, 2870–2879.

Charles-Schoeman, C., Yin Lee, Y., Shahbazian, A., Wang, X., Elashoff, D., Curtis, J.R., Navarro-Millán, I., Yang, S., Chen, L., Cofield, S.S., Moreland, L.W., Paulus, H., O'Dell, J., Bathon, J., Bridges, S.L., Reddy, S.T., 2017. Improvement of High-Density Lipoprotein Function in Patients With Early Rheumatoid Arthritis Treated With Methotrexate Monotherapy or Combination Therapies in a Randomized Controlled Trial. Arthritis Rheumatol. 69, 46–57.

Chen, D.-Y., Chen, Y., Hsieh, T.-Y., Hsieh, C., Lin, C., Lan, J., 2015. Significant effects of biologic therapy on lipid profiles and insulin resistance in patients with rheumatoid arthritis. Arthritis Res. Ther. 17, 52.

Chen, X., Xun, K., Chen, L., Wang, Y., 2009. TNF- α , a potent lipid metabolism regulator. Cell Biochem. Funct. 27, 407–416.

Chistiakov, D.A., Bobryshev, Y. V., Orekhov, A.N., 2016. Macrophage-mediated cholesterol handling in atherosclerosis. J. Cell. Mol. Med. 20, 17–28.

Choi, Hafiane, A., Schwertani, A., Genest, J., 2017a. High-Density Lipoproteins: Biology, Epidemiology, and Clinical Management. Can. J. Cardiol. 33, 325–333.

Choi, Sviridov, D., Miller, Y.I., 2017b. Oxidized cholesteryl esters and inflammation. Biochim. Biophys. Acta - Mol. Cell Biol. Lipids 1862, 393–397.

Choy, E., Ganeshalingam, K., Semb, A.G., Szekanecz, Z., Nurmohamed, M., 2014. Cardiovascular risk in rheumatoid arthritis: recent advances in the understanding of the pivotal role of inflammation, risk predictors and the impact of treatment. Rheumatology

53, 2143–2154.

Choy, E., Sattar, N., 2009. Interpreting lipid levels in the context of high-grade inflammatory states with a focus on rheumatoid arthritis: a challenge to conventional cardiovascular risk actions. Ann. Rheum. Dis. 68, 460–469.

Chung, C.P., Oeser, A., Raggi, P., Sokka, T., Pincus, T., Solus, J.F., Linton, M.F., Fazio, S., Stein, C.M., 2010. Lipoprotein Subclasses Determined by Nuclear Magnetic Resonance Spectroscopy and Coronary Atherosclerosis in Patients with Rheumatoid Arthritis. J. Rheumatol. 37, 1633–1638.

Cigliano, L., Spagnuolo, M.S., Cuomo, G., Valentini, G., Niglio, A., Abrescia, P., 2005. Apolipoprotein A-I-dependent cholesterol esterification in patients with rheumatoid arthritis. Life Sci. 77, 108–120.

Cojocaru, M., Cojocaru, I.M., Silosi, I., Vrabie, C.D., Tanasescu, R., 2010. Extra-articular Manifestations in Rheumatoid Arthritis. Maedica (Buchar). 5, 286–91.

Cronstein, B.N., 2007. Interleukin-6 A Key Mediator of Systemic and Local Symptoms in Rheumatoid Arthritis. Bull. NYU Hosp. Jt. Dis. 65, S11-15.

Crowson, C.S., Rollefstad, S., Ikdahl, E., Kitas, G.D., van Riel, P.L.C.M., Gabriel, S.E., Matteson, E.L., Kvien, T.K., Douglas, K., Sandoo, A., Arts, E., Wållberg-Jonsson, S., Innala, L., Karpouzas, G., Dessein, P.H., Tsang, L., El-Gabalawy, H., Hitchon, C., Ramos, V.P., Yáñez, I.C., Sfikakis, P.P., Zampeli, E., Gonzalez-Gay, M.A., Corrales, A., Laar, M. van de, Vonkeman, H.E., Meek, I., Semb, A.G., 2018. Impact of risk factors associated with cardiovascular outcomes in patients with rheumatoid arthritis. Ann. Rheum. Dis. 77, 48–54.

Cuchel, M., Rader, D.J., 2006. Macrophage Reverse Cholesterol Transport. Circulation 113,

2548–2555.

Curtis, J.R., Beukelman, T., Onofrei, A., Cassell, S., Greenberg, J.D., Kavanaugh, A., Reed, G., Strand, V., Kremer, J.M., 2010. Elevated liver enzyme tests among patients with rheumatoid arthritis or psoriatic arthritis treated with methotrexate and/or leflunomide. Ann. Rheum. Dis. 69, 43–47.

Curtis, J.R., John, A., Baser, O., 2012. Dyslipidemia and changes in lipid profiles associated with rheumatoid arthritis and initiation of anti-tumor necrosis factor therapy. Arthritis Care Res. (Hoboken). 64, 1282–1291.

Dabeer, S., Sharma, N., Nagdeote, A., 2019. Correlation between CRP and Atherogenic Index and Assessment of Cardiovascular Disease Risk in Patients with Rheumatic Arthritis. Int. J. Res. Rev. 6, 12.

Daffner, S.D., Watkins, C.M., 2014. Rheumatoid Arthritis. Spine Surg. Basics 376, 465–474.

Daïen, C.I., Duny, Y., Barnetche, T., Daurès, J., Combe, B., Morel, J., 2012. Effect of TNF inhibitors on lipid profile in rheumatoid arthritis: a systematic review with meta-analysis. Ann. Rheum. Dis. 71, 862–868.

de La Forest Divonne, M., Gottenberg, J.E., Salliot, C., 2017. Safety of biologic DMARDs in RA patients in real life: A systematic literature review and meta-analyses of biologic registers. Jt. Bone Spine 84, 133–140.

De La, M., Moya, L., Mcgillicuddy, F.C., Hinkle, C.C., Byrne, M., Joshi, M.R., Nguyen, V., Tabita-Martinez, J., Wolfe, M.L., Badellino, K., Pruscino, L., Mehta, N.N., Asztalos, B.F., Reilly, M.P., 2012. Inflammation Modulates Human HDL Composition and Function in vivo. Atherosclerosis 222, 390–394.

De Sanctis, S., Marcovecchio, M.L., Gaspari, S., Del Torto, M., Mohn, A., Chiarelli, F., Breda, L., 2013. Etanercept Improves Lipid Profile and Oxidative Stress Measures in Patients with Juvenile Idiopathic Arthritis. J. Rheumatol. 40, 943–948.

Dehestani, V., Shariati-Sarabi, Z., Mohiti, S., 2015. Liver toxicity in rheumatoid arthritis patients treated with methotrexate. Asia Pacific J. Med. Toxicol. 4, 102–105.

Deora, A., Hegde, S., Lee, J., Choi, C.H., Chang, Q., Lee, C., Eaton, L., Tang, H., Wang, D., Lee, D., Michalak, M., Tomlinson, M., Tao, Q., Gaur, N., Harvey, B., McLoughlin, S., Labkovsky, B., Ghayur, T., 2017. Transmembrane TNF-dependent uptake of anti-TNF antibodies. MAbs 9, 680–695.

Dessein, P.H., Solomon, A., Hollan, I., 2016. Metabolic abnormalities in patients with inflammatory rheumatic diseases. Best Pract. Res. Clin. Rheumatol. 30, 901–915.

Dragoljevic, D., Kraakman, M.J., Nagareddy, P.R., Ngo, D., Shihata, W., Kammoun, H.L., Whillas, A., Lee, M.K.S., Al-Sharea, A., Pernes, G., Flynn, M.C., Lancaster, G.I., Febbraio, M.A., Chin-Dusting, J., Hanaoka, B.Y., Wicks, I.P., Murphy, A.J., 2018. Defective cholesterol metabolism in haematopoietic stem cells promotes monocyte-driven atherosclerosis in rheumatoid arthritis. Eur. Heart J. 39, 2158–2167.

Dubey, L., Chatterjee, S., Ghosh, A., 2016. Hepatic and hematological adverse effects of long-term low-dose methotrexate therapy in rheumatoid arthritis: An observational study. Indian J. Pharmacol. 48, 591.

Edgel, K.A., LeBoeuf, R.C., Oram, J.F., 2010. Tumor necrosis factor-α and lymphotoxin-α increase macrophage ABCA1 by gene expression and protein stabilization via different receptors. Atherosclerosis 209, 387–392.

El-Ashmawy, N.E., Khedr, E.G., Shamloula, M.M., Kamel, M.M., 2019. Evaluation of the

antirheumatic effects of isoflavone-free soy protein isolate and etanercept in rats with adjuvant-induced arthritis. Exp. Biol. Med. 244, 545–553.

El-Gaphar, O.A.M.A., Abo-Youssef, A.M., Abo-Saif, A.A., 2018. Effect of Losartan in Complete Freund's Adjuvant-Induced Arthritis in Rats. Iran. J. Pharm. Res. 17, 1420–1430.

Elshafie, A.I., Elkhalifa, A.D., Elbagir, S., Aledrissy, M.I.E., Elagib, E.M., Nur, M.A.M., Weitoft, T., Rönnelid, J., 2016. Active Rheumatoid Arthritis in Central Africa: A Comparative Study Between Sudan and Sweden. J. Rheumatol. 43, 1777–1786.

Emery, P., Keystone, E., Tony, H.P., Cantagrel, A., van Vollenhoven, R., Sanchez, A., Alecock, E., Lee, J., Kremer, J., 2008. IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. Ann. Rheum. Dis. 67, 1516–1523.

Ettinger, W.H., Miller, L.A., Smith, T.K., Parks, J.S., 1992. Effect of interleukin-1 alpha on lipoprotein lipids in cynomolgus monkeys: Comparison to tumor necrosis factor. Biochim. Biophys. Acta - Lipids Lipid Metab. 1128, 186–192.

Ettinger, W.H., Varma, V.K., Sorci-Thomas, M., Parks, J.S., Sigmon, R.C., Smith, T.K., Verdery, R.B., 1994. Cytokines Decrease Apolipoprotein Accumulation in Medium From Hep G2 Cells. Arterioscler. Thromb. 14, 8–13.

Farah, Z., Ali, S., Price-Kuehne, F., Mackworth-Young, C.G., 2016. Tocilizumab in refractory rheumatoid arthritis: Long-term efficacy, safety, and tolerability beyond 2 years. Biol. Targets Ther. 10, 59–66.

Field, F.J., Watt, K., Mathur, S.N., 2010. TNF-α decreases ABCA1 expression and

attenuates HDL cholesterol efflux in the human intestinal cell line Caco-2. J. Lipid Res. 51, 1407–1415.

Firestein, G.S., McInnes, I.B., 2017. Immunopathogenesis of Rheumatoid Arthritis. Immunity 46, 183–196.

Fisher, K., Vuppalanchi, R., Saxena, R., 2015. Drug-Induced Liver Injury. Arch. Pathol. Lab. Med. 139, 876–887.

Franzén, L.E., Ekstedt, M., Kechagias, S., Bodin, L., 2005. Semiquantitative evaluation overestimates the degree of steatosis in liver biopsies: A comparison to stereological point counting. Mod. Pathol. 18, 912–916.

Frayn, K.N., Arner, P., Yki-Järvinen, H., 2006. Fatty acid metabolism in adipose tissue, muscle and liver in health and disease. Essays Biochem. 42, 89–103.

French, J.B., Bonacini, M., Ghabril, M., Foureau, D., Bonkovsky, H.L., 2016. Hepatotoxicity Associated with the Use of Anti-TNF-α Agents. Drug Saf. 39, 199–208.

Frider, B., Bruno, A., Ponte, M., Amante, M., 2013. Drug-Induced Liver Injury Caused by Adalimumab: A Case Report and Review of the Bibliography. Case Reports Hepatol. 2013, 1–3.

Frisdal, E., Lesnik, P., Olivier, M., Robillard, P., Chapman, M.J., Huby, T., Guerin, M., Le Goff, W., 2011. Interleukin-6 Protects Human Macrophages from Cellular Cholesterol Accumulation and Attenuates the Proinflammatory Response. J. Biol. Chem. 286, 30926–30936.

Gabay, C., McInnes, I.B., Kavanaugh, A., Tuckwell, K., Klearman, M., Pulley, J., Sattar, N., 2016. Comparison of lipid and lipid-associated cardiovascular risk marker changes after

treatment with tocilizumab or adalimumab in patients with rheumatoid arthritis. Ann. Rheum. Dis. 75, 1806–1812.

Gaffo, A., Saag, K.G., Curtis, J.R., 2006. Treatment of rheumatoid arthritis. Am. J. Heal. Pharm. 63, 2451–2465.

García-Gómez, C., Bianchi, M., de la Fuente, D., Badimon, L., Padró, T., Corbella, E., Pintó, X., 2014. Inflammation, lipid metabolism and cardiovascular risk in rheumatoid arthritis: A qualitative relationship? World J. Orthop. 5, 304–11.

Garrison, L., McDonnell, N.D., 1999. Etanercept: therapeutic use in patients with rheumatoid arthritis. Ann. Rheum. Dis. 58, 165–169.

Genest, J., Choi, H.Y., 2017. Novel Approaches for HDL-Directed Therapies. Curr. Atheroscler. Rep. 19, 55.

Genovese, M.C., Kremer, J.M., van Vollenhoven, R.F., Alten, R., Scali, J.J., Kelman, A., Dimonaco, S., Brockwell, L., 2017. Transaminase Levels and Hepatic Events During Tocilizumab Treatment: Pooled Analysis of Long-Term Clinical Trial Safety Data in Rheumatoid Arthritis. Arthritis Rheumatol. 69, 1751–1761.

Genovese, M.C., McKay, J.D., Nasonov, E.L., Mysler, E.F., da Silva, N.A., Alecock, E., Woodworth, T., Gomez-Reino, J.J., 2008. Interleukin-6 receptor inhibition with tocilizumab reduces disease activity in rheumatoid arthritis with inadequate response to disease-modifying antirheumatic drugs: The tocilizumab in combination with traditional disease-modifying antirheumatic drug the. Arthritis Rheum. 58, 2968–2980.

Georgiadis, A.N., Papavasiliou, E.C., Lourida, E.S., Alamanos, Y., Kostara, C., Tselepis, A.D., Drosos, A.A., 2006. Atherogenic lipid profile is a feature characteristic of patients with early rheumatoid arthritis : effect of early treatment – a prospective , controlled

study. Arthritis Res. Ther. 8, 1–7.

Gerbod-Giannone, M.C., Li, Y., Holleboom, A., Han, S., Hsu, L.C., Tabas, I., Tall, A.R., 2006. TNF induces ABCA1 through NF- B in macrophages and in phagocytes ingesting apoptotic cells. Proc. Natl. Acad. Sci. 103, 3112–3117.

Ghabril, M., Bonkovsky, H.L., Kum, C., Davern, T., Hayashi, P.H., Kleiner, D.E., Serrano, J., Rochon, J., Fontana, R.J., Bonacini, M., 2013. Liver Injury From Tumor Necrosis Factor-α Antagonists: Analysis of Thirty-four Cases. Clin. Gastroenterol. Hepatol. 11, 558–564.

Ghosh, A., Gao, L., Thakur, A., Siu, P.M., Lai, C.W.K., 2017. Role of free fatty acids in endothelial dysfunction. J. Biomed. Sci. 24, 50.

Ghosh, U.C., Roy, A., Sen, K., Kundu, A.K., Saha, I., Biswas, A., 2009. Dyslipidaemia in rheumatoid arthritis in a tertiary care centre in Eastern India--a non-randomised trial. J. Indian Med. Assoc. 107, 427–30.

Gianazza, E., Brioschi, M., Fernandez, A.M., Banfi, C., 2019. Lipoxidation in cardiovascular diseases. Redox Biol. 23, 101119.

Gibiansky, L., Frey, N., 2012. Linking interleukin-6 receptor blockade with tocilizumab and its hematological effects using a modeling approach. J. Pharmacokinet. Pharmacodyn. 39, 5–16.

Ginosyan, K., 2019. Benefits and disadvantages of biologic agents in chronic inflammatory arthritis. Int. J. Clin. Rheumtol. 15, 6–9.

Gliozzi, M., Scicchitano, M., Bosco, F., Musolino, V., Carresi, C., Scarano, F., Maiuolo, J., Nucera, S., Maretta, A., Paone, S., Mollace, R., Ruga, S., Zito, M.C., Macrì, R.,

Oppedisano, F., Palma, E., Salvemini, D., Muscoli, C., Mollace, V., 2019. Modulation of Nitric Oxide Synthases by Oxidized LDLs: Role in Vascular Inflammation and Atherosclerosis Development. Int. J. Mol. Sci. 20, 3294.

Godo, S., Shimokawa, H., 2017. Endothelial Functions. Arterioscler. Thromb. Vasc. Biol. 37, e108–e114.

Gong, M., Wilson, M., Kelly, T., Su, W., Dressman, J., Kincer, J., Matveev, S. V., Guo, L., Guerin, T., Li, X.-A., Zhu, W., Uittenbogaard, A., Smart, E.J., 2003. HDL-associated estradiol stimulates endothelial NO synthase and vasodilation in an SR-BI–dependent manner. J. Clin. Invest. 111, 1579–1587.

González-Gay, M.A., González-Juanatey, C., 2012. Inflammation, endothelial function and atherosclerosis in rheumatoid arthritis. Arthritis Res. Ther. 14, 122.

González-Gay, M.A., González-Juanatey, C., 2014. Inflammation and lipid profile in rheumatoid arthritis: bridging an apparent paradox. Ann. Rheum. Dis. 73, 1281–1283.

Gonzalez-Gay, M.A., Gonzalez-Juanatey, C., Martin, J., 2005. Rheumatoid Arthritis: A Disease Associated with Accelerated Atherogenesis. Semin. Arthritis Rheum. 35, 8–17.

González-Juanatey, C., Llorca, J., González-Gay, M.A., 2011. Correlation between endothelial function and carotid atherosclerosis in rheumatoid arthritis patients with long-standing disease. Arthritis Res. Ther. 13, R101.

Greco, D., Gualtierotti, R., Agosti, P., Adorni, M.P., Ingegnoli, F., Rota, M., Bernini, F., Meroni, P.L., Ronda, N., 2020. Anti-atherogenic Modification of Serum Lipoprotein Function in Patients with Rheumatoid Arthritis after Tocilizumab Treatment, a Pilot Study. J. Clin. Med. 9, 2157.

Greenberg, A.S., Nurdan, R.P., Mclntosh, J., Calvo, J.C., Scow, R.O., Jablons2, D., 1992. Interleukin 6 Reduces Lipoprotein Lipase Activity in Adipose Tissue of Mice in Vivo and in 3T3-L1 Adipocytes: A Possible Role for Interleukin 6 in Cancer Cachexia. Cancer Res. 52, 4113–4116.

Grove, R.I., Mazeucco, C., Allegretto, N., Kiener, P.A., Spitalny, G., Radka, S.F., Shoyab, M., Antonaccio, M., Warr, G.A., 1991. Macrophage-derived factors increase low density lipoprotein uptake and receptor number in cultured human liver cells. J. Lipid Res. 32, 1889–1897.

Guo, S., Wang, R., Jiang, T., Bai, F., Ding, Q., Ma, Y., Wang, Y., Yang, Y., Wang, X., Tan, L., Li, J., Nie, H., Wang, J., He, D., 2017. Alterations and diagnosis potential of serum lipid profiles in rheumatoid arthritis patients. Int. J. Clin. Exp. Pathol. 10, 3503–3509.

Gupta, R., Bhatia, J., Gupta, S., 2011. Risk of hepatotoxicity with add-on leflunomide in rheumatoid arthritis patients. Arzneimittelforschung 61, 312–316.

Haas, M.J., Horani, M., Mreyoud, A., Plummer, B., Wong, N.C.W., Mooradian, A.D., 2003. Suppression of apolipoprotein AI gene expression in HepG2 cells by TNF α and IL-1β. Biochim. Biophys. Acta - Gen. Subj. 1623, 120–128.

Hadi, N.R., Yousif, N.G., Abdulzahra, M.S., Mohammad, B.I., Al-amran, F.G., Majeed, M.L., Yousif, M.G., 2013. Role of NF-κβ and Oxidative Pathways in Atherosclerosis: Cross-Talk Between Dyslipidemia and Candesartan. Cardiovasc. Ther. 31, 381–387.

Hameed, S., Al-Barzinjy, N., 2017. Prevalence of dyslipidemia and its association with disease activity in patients with rheumatoid arthritis attending Rizgary Teaching Hospital in Erbil City. Zanco J. Med. Sci. 21, 1781–1788.

Harmse, L., Reuter, H., 2016. An overview of the biological disease modifying drugs

available for arthritic conditions in South Africa. South African Fam. Pract. 58, 6–10.

Hasan, H., Ismail, H., El-Orfali, Y., Khawaja, G., 2018. Therapeutic benefits of Indole-3-Carbinol in adjuvant-induced arthritis and its protective effect against methotrexate induced-hepatic toxicity. BMC Complement. Altern. Med. 18, 337.

Hashizume, M., Mihara, M., 2011. The Roles of Interleukin-6 in the Pathogenesis of Rheumatoid Arthritis. Arthritis 2011, 1–8.

Hassen Zrour, S., Hassine Neffeti, F., Sakly, N., Jguirim, M., Korbaa, W., Younes, M., Bejia, I., Touzi, M., Fadel, N.M., Bergaoui, N., 2011. Lipid profile in Tunisian patients with rheumatoid arthritis. Clin. Rheumatol. 30, 1325–1331.

He, M., Liang, X., He, L., Wen, W., Zhao, S., Wen, L., Liu, Y., Shyy, J.Y.-J., Yuan, Z., 2013. Endothelial Dysfunction in Rheumatoid Arthritis. Arterioscler. Thromb. Vasc. Biol. 33, 1384–1391.

Hedayatnia, M., Asadi, Z., Zare-Feyzabadi, R., Yaghooti-Khorasani, M., Ghazizadeh, H., Ghaffarian-Zirak, R., Nosrati-Tirkani, A., Mohammadi-Bajgiran, M., Rohban, M., Sadabadi, F., Rahimi, H.-R., Ghalandari, M., Ghaffari, M.-S., Yousefi, A., Pouresmaeili, E., Besharatlou, M.-R., Moohebati, M., Ferns, G.A., Esmaily, H., Ghayour-Mobarhan, M., 2020. Dyslipidemia and cardiovascular disease risk among the MASHAD study population. Lipids Health Dis. 19, 42.

Hennigan, S., Kavanaugh, A., 2008. Interleukin-6 inhibitors in the treatment of rheumatoid arthritis. Ther. Clin. Risk Manag. 4, 767–75.

Hernáez, Á., Soria-Florido, M.T., Schröder, H., Ros, E., Pintó, X., Estruch, R., Salas-Salvadó, J., Corella, D., Arós, F., Serra-Majem, L., Martínez-González, M.Á., Fiol, M., Lapetra, J., Elosua, R., Lamuela-Raventós, R.M., Fitó, M., 2019. Role of HDL function

and LDL atherogenicity on cardiovascular risk: A comprehensive examination. PLoS One 14, e0218533.

Herrington, W., Lacey, B., Sherliker, P., Armitage, J., Lewington, S., 2016. Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. Circ. Res. 118, 535–546.

Horiuchi, T., Mitoma, H., Harashima, S.I., Tsukamoto, H., Shimoda, T., 2010. Transmembrane TNF-α: Structure, function and interaction with anti-TNF agents. Rheumatology 49, 1215–1228.

Howes, L.G., Abbott, D., Straznicky, N.E., 1997. Lipoproteins and cardiovascular reactivity. Br J Clin Pharmacol 44, 319–324.

Huang, J., Qian, H.-Y., Li, Z.-Z., Zhang, J.-M., Wang, S., Tao, Y., Gao, Y.-L., Yin, C.-Q., Que, B., Sun, T., Zhao, Z.-Y., Li, Z., 2010. Role of endothelial lipase in atherosclerosis. Transl. Res. 156, 1–6.

Huang, L., Fan, B., Ma, A., Shaul, P.W., Zhu, H., 2015. Inhibition of ABCA1 protein degradation promotes HDL cholesterol efflux capacity and RCT and reduces atherosclerosis in mice. J. Lipid Res. 56, 986–997.

Hui, N., Barter, P.J., Ong, K.-L., Rye, K.-A., 2019. Altered HDL metabolism in metabolic disorders: insights into the therapeutic potential of HDL. Clin. Sci. 133, 2221–2235.

Hurt-Camejo, E., Paredes, S., Masana, L., Camejo, G., Sartipy, P., Rosengren, B., Pedreno, J., Vallve, J.C., Benito, P., Wiklund, O., 2001. Elevated levels of small, low-density lipoprotein with high affinity for arterial matrix components in patients with rheumatoid arthritis: possible contribution of phospholipase A2 to this atherogenic profile. Arthritis Rheum. 44, 2761–7.

Hussain, M.M., 2014. Intestinal lipid absorption and lipoprotein formation. Curr. Opin. Lipidol. 25, 200–206.

Ismaili, H., Ismaili, L., Rexhepi, M., 2019. Values and Correlations between C-Reactive Protein and Apolipoprotein B after Treatment with Methotrexate at Patients with Rheumatoid Arthritis. Open Access Maced. J. Med. Sci. 7, 1293–1298.

Jagpal, A., Navarro-Millán, I., 2018. Cardiovascular co-morbidity in patients with rheumatoid arthritis: a narrative review of risk factors, cardiovascular risk assessment and treatment. BMC Rheumatol. 2, 10.

Jaipersad, A.S., Lip, G.Y.H., Silverman, S., Shantsila, E., 2014. The Role of Monocytes in Angiogenesis and Atherosclerosis. J. Am. Coll. Cardiol. 63, 1–11.

Jang, M.H., Piao, X.L., Kim, J.M., Kwon, S.W., Park, J.H., 2008. Inhibition of cholinesterase and amyloid-&bgr; aggregation by resveratrol oligomers from Vitis amurensis. Phyther. Res. 22, 544–549.

Jia, Q., Cao, H., Shen, D., Li, S., Yan, L., Chen, C., Xing, S., Dou, F., 2019. Quercetin protects against atherosclerosis by regulating the expression of PCSK9, CD36, PPARγ, LXRα and ABCA1. Int. J. Mol. Med. 44, 893–902.

Jones, G., Sebba, A., Gu, J., Lowenstein, M.B., Calvo, A., Gomez-Reino, J.J., Siri, D.A., Tomsic, M., Alecock, E., Woodworth, T., Genovese, M.C., 2010. Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: the AMBITION study. Ann. Rheum. Dis. 69, 88–96.

Jones, S.M., 2000. Lipoproteins and their subfractions in psoriatic arthritis: identification of an atherogenic profile with active joint disease. Ann. Rheum. Dis. 59, 904–909.

Jukema, R.A., Ahmed, T.A.N., Tardif, J.-C., 2019. Does low-density lipoprotein cholesterol induce inflammation? If so, does it matter? Current insights and future perspectives for novel therapies. BMC Med. 17, 197.

Kajani, S., Curley, S., McGillicuddy, F., 2018. Unravelling HDL—Looking beyond the Cholesterol Surface to the Quality Within. Int. J. Mol. Sci. 19, 1971.

Kaneko, K., Williams, R.O., Dransfield, D.T., Nixon, A.E., Sandison, A., Itoh, Y., 2016. Selective Inhibition of Membrane Type 1 Matrix Metalloproteinase Abrogates Progression of Experimental Inflammatory Arthritis: Synergy With Tumor Necrosis Factor Blockade. Arthritis Rheumatol. 68, 521–531.

Kang, M.H., Zhang, L.-H., Wijesekara, N., de Haan, W., Butland, S., Bhattacharjee, A., Hayden, M.R., 2013. Regulation of ABCA1 Protein Expression and Function in Hepatic and Pancreatic Islet Cells by miR-145. Arterioscler. Thromb. Vasc. Biol. 33, 2724–2732.

Kaptoge, S., Di Angelantonio, Emanuele Lowe, G., Pepys, M.B., Thompson, S.G., Collins, R., Danesh, J., 2010. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet. 375, 132–40.

Kapwata, T., Manda, S., 2018. Geographic assessment of access to health care in patients with cardiovascular disease in South Africa. BMC Health Serv. Res. 18, 197.

Karimifar, Mansoor, Sepehrifar, M.S., Moussavi, H., Sepehrifar, M.B., Mottaghi, P., Siavash, M., Karimifar, Mozhgan, 2018. The effects of conventional drugs in the treatment of rheumatoid arthritis on the serum lipids. J. Res. Med. Sci. 23, 105.

Kawashiri, S., Kawakami, A., Yamasaki, S., Imazato, T., Iwamoto, N., Fujikawa, K., Aramaki, T., Tamai, M., Nakamura, H., Ida, H., Origuchi, T., Ueki, Y., Eguchi, K., 2011. Effects of

the anti-interleukin-6 receptor antibody, tocilizumab, on serum lipid levels in patients with rheumatoid arthritis. Rheumatol. Int. 31, 451–456.

Khera, A. V., Cuchel, M., de la Llera-Moya, M., Rodrigues, A., Burke, M.F., Jafri, K., French, B.C., Phillips, J.A., Mucksavage, M.L., Wilensky, R.L., Mohler, E.R., Rothblat, G.H., Rader, D.J., 2011. Cholesterol Efflux Capacity, High-Density Lipoprotein Function, and Atherosclerosis. N. Engl. J. Med. 364, 127–135.

Khera, A. V., Demler, O. V., Adelman, S.J., Collins, H.L., Glynn, R.J., Ridker, P.M., Rader, D.J., Mora, S., 2017. Cholesterol Efflux Capacity, High-Density Lipoprotein Particle Number, and Incident Cardiovascular Events. Circulation 135, 2494–2504.

Khovidhunkit, W., Memon, R.A., Feingold, K.R., Grunfeld, C., 2000. Infection and Inflammation-Induced Proatherogenic Changes of Lipoproteins. J. Infect. Dis. 181, S462–S472.

Khovidhunkit, W., Kim, M.-S., Memon, R.A., Shigenaga, J.K., Moser, A.H., Feingold, K.R., Grunfeld, C., 2004. Thematic review series: The Pathogenesis of Atherosclerosis. Effects of infection and inflammation on lipid and lipoprotein metabolism mechanisms and consequences to the host. J. Lipid Res. 45, 1169–1196.

Kim, J.-Y., Lee, E.-Y., Park, J.K., Song, Y.W., Kim, J.-R., Cho, K.-H., 2016. Patients with Rheumatoid Arthritis Show Altered Lipoprotein Profiles with Dysfunctional High-Density Lipoproteins that Can Exacerbate Inflammatory and Atherogenic Process. PLoS One 11, e0164564.

Kim, K.-R., Jeong, C.-K., Park, K.-K., Choi, J.-H., Park, J.H.Y., Lim, S.S., Chung, W.-Y., 2010. Anti-Inflammatory Effects of Licorice and Roasted Licorice Extracts on TPA-Induced Acute Inflammation and Collagen-Induced Arthritis in Mice. J. Biomed.

Biotechnol. 2010, 1–8.

Kingwell, B.A., Chapman, M.J., Kontush, A., Miller, N.E., 2014. HDL-targeted therapies: progress, failures and future. Nat. Rev. Drug Discov. 13, 445–464.

Kisilevsky, R., Manley, P.N., 2012. Acute-phase serum amyloid A: Perspectives on its physiological and pathological roles. Amyloid 19, 5–14.

Kitas, G.D., Gabriel, S.E., 2011. Cardiovascular disease in rheumatoid arthritis: state of the art and future perspectives. Ann. Rheum. Dis. 70, 8–14.

Klimiuk, P.A., Domysławska, I., Sierakowski, S., Chwiećko, J., 2015. Regulation of serum matrix metalloproteinases and tissue inhibitor of metalloproteinases-1 following rituximab therapy in patients with rheumatoid arthritis refractory to anti-tumor necrosis factor blockers. Rheumatol. Int. 35, 749–755.

Klimiuk, P.A., Sierakowski, S., Domyslawska, I., Chwiecko, J., 2004. Effect of repeated infliximab therapy on serum matrix metalloproteinases and. J. Rheumatol. Rheumatol. 31, 238–242.

Kojma, Y., Hirata, K.I., Ishida, T., Shimokawa, Y., Inoue, N., Kawashima, S., Quertermous, T., Yokoyama, M., 2004. Endothelial lipase modulates monocyte adhesion to the vessel wall: A potential role in inflammation. J. Biol. Chem. 279, 54032–54038.

Kontush, A., Lhomme, M., Chapman, M.J., 2013. Unraveling the complexities of the HDL lipidome. J. Lipid Res. 54, 2950–2963.

Kremer, J.M., Alarcn, G.S., Lightfoot, R.W., Willkens, R.F., Furst, D.E., Williams, H.J., Dent, P.B., Weinblatt, M.E., 1994. Methotrexate for rheumatoid arthritis. Arthritis Rheum. 37, 316–328.

Kremer, J.M., Genovese, M.C., Keystone, E., Taylor, P.C., Zuckerman, S.H., Ruotolo, G., Schlichting, D.E., Crotzer, V.L., Nantz, E., Beattie, S.D., Macias, W.L., 2017. Effects of Baricitinib on Lipid, Apolipoprotein, and Lipoprotein Particle Profiles in a Phase IIb Study of Patients With Active Rheumatoid Arthritis. Arthritis Rheumatol. 69, 943–952.

Kunitomo, M., Yamaguchi, Y., Futagawa, Y., Hamaguchi, Y., Bando, Y., 1987. Suppression of Adjuvant Arthritis in Rats by Cholesterol. J. Pharmacol 44, 15–22.

Kunitomo, M., Yamaguchi, Y., Futagawa, Y., Hamaguchi, Y., Do, Y.B., 1986. Lipid Deposition in the Aorta of Adjuvant Arthritic Rats with Hypercholesterolemia. Japan J. Pharmacol 42, 261–267.

Lala, V., Goyal, A., Bansal, P., Minter, D.A., 2020. Liver Function Tests, StatPearls. StatPearls Publishing.

Lazarevic, M.B., Vitic, J., Mladenovic, V., Lee Myones, B., Skosey, J.L., Swedler, W.I., 1992. Dyslipoproteinemia in the course of active rheumatoid arthritis. Semin. Arthritis Rheum. 22, 172–180.

Leise, M.D., Poterucha, J.J., Talwalkar, J.A., 2014. Drug-Induced Liver Injury. Mayo Clin. Proc. 89, 95–106.

Levitan, I., Volkov, S., Subbaiah, P. V, 2010. Oxidized LDL: Diversity, Patterns of Recognition, and Pathophysiology. Antioxid. Redox Signal. 13, 39–75.

Lewis, G.F., Rader, D.J., 2005. New Insights Into the Regulation of HDL Metabolism and Reverse Cholesterol Transport. Circ. Res. 96, 1221–1232.

Li, S., Cao, H., Shen, D., Chen, C., Xing, S., Dou, F., Jia, Q., 2020. Effect of Quercetin on Atherosclerosis Based on Expressions of ABCA1, LXR-α and PCSK9 in ApoE-/- Mice.

Chin. J. Integr. Med. 26, 114–121.

Liao, Liu, J., Lu, B., Solomon, D.H., Kim, S.C., 2015. Association Between Lipid Levels and Major Adverse Cardiovascular Events in Rheumatoid Arthritis Compared to Non-Rheumatoid Arthritis Patients. Arthritis Rheumatol. 67, 2004–2010.

Liao, Solomon, D.H., 2013. Traditional cardiovascular risk factors, inflammation and cardiovascular risk in rheumatoid arthritis. Rheumatology 52, 45–52.

Liu, S., Li, Y., Zeng, X., Wang, H., Yin, P., Wang, L., Liu, Y., Liu, J., Qi, J., Ran, S., Yang, S., Zhou, M., 2019. Burden of Cardiovascular Diseases in China, 1990-2016: Findings from the 2016 Global Burden of Disease Study. JAMA Cardiol. 4, 342–352.

Lopetuso, L.R., Mocci, G., Marzo, M., D'Aversa, F., Rapaccini, G.L., Guidi, L., Armuzzi, A., Gasbarrini, A., Papa, A., 2018. Harmful Effects and Potential Benefits of Anti-Tumor Necrosis Factor (TNF)-α on the Liver. Int. J. Mol. Sci. 19, 2199.

López-Mejías, R., Genre, F., Remuzgo-Martínez, S., González-Juanatey, C., Robustillo-Villarino, M., Llorca, J., Corrales, A., Vicente, E., Miranda-Filloy, J.A., Magro, C., Tejera-Segura, B., Ramírez Huaranga, M.A., Pina, T., Blanco, R., Alegre-Sancho, J.J., Raya, E., Mijares, V., Ubilla, B., Mínguez Sánchez, M.D., Gómez-Vaquero, C., Balsa, A., Pascual-Salcedo, D., López-Longo, F.J., Carreira, P., González-Álvaro, I., Rodríguez-Rodríguez, L., Fernández-Gutiérrez, B., Ferraz-Amaro, I., Castañeda, S., Martín, J., González-Gay, M.A., 2016. Influence of elevated-CRP level-related polymorphisms in non-rheumatic Caucasians on the risk of subclinical atherosclerosis and cardiovascular disease in rheumatoid arthritis. Sci. Rep. 6, 31979.

Lubrano, V., Gabriele, M., Puntoni, M.R., Longo, V., Pucci, L., 2015. Relationship among IL-6, LDL cholesterol and lipid peroxidation. Cell. Mol. Biol. Lett. 20, 310–322.

Lüscher, T.F., Landmesser, U., von Eckardstein, A., Fogelman, A.M., 2014. High-Density Lipoprotein. Circ. Res. 114, 171–182.

Ly, H., Francone, O.L., Fielding, Christopher J, Shigenaga, Judy K, Moser, Arthur H, Grunfeld, Carl, Feingold', K.R., Francone, L., Fielding, C J, Shigenaga, J K, Moser, A H, Grunfeld, C, Feingold, K.R., 1995. Endotoxin and TNF lead to reduced plasma LCAT activity and decreased hepatic LCAT mRNA levels in Syrian hamsters. J. Lipid Res. 36, 1254–1263.

Ma, K.L., Ruan, X.Z., Powis, S.H., Chen, Y., Moorhead, J.F., Varghese, Z., 2008. Inflammatory stress exacerbates lipid accumulation in hepatic cells and fatty livers of apolipoprotein E knockout mice. Hepatology 48, 770–781.

Ma, S., Zhang, Q., Song, Z.Y., 2013. TNFa alter cholesterol metabolism in human macrophages via PKC-theta-dependent pathway. BMC Biochem. 14, 20.

Machado, R.M., Nakandakare, E.R., Quintao, E.C.R., Cazita, P.M., Koike, M.K., Nunes, V.S., Ferreira, F.D., Afonso, M.S., Bombo, R.P.A., Machado-Lima, A., Soriano, F.G., Catanozi, S., Lottenberg, A.M., 2012. Omega-6 polyunsaturated fatty acids prevent atherosclerosis development in LDLr-KO mice, in spite of displaying a pro-inflammatory profile similar to trans fatty acids. Atherosclerosis 224, 66–74.

Madhusudan, S., Muthuramalingam, S.R., Braybrooke, J.P., Wilner, S., Kaur, K., Han, C., Hoare, S., Balkwill, F., Ganesan, T.S., 2005. Study of Etanercept, a Tumor Necrosis Factor-Alpha Inhibitor, in Recurrent Ovarian Cancer. J. Clin. Oncol. 23, 5950–5959.

Madsen, C.M., Varbo, A., Nordestgaard, B.G., 2017. Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. Eur. Heart J. 38, 2478–2486.

Mahmoudi, M., Aslani, S., Fadaei, R., Jamshidi, A.R., 2017. New insights to the mechanisms underlying atherosclerosis in rheumatoid arthritis. Int. J. Rheum. Dis. 20, 287–297.

Maini, R.N., Taylor, P.C., Szechinski, J., Pavelka, K., Bröll, J., Balint, G., Emery, P., Raemen, F., Petersen, J., Smolen, J., Thomson, D., Kishimoto, T., 2006. Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. Arthritis Rheum. 54, 2817–2829.

Mann, D.L., Bozkurt, B., Torre-Amione, G., Soran, O.Z., Sivasubramanian, N., 2008. Effect of the Soluble TNF-Antagonist Etanercept on Tumor Necrosis Factor Bioactivity and Stability. Clin. Transl. Sci. 1, 142–145.

Mao, M., Lei, H., Liu, Q., Chen, Y., Zhao, L., Li, Q., Luo, S., Zuo, Z., He, Q., Huang, W., Zhang, N., Zhou, C., Ruan, X.Z., 2014. Effects of miR-33a-5P on ABCA1/G1-Mediated Cholesterol Efflux under Inflammatory Stress in THP-1 Macrophages. PLoS One 9, e109722.

Marchio, P., Guerra-Ojeda, S., Vila, J.M., Aldasoro, M., Victor, V.M., Mauricio, M.D., 2019. Targeting Early Atherosclerosis: A Focus on Oxidative Stress and Inflammation. Oxid. Med. Cell. Longev. 2019, 1–32.

März, W., Kleber, M.E., Scharnagl, H., Speer, T., Zewinger, S., Ritsch, A., Parhofer, K.G., von Eckardstein, A., Landmesser, U., Laufs, U., 2017. HDL cholesterol: reappraisal of its clinical relevance. Clin. Res. Cardiol. 106, 663–675.

McFarlane, I.M., Zhaz Leon, S.Y., Bhamra, M.S., Burza, A., Waite, S.A., Rodriguez Alvarez, M., Koci, K., Taklalsingh, N., Kaplan, I., Pathiparampil, J., Kabani, N., Watler, E., Sorrento, C.S., Frefer, M., Vaitkus, V., Green, J., Matthew, K., Arroyo-Mercado, F., Lyo,

H., Soliman, F., Sanchez, R.A., Reyes, F.M., Ozeri, D.J., Dronamraju, V., Trevisonno, M., Grant, C., Clerger, G., Amin, K., Freeman, L., Dawkins, M., Lopez, D.L., Smerling, J., Gondal, I., Dellinger, E., Paltoo, K., Bhat, H., Kolla, S., 2019. Assessment of Cardiovascular Disease Risk and Therapeutic Patterns among Urban Black Rheumatoid Arthritis Patients. Med. Sci. 7, 31.

McGrath, C.M., Young, S.P., 2015. Lipid and Metabolic Changes in Rheumatoid Arthritis. Curr. Rheumatol. Rep. 17, 57.

McInnes, I.B., Thompson, L., Giles, J.T., Bathon, J.M., Salmon, J.E., Beaulieu, A.D., Codding, C.E., Carlson, T.H., Delles, C., Lee, J.S., Sattar, N., 2015. Effect of interleukin-6 receptor blockade on surrogates of vascular risk in rheumatoid arthritis: MEASURE, a randomised, placebo-controlled study. Ann. Rheum. Dis. 74, 694–702.

Mehta, J.L., Li, D.Y., 1999. Inflammation in ischemic heart disease: Response to tissue injury or a pathogenetic villain? Cardiovasc. Res. 43, 291–299.

Mei, C., Chen, Z., Liao, Y., Wang, Y., Peng, H., Chen, Y., 2007. Interleukin-10 inhibits the down-regulation of ATP binding cassette transporter A1 by tumour necrosis factor-alpha in THP-1 macrophage-derived foam cells. Cell Biol. Int. 31, 1456–1461.

Monti, S., Klersy, C., Gorla, R., Sarzi-Puttini, P., Atzeni, F., Pellerito, R., Fusaro, E., Paolazzi, G., Rocchetta, P.A., Favalli, E.G., Marchesoni, A., Caporali, R., 2017. Factors influencing the choice of first- and second-line biologic therapy for the treatment of rheumatoid arthritis: real-life data from the Italian LORHEN Registry. Clin. Rheumatol. 36, 753–761.

Morita, S.-Y., 2016. Metabolism and Modification of Apolipoprotein B-Containing Lipoproteins Involved in Dyslipidemia and Atherosclerosis. Biol. Pharm. Bull 39, 1–24.

Mudter, J., Neurath, M.F., 2007. Il-6 signaling in inflammatory bowel disease: Pathophysiological role and clinical relevance. Inflamm. Bowel Dis. 13, 1016–1023.

Myasoedova, E., Crowson, C.S., Kremers, H.M., Roger, V.L., Fitz-Gibbon, P.D., Therneau, T.M., Gabriel, S.E., 2011. Lipid paradox in rheumatoid arthritis: the impact of serum lipid measures and systemic inflammation on the risk of cardiovascular disease. Ann. Rheum. Dis. 70, 482–487.

Naerr, G.W., Rein, P., Saely, C.H., Drexel, H., 2016. Effects of synthetic and biological disease modifying antirheumatic drugs on lipid and lipoprotein parameters in patients with rheumatoid arthritis. Vascul. Pharmacol. 81, 22–30.

Naka, T., Nishimoto, N., Kishimoto, T., 2002. The paradigm of IL-6: from basic science to medicine. Arthritis Res. 4, S233–S242.

Nanau, R.M., Neuman, M.G., 2014. Safety of Anti-Tumor Necrosis Factor Therapies in Arthritis Patients. J Pharm Pharm Sci 17, 324–361.

Nassir, S.F., Jasim, Z.M., Ewadh, R.S., Anoze, A., 2007. Serum Lipid Profile in Early Rheumatoid Arthritis. Med. J. Babylon 4, 3–10.

Navab, M., Reddy, S.T., Van Lenten, B.J., Anantharamaiah, G.M., Fogelman, A.M., 2009. The role of dysfunctional HDL in atherosclerosis. J. Lipid Res. 50, S145–S149.

Navarro, M., Carpintero, R., Acín, S., Arbonés-Mainar, J.M., Calleja, L., Carnicer, R., Surra, J.C., Guzmán-García, M.A., González-Ramón, N., Iturralde, M., Lampreave, F., Piñeiro, A., Osada, J., 2005. Immune-regulation of the apolipoprotein A-I/C-III/A-IV gene cluster in experimental inflammation. Cytokine 31, 52–63.

Nishida, K., Okada, Y., Nawata, M., Saito, K., Tanaka, Y., 2008. Induction of

hyperadiponectinemia following long-term treatment of patients with rheumatoid arthritis with infliximab (IFX), an anti-TNF-alpha antibody. Endocr. J. 55, 213–216.

Nishimoto, N., Terao, K., Mima, T., Nakahara, H., Takagi, N., Kakehi, T., 2008. Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti–IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. Blood 112, 3959–3964.

Nojilana, B., Bradshaw, D., Pillay-van Wyk, V., Msemburi, W., Somdyala, N., Joubert, J.D., Groenewald, P., Laubscher, R., Dorrington, R.E., 2016. Persistent burden from non-communicable diseases in South Africa needs strong action. South African Med. J. 106, 436–437.

Norata, G.D., Catapano, A.L., 2005. Molecular mechanisms responsible for the antiinflammatory and protective effect of HDL on the endothelium. Vasc. Health Risk Manag. 1, 119–129.

Nordestgaard, B.G., 2017. A Test in Context: Lipid Profile, Fasting Versus Nonfasting. J. Am. Coll. Cardiol. 70, 1637–1646.

Nurmohamed, M.T., 2007. Atherogenic lipid profiles and its management in patients with rheumatoid arthritis. Vasc. Health Risk Manag. 3, 845–852.

Oan, J., Athon, M.B., Artin, I.W.M., Leischmann, O.M.F., Esser, O.R.T., Chiff, I.H.S., Dward, E., Eystone, C.K., Ark, M., Enovese, C.G., Ary, M., Hester, C., Asko, W., Oreland, A.W.M., Rthur, A., Eaver, L.W., Oseph, J., Arkenson, M., Inck, A.K.F., 2000. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. N. Engl. J. Med. 343, 1586–1593.

Ogata, A., Kato, Y., Higa, S., Yoshizaki, K., 2019. IL-6 inhibitor for the treatment of

rheumatoid arthritis: A comprehensive review. Mod. Rheumatol. 29, 258–267.

Ogdie, A., Assistant Professor of Medicine, M., Shin, D., Takeshita, J., Assistant Professor of Dermatology, M., Chiesa Fuxench, Z.C., Carr, R.M., Professor of Medicine, A., Gelfand, J.M., Professor of Dermatology, M., 2018. Risk of incident liver disease in patients with psoriasis, psoriatic arthritis, and rheumatoid arthritis: a population-based study. HHS Public Access. J Invest Dermatol 138, 760–767.

Olivecrona, G., 2016. Role of lipoprotein lipase in lipid metabolism. Curr. Opin. Lipidol. 27, 233–241.

Oliviero, F., Sfriso, P., Baldo, G., Dayer, J.-M., Giunco, S., Scanu, A., Bernardi, D., Ramonda, R., Plebani, M., Punzi, L., 2009. Apolipoprotein A-I and cholesterol in synovial fluid of patients with rheumatoid arthritis, psoriatic arthritis and osteoarthritis. Clin. Exp. Rheumatol. 27, 79–83.

Oram, J.F., Lawn, R.M., Garvin, M.R., Wade, D.P., 2000. ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. J. Biol. Chem. 275, 34508–11.

Ormseth, M.J., Stein, C.M., 2016. High-density lipoprotein function in rheumatoid arthritis. Curr. Opin. Lipidol. 27, 67–75.

Ormseth, M.J., Yancey, P.G., Yamamoto, S., Oeser, A.M., Gebretsadik, T., Shintani, A., Linton, M.F., Fazio, S., Davies, S.S., Roberts, L.J., Vickers, K.C., Raggi, P., Kon, V., Stein, C.M., 2016. Net cholesterol efflux capacity of HDL enriched serum and coronary atherosclerosis in rheumatoid arthritis. IJC Metab. Endocr. 13, 6–11.

Osuga, T., Ikura, Y., Kadota, C., Hirano, S., Iwai, Y., Hayakumo, T., 2015. Significance of liver biopsy for the evaluation of methotrexate-induced liver damage in patients with

rheumatoid arthritis. Int J Clin Exp Pathol 8, 1961–1966.

Pandit, 2012. Drug-Induced Hepatotoxicity: A Review. J. Appl. Pharm. Sci. 2012, 13–25.

Papanicolaou, D.A., 2000. Interleukin-6: The Endocrine Cytokine. J. Clin. Endocrinol. Metab. 85, 1331–1333.

Paradis, M.-E., Badellino, K.O., Rader, D.J., Deshaies, Y., Couture, P., Archer, W.R., Bergeron, N., Lamarche, B., 2006. Endothelial lipase is associated with inflammation in humans. J. Lipid Res. 47, 2808–2813.

Parameswaran, N., Patial, S., 2010. Tumor Necrosis Factor-α Signaling in Macrophages. Crit. Rev. Eukaryot. Gene Expr. 20, 87–103.

Parekh, R., Kaur, N., 2014. Liver Injury Secondary to Anti-TNF-Alpha Therapy in Inflammatory Bowel Disease: A Case Series and Review of the Literature. Case Rep. Gastrointest. Med. 2014, 1–5.

Park, Y.B., Lee, S.K., Lee, W.K., Suh, C.H., Lee, C.W., Lee, C.H., Song, C.H., Lee, J., 1999. Lipid profiles in untreated patients with rheumatoid arthritis. J. Rheumatol. 26, 1701–1704.

Parseghian, S., Onstead-Haas, L.M., Wong, N.C.W., Mooradian, A.D., Haas, M.J., 2014. Inhibition of Apolipoprotein A-I Expression by TNF-Alpha in HepG2 Cells: Requirement for c- jun. J. Cell. Biochem. 115, 253–260.

Parthasarathy, S., 2010. Oxidized Low-Density Lipoprotein. Methods Mol Biol 403–417.

Parveen, S., Jacob, R., Rajasekhar, L., Srinivasa, C., Mohan, I.K., 2017. Serum Lipid Alterations in Early Rheumatoid Arthritis Patients on Disease Modifying Anti Rheumatoid Therapy. Indian J. Clin. Biochem. 32, 26–32.

Patel, M.G., Pundarikakshudu, K., 2016. Anti-arthritic activity of a classical Ayurvedic formulation Vatari Guggulu in rats. J. Tradit. Complement. Med. 6, 389–394.

Paulus, W.J., Tschöpe, C., 2013. A Novel Paradigm for Heart Failure With Preserved Ejection Fraction. J. Am. Coll. Cardiol. 62, 263–271.

Peer, N., Balakrishna, Y., de Villiers, A., Crickmore, C., Mungal-Singh, V., 2018. Effectiveness of a screening programme in identifying individuals with increased risk of cardiovascular disease in South Africa. J. Public Health (Bangkok). 40, e34–e45.

Pegoretti, V., Baron, W., Laman, J.D., Eisel, U.L.M., 2018. Selective Modulation of TNF–TNFRs Signaling: Insights for Multiple Sclerosis Treatment. Front. Immunol. 9, 925.

Pérez-Baos, Barrasa, J.I., Gratal, P., Larrañaga-Vera, A., Prieto-Potin, I., Herrero-Beaumont, G., Largo, R., 2017. Tofacitinib restores the inhibition of reverse cholesterol transport induced by inflammation: understanding the lipid paradox associated with rheumatoid arthritis. Br. J. Pharmacol. 174, 3018–3031.

Peters, M.J.L., Vis, M., van Halm, V.P., Wolbink, G.J., Voskuyl, A.E., Lems, W.F., Dijkmans, B.A.C., Twisk, J.W.R., de Koning, M.H.M.T., van de Stadt, R.J., Nurmohamed, M.T., 2007. Changes in lipid profile during infliximab and corticosteroid treatment in rheumatoid arthritis. Ann. Rheum. Dis. 66, 958–961.

Peters, M.J.L., Voskuyl, A.E., Sattar, N., Dijkmans, B.A.C., Smulders, Y.M., Nurmohamed, M.T., 2010. The interplay between inflammation, lipids and cardiovascular risk in rheumatoid arthritis: why ratios may be better. Int. J. Clin. Pract. 64, 1440–1443.

Pi, X., Xie, L., Patterson, C., 2018. Emerging Roles of Vascular Endothelium in Metabolic Homeostasis. Circ. Res. 123, 477–494.

Pignatelli, P., Menichelli, D., Pastori, D., Violi, F., 2018. Oxidative stress and cardiovascular disease: new insights. Kardiol. Pol. 76, 713–722.

Popa, Netea, M.G., Radstake, T., Van der Meer, J., Stalenhoef, A., van Riel, P., Barerra, P., 2005. Influence of anti-tumour necrosis factor therapy on cardiovascular risk factors in patients with active rheumatoid arthritis. Ann. Rheum. Dis. 64, 303–305.

Popa, Netea, M.G., van Riel, P.L.C.M., van der Meer, J.W.M., Stalenhoef, A.F.H., 2007a. The role of TNF-α in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. J. Lipid Res. 48, 751–762.

Popa, van den Hoogen, F.H.J., Radstake, T.R.D.J., Netea, M.G., Eijsbouts, A.E., Heijer, M. d., van der Meer, J.W.M., van Riel, P.L.C.M., Stalenhoef, A.F.H., Barrera, P., 2007b. Modulation of lipoprotein plasma concentrations during long-term anti-TNF therapy in patients with active rheumatoid arthritis. Ann. Rheum. Dis. 66, 1503–1507.

Provan, S.A., Berg, I.J., Hammer, H.B., Mathiessen, A., Kvien, T.K., Semb, A.G., 2015. The impact of newer biological disease modifying anti-rheumatic drugs on cardiovascular risk factors: A 12-month longitudinal study in rheumatoid arthritis patients treated with rituximab, abatacept and tociliziumab. PLoS One 10, 1–8.

Prüfer, N., Kleuser, B., van der Giet, M., 2015. The role of serum amyloid A and sphingosine-1-phosphate on high-density lipoprotein functionality. Biol. Chem. 396, 573–583.

Qian, H., Zhao, X., Cao, P., Lei, J., Yan, N., Gong, X., 2017. Structure of the Human Lipid Exporter ABCA1. Cell 169, 1228-1239.e10.

Rein, P., Mueller, R.B., 2017. Treatment with Biologicals in Rheumatoid Arthritis: An Overview. Rheumatol. Ther. 4, 247–261.

Remmerie, A., Scott, C.L., 2018. Macrophages and lipid metabolism. Cell. Immunol. 330, 27–42.

Ridgley, L.A., Anderson, A.E., Pratt, A.G., 2018. What are the dominant cytokines in early rheumatoid arthritis? Curr. Opin. Rheumatol. 30, 207–214.

Ridker, P.M., Everett, B.M., Thuren, T., MacFadyen, J.G., Chang, W.H., Ballantyne, C., Fonseca, F., Nicolau, J., Koenig, W., Anker, S.D., Kastelein, J.J.P., Cornel, J.H., Pais, P., Pella, D., Genest, J., Cifkova, R., Lorenzatti, A., Forster, T., Kobalava, Z., Vida-Simiti, L., Flather, M., Shimokawa, H., Ogawa, H., Dellborg, M., Rossi, P.R.F., Troquay, R.P.T., Libby, P., Glynn, R.J., 2017. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N. Engl. J. Med. 377, 1119–1131.

Rigby, W.F.C., Lampl, K., Low, J.M., Furst, D.E., 2017. Review of Routine Laboratory Monitoring for Patients with Rheumatoid Arthritis Receiving Biologic or Nonbiologic DMARDs. Int. J. Rheumatol. 2017, 1–15.

Robert, J., Lehner, M., Frank, S., Perisa, D., von Eckardstein, A., Rohrer, L., 2013. Interleukin 6 Stimulates Endothelial Binding and Transport of High-Density Lipoprotein Through Induction of Endothelial Lipase. Arterioscler. Thromb. Vasc. Biol. 33, 2699–2706.

Robertson, J., Peters, M.J., McInnes, I.B., Sattar, N., 2013. Changes in lipid levels with inflammation and therapy in RA: a maturing paradigm. Nat. Rev. Rheumatol. 9, 513–523.

Robertson, J., Porter, D., Sattar, N., Packard, C.J., Caslake, M., McInnes, I., McCarey, D., 2017. Interleukin-6 blockade raises LDL via reduced catabolism rather than via increased synthesis: a cytokine-specific mechanism for cholesterol changes in

rheumatoid arthritis. Ann. Rheum. Dis. 76, 1949–1952.

Rodríguez-Carrio, J., Alperi-López, M., López, P., López-Mejías, R., Alonso-Castro, S., Abal, F., Ballina-García, F.J., González-Gay, M.Á., Suárez, A., 2017. High triglycerides and low high-density lipoprotein cholesterol lipid profile in rheumatoid arthritis: A potential link among inflammation, oxidative status, and dysfunctional high-density lipoprotein. J. Clin. Lipidol. 11, 1043–1054.

Rohatgi, A., Khera, A., Berry, J.D., Givens, E.G., Ayers, C.R., Wedin, K.E., Neeland, I.J., Yuhanna, I.S., Rader, D.R., de Lemos, J.A., Shaul, P.W., 2014. HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events. N. Engl. J. Med. 371, 2383–2393.

Ronda, N., Favari, E., Borghi, M.O., Ingegnoli, F., Gerosa, M., Chighizola, C., Zimetti, F., Adorni, M.P., Bernini, F., Meroni, P.L., 2014. Impaired serum cholesterol efflux capacity in rheumatoid arthritis and systemic lupus erythematosus. Ann. Rheum. Dis. 73, 609–615.

Ronda, N., Greco, D., Adorni, M.P., Zimetti, F., Favari, E., Hjeltnes, G., Mikkelsen, K., Borghi, M.O., Favalli, E.G., Gatti, R., Hollan, I., Meroni, P.L., Bernini, F., 2015. Newly Identified Antiatherosclerotic Activity of Methotrexate and Adalimumab: Complementary Effects on Lipoprotein Function and Macrophage Cholesterol Metabolism. Arthritis Rheumatol. 67, 1155–1164.

Ronsein, G.E., Vaisar, T., 2017. Inflammation, remodeling, and other factors affecting HDL cholesterol efflux. Curr. Opin. Lipidol. 28, 52–59.

Rosenson, R.S., Brewer, H.B., Davidson, W.S., Fayad, Z.A., Fuster, V., Goldstein, J., Hellerstein, M., Jiang, X.-C., Phillips, M.C., Rader, D.J., Remaley, A.T., Rothblat, G.H., Tall, A.R., Yvan-Charvet, L., 2012. Cholesterol Efflux and Atheroprotection. Circulation

125, 1905–1919.

Roy, A., Das, S., 2016. A study of lipid profile and vitamin E status in Rheumatoid Arthritis patients. Sch. J. Appl. Med. Sci. 4, 1338–1345.

Rubbert-Roth, A., 2012. Assessing the safety of biologic agents in patients with rheumatoid arthritis. Rheumatology 51, v38–v47.

Ruder, B., Atreya, R., Becker, C., 2019. Tumour Necrosis Factor Alpha in Intestinal Homeostasis and Gut Related Diseases. Int. J. Mol. Sci. 20, 1887.

Ruderman, E.M., Crawford, J.M., Maier, A., Liu, J.J., Gravallese, E.M., Weinblatt, M.E., 1997. Histologic liver abnormalities in an autopsy series of patients with rheumatoid arthritis. Br. J. Rheumatol. 36, 210–213.

Saiki, O., Takao, R., Naruse, Y., Kuhara, M., Imai, S., Uda, H., 2007. Infliximab but not methotrexate induces extra-high levels of VLDL-triglyceride. J. Rheumatol. Rheumatol. 34, 1997–2004.

Sakkas, L., 2016. Spotlight on tocilizumab and its potential in the treatment of systemic sclerosis. Drug Des. Devel. Ther. 10, 2723–2728.

Salisbury, D., Bronas, U., 2014. Inflammation and Immune System Contribution to the Etiology of Atherosclerosis. Nurs. Res. 63, 375–385.

Sato, S., Imachi, H., Yu, J., Miyai, Y., Fukunaga, K., 2018. Effect of TNF-α on the expression of ABCA1 in pancreatic β-cells. J. Mol. Endocrinol. 61, 185–193.

Schectman, G., Kaul, S., Mueller, R.A., Borden, E.C., Kissebah, A.H., 1992. The Effect of Interferon on the Metabolism of LDLs. Arterioscler. Thromb. 12, 1053–1062.

Schiff, M.H., Kremer, J.M., Jahreis, A., Vernon, E., Isaacs, J.D., van Vollenhoven, R.F., 2011. Integrated safety in tocilizumab clinical trials. Arthritis Res. Ther. 13, R141.

Schimmel, E.K., Yazici, Y., 2009. Increased lipid levels but unchanged atherogenic index in rheumatoid arthritis patients treated with biologic disease modifying antirheumatic drugs: published experience. Clin. Exp. Rheumatol. 27, 446–51.

Schmidt-Arras, D., Rose-John, S., 2016. IL-6 pathway in the liver: From physiopathology to therapy. J. Hepatol. 64, 1403–1415.

Schultz, O., Oberhauser, F., Saech, J., Rubbert-Roth, A., Hahn, M., Krone, W., Laudes, M., 2010. Effects of Inhibition of Interleukin-6 Signalling on Insulin Sensitivity and Lipoprotein (A) Levels in Human Subjects with Rheumatoid Diseases. PLoS One 5, e14328.

Schumacher, S.M., Naga Prasad, S. V, 2018. Tumor Necrosis Factor-α in Heart Failure: an Updated Review. Curr. Cardiol. Rep. 20, 117.

Schumacher, T., Benndorf, R.A., 2017. ABC Transport Proteins in Cardiovascular Disease—A Brief Summary. Molecules 22, 589.

Scott, L.J., 2017. Tocilizumab: A Review in Rheumatoid Arthritis. Drugs 77, 1865–1879.

Sebba, A., 2008. Tocilizumab: The first interleukin-6-receptor inhibitor. Am. J. Heal. Pharm. 65, 1413–1418.

Segrest, J.P., Jones, M.K., Catte, A., Manchekar, M., Datta, G., Zhang, L., Zhang, R., Li, L., Patterson, J.C., Palgunachari, M.N., Oram, J.F., Ren, G., 2015. Surface Density-Induced Pleating of a Lipid Monolayer Drives Nascent High-Density Lipoprotein Assembly. Structure 23, 1214–1226.

Seki, I., Takai-Imamura, M., Kohara-Tanaka, T., Shirae, S., Sasano, M., Matsuno, H., Aono, H., 2019. Concomitant Treatment with Etanercept and Tacrolimus Synergistically Attenuates Arthritis Progression via Inhibition of Matrix Metalloproteinase-3 Production and Osteoclastogenesis in Human TNF- α Transgenic Mice. Mediators Inflamm. 2019, 1–12.

Serhan, C.N., Chiang, N., Dalli, J., Levy, B.D., 2015. Lipid Mediators in the Resolution of Inflammation. Cold Spring Harb. Perspect. Biol. 7, a016311.

Seriolo, B., 2006. Effects of Anti-TNF- Treatment on Lipid Profile in Patients with Active Rheumatoid Arthritis. Ann. N. Y. Acad. Sci. 1069, 414–419.

Sha Ma, A., Zhang, Q., Song, Z.Y., 2013. TNFa alter cholesterol metabolism in human macrophages via PKC-theta-dependent pathway. BMC Biochem. 14, 20.

Shi, Y., Lüscher, T.F., Camici, G.G., 2014. Dual Role of Endothelial Nitric Oxide Synthase in Oxidized LDL-Induced, p66Shc-Mediated Oxidative Stress in Cultured Human Endothelial Cells. PLoS One 9, e107787.

Singaraja, R.R., Fievet, C., Castro, G., James, E.R., Hennuyer, N., Clee, S.M., Bissada, N., Choy, J.C., Fruchart, J.-C., McManus, B.M., Staels, B., Hayden, M.R., 2002. Increased ABCA1 activity protects against atherosclerosis. J. Clin. Invest. 110, 35–42.

Singh, S., Fumery, M., Singh, A.G., Singh, N., Prokop, L.J., Dulai, P.S., Sandborn, W.J., Curtis, J.R., 2020. Comparative Risk of Cardiovascular Events With Biologic and Synthetic Disease-Modifying Antirheumatic Drugs in Patients With Rheumatoid Arthritis: A Systematic Review and Meta-Analysis. Arthritis Care Res. (Hoboken). 72, 561–576.

Sizova, L., 2008. Approaches to the treatment of early rheumatoid arthritis with disease-modifying antirheumatic drugs. Br. J. Clin. Pharmacol. 66, 173–8.

Smolen, J.S., Aletaha, D., McInnes, I.B., 2016. Rheumatoid arthritis. Lancet 388, 2023–2038.

Smolen, J.S., Landewé, R., Bijlsma, J., Burmester, G., Chatzidionysiou, K., Dougados, M., Nam, J., Ramiro, S., Voshaar, M., van Vollenhoven, R., Aletaha, D., Aringer, M., Boers, M., Buckley, C.D., Buttgereit, F., Bykerk, V., Cardiel, M., Combe, B., Cutolo, M., van Eijk-Hustings, Y., Emery, P., Finckh, A., Gabay, C., Gomez-Reino, J., Gossec, L., Gottenberg, J.-E., Hazes, J.M.W., Huizinga, T., Jani, M., Karateev, D., Kouloumas, M., Kvien, T., Li, Z., Mariette, X., McInnes, I., Mysler, E., Nash, P., Pavelka, K., Poór, G., Richez, C., van Riel, P., Rubbert-Roth, A., Saag, K., da Silva, J., Stamm, T., Takeuchi, T., Westhovens, R., de Wit, M., van der Heijde, D., 2017. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. Ann. Rheum. Dis. 76, 960–977.

Sokolove, J., Strand, V., Greenberg, J.D., Curtis, J.R., Kavanaugh, A., Kremer, J.M., Anofrei, A., Reed, G., Calabrese, L., Hooper, M., Baumgartner, S., Furst, D.E., 2010. Risk of elevated liver enzymes associated with TNF inhibitor utilisation in patients with rheumatoid arthritis. Ann. Rheum. Dis. 69, 1612–1617.

Sookoian, S., Castaño, G.O., Scian, R., Fernández Gianotti, T., Dopazo, H., Rohr, C., Gaj, G., San Martino, J., Sevic, I., Flichman, D., Pirola, C.J., 2016. Serum aminotransferases in nonalcoholic fatty liver disease are a signature of liver metabolic perturbations at the amino acid and Krebs cycle level1,2. Am. J. Clin. Nutr. 103, 422–434.

Sorci-Thomas, M.G., Thomas, M.J., 2013. Why Targeting HDL Should Work as a Therapeutic Tool, but Has Not. J. Cardiovasc. Pharmacol. 62, 239–246.

Sotoudehmanesh, R, Anvari, B., Akhlaghi, M., Shahraeeni, S., Kolahdoozan, S.,

Sotoudehmanesh, Rasoul, 2010. Methotrexate Hepatotoxicity in Patients with Rheumatoid Arthritis. Middle East J. Dig. Dis. 2, 104–109.

Soubrier, M., Jouanel, P., Mathieu, S., Poujol, D., Claus, D., Dubost, J.J., Ristori, J.M., 2008. Effects of anti-tumor necrosis factor therapy on lipid profile in patients with rheumatoid arthritis. Jt. Bone Spine 75, 22–24.

Souto, A., Salgado, E., Maneiro, J.R., Mera, A., Carmona, L., Gómez-Reino, J.J., 2015. Lipid Profile Changes in Patients With Chronic Inflammatory Arthritis Treated With Biologic Agents and Tofacitinib in Randomized Clinical Trials: A Systematic Review and Meta-Analysis. Arthritis Rheumatol. 67, 117–127.

Spanakis, E., Sidiropoulos, P., Papadakis, J., Ganotakis, E., Katsikas, George, Karvounaris, S., Bizaki, A., Kritikos, H., Boumpas, D.T., Spanakis, E.K., Katsikas, G, Karvounaris, ; S, Bizaki, ; A, Kritikos, ; H, 2006. Modest But Sustained Increase of Serum High Density Lipoprotein Cholesterol Levels in Patients with Inflammatory Arthritides Treated with Infliximab. J. Rheumatol. 33, 2440–2446.

Srivastava, N.K., Sharma, S., Purusottam, R.N., Sinha, N., Singh, R., Sharma, D., 2014. Abnormal lipid metabolism in collagen-induced arthritis rat model: In vitro, high resolution NMR spectroscopy based analysis. Indian J. Exp. Biol. 52, 673–682.

Srivastava, N.K., Sharma, S., Sinha, N., Mandal, S.K., Sharma, D., 2018. Abnormal lipid metabolism in a rat model of arthritis: one possible pathway. Mol. Cell. Biochem. 448, 107–124.

Stamatikos, A., Dronadula, N., Ng, P., Palmer, D., Knight, E., Wacker, B.K., Tang, C., Kim, F., Dichek, D.A., 2019. ABCA1 Overexpression in Endothelial Cells In Vitro Enhances ApoAI-Mediated Cholesterol Efflux and Decreases Inflammation. Hum. Gene Ther. 30,

236–248.

Steinberg, D., 1997. Low Density Lipoprotein Oxidation and Its Pathobiological Significance*. J. Biol. Chem. 272, 20963–20966.

Steiner, G., Urowitz, M.B., 2009. Lipid Profiles in Patients with Rheumatoid Arthritis: Mechanisms and the Impact of Treatment. Semin. Arthritis Rheum. 38, 372–381.

Stoffel, W., 1984. Synthesis, transport, and processing of apolipoproteins of high density lipoproteins. J. Lipid Res. 25, 1586–1592.

Strang, A.C., Bisoendial, R.J., Kootte, R.S., Schulte, D.M., Dallinga-Thie, G.M., Levels, J.H.M., Kok, M., Vos, K., Tas, S.W., Tietge, U.J.F., Müller, N., Laudes, M., Gerlag, D.M., Stroes, E.S.G., Tak, P.P., 2013. Pro-atherogenic lipid changes and decreased hepatic LDL receptor expression by tocilizumab in rheumatoid arthritis. Atherosclerosis 229, 174–181.

Streetz, K.L., 2000. Interleukin 6 and liver regeneration. Gut 47, 309–312.

Takeuchi, T., Thorne, C., Karpouzas, G., Sheng, S., Xu, W., Rao, R., Fei, K., Hsu, B., Tak, P.P., 2017. Sirukumab for rheumatoid arthritis: the phase III SIRROUND-D study. Ann. Rheum. Dis. 76, 2001–2008.

Talayero, B.G., Sacks, F.M., 2011. The Role of Triglycerides in Atherosclerosis. Curr. Cardiol. Rep. 13, 544–552.

Tall, A.R., Yvan-Charvet, L., 2015. Cholesterol, inflammation and innate immunity. Nat Rev Immunol 15, 104–116.

Tang, C.-K., 2012. Epigallocatechin-3-gallate prevents TNF-α-induced NF-κB activation thereby upregulating ABCA1 via the Nrf2/Keap1 pathway in macrophage foam cells.

Int. J. Mol. Med. 29, 946–956.

Tang, K.-T., Dufour, J.-F., Chen, P.-H., Hernaez, R., Hutfless, S., 2020. Antitumour necrosis factor-α agents and development of new-onset cirrhosis or non-alcoholic fatty liver disease: a retrospective cohort. BMJ Open Gastroenterol. 7, e000349.

Tanimoto, N., Kumon, Y., Suehiro, T., Ohkubo, S., Ikeda, Y., Nishiya, K., Hashimoto, K., 2003. Serum paraoxonase activity decreases in rheumatoid arthritis. Life Sci. 72, 2877–2885.

Taylor, P.C., 2003. Anti-TNFa Therapy for Rheumatoid Arthritis: an Update. Intern. Med. 42, 15–20.

Testa, M., Yeh, M., Lee, P., Berman, J.W., Lejemtel, T.H., Fanelli, R., Loperfido, F., 1996. Circulating Levels of Cytokines and Their Endogenous Modulators in Patients With Mild to Severe Congestive Heart Failure Due to Coronary Artery Disease or Hypertension. J. Am. Coll. Cardiol. 28, 964–971.

The Lancet Global Health, 2018. Getting to the heart of non-communicable diseases. Lancet Glob. Heal. 6, e933.

Tocci, G., Goletti, D., Marino, V., Matucci, A., Milano, G.M., Cantini, F., Scarpa, R., 2016. Cardiovascular outcomes and tumour necrosis factor antagonists in chronic inflammatory rheumatic disease: a focus on rheumatoid arthritis. Expert Opin. Drug Saf. 15, 55–61.

Toms, T.E., Panoulas, V.F., Kitas, G.D., 2011. Dyslipidaemia in Rheumatological Autoimmune Diseases. Open Cardiovasc. Med. J. 5, 64–75.

Totoson, P., Maguin-Gaté, K., Prigent-Tessier, A., Monnier, A., Verhoeven, F., Marie, C.,

Wendling, D., Demougeot, C., 2016. Etanercept improves endothelial function via pleiotropic effects in rat adjuvant-induced arthritis. Rheumatology 55, 1308–1317.

Tournadre, A., Pereira, B., Dutheil, F., Giraud, C., Courteix, D., Sapin, V., Frayssac, T., Mathieu, S., Malochet-Guinamand, S., Soubrier, M., 2017. Changes in body composition and metabolic profile during interleukin 6 inhibition in rheumatoid arthritis. J. Cachexia. Sarcopenia Muscle 8, 639–646.

Tran-Dinh, A., Diallo, D., Delbosc, S., Varela-Perez, L.M., Dang, Q., Lapergue, B., Burillo, E., Michel, J., Levoye, A., Martin-Ventura, J., Meilhac, O., 2013. HDL and endothelial protection. Br. J. Pharmacol. 169, 493–511.

Tschöpe, C., Van Linthout, S., 2014. New Insights in (Inter)Cellular Mechanisms by Heart Failure with Preserved Ejection Fraction. Curr. Heart Fail. Rep. 11, 436–444.

Urman, A., Taklalsingh, N., Sorrento, C., Mcfarlane, I.M., 2018. Inflammation beyond the Joints: Rheumatoid Arthritis and Cardiovascular Disease. Scifed J Cardiol 2, 1–16.

Usenbo, A., Kramer, V., Young, T., Musekiwa, A., 2015. Prevalence of Arthritis in Africa: A Systematic Review and Meta-Analysis. PLoS One 10, e0133858.

Vaisar, T., Tang, C., Babenko, I., Hutchins, P., Wimberger, J., Suffredini, A.F., Heinecke, J.W., 2015. Inflammatory remodeling of the HDL proteome impairs cholesterol efflux capacity. J. Lipid Res. 56, 1519–1530.

van den Oever, I.A.M., van Sijl, A.M., Nurmohamed, M.T., 2013. Management of cardiovascular risk in patients with rheumatoid arthritis: evidence and expert opinion. Ther. Adv. Musculoskelet. Dis. 5, 166–181.

van Halm, V.P., Nielen, M.M.J., Nurmohamed, M.T., van Schaardenburg, D., Reesink, H.W.,

Voskuyl, A.E., Twisk, J.W.R., van de Stadt, R.J., de Koning, M.H.M.T., Habibuw, M.R., van der Horst-Bruinsma, I.E., Dijkmans, B.A.C., 2006. Lipids and inflammation: serial measurements of the lipid profile of blood donors who later developed rheumatoid arthritis. Ann. Rheum. Dis. 66, 184–188.

Vis, M., Nurmohamed, M.T., Wolbink, G., Voskuyl, A.E., Koning, M.H.M.T. De, Van De Stadt, R.J., Twisk, J.W.R., Dijkmans, B.A.C., Lems, W.F., 2005. Short Term Effects of Infliximab on the Lipid Profile in Patients with Rheumatoid Arthritis. J. Rheumatol. 32, 252–255.

Voloshyna, I., Modayil, S., Littlefield, M.J., Belilos, E., Belostocki, K., Bonetti, L., Rosenblum, G., Carsons, S.E., Reiss, A.B., 2013. Plasma from rheumatoid arthritis patients promotes pro-atherogenic cholesterol transport gene expression in THP-1 human macrophages. Exp. Biol. Med. 238, 1192–1197.

Voloshyna, I., Seshadri, S., Anwar, K., Littlefield, M.J., Belilos, E., Carsons, S.E., Reiss, A.B., 2014. Infliximab Reverses Suppression of Cholesterol Efflux Proteins by TNF- α : A Possible Mechanism for Modulation of Atherogenesis. Biomed Res. Int. 2014, 1–8.

Wagan, D.A.A., Bhutto, A.Q., Khan, D., Rahim, A., 2020. Fatty liver in Pakistani cohort with rheumatoid arthritis. Pakistan J. Med. Sci. 36, 723–728.

Wallberg-Jonsson, S., Dahlen, G., Johnson, O., Olivecrona, G., Rantappa-Dahlqvist, S., 1996. Lipoprotein lipase in relation to inflammatory activity in rheumatoid arthritis. J. Intern. Med. 240, 373–380.

Wang, Garruti, G., Liu, M., Portincasa, P., Wang, D.Q.H., 2017a. Cholesterol and Lipoprotein Metabolism and Atherosclerosis: Recent Advances in Reverse Cholesterol Transport. Ann. Hepatol. 16, S27–S42.

Wang, H., Eckel, R.H., 2009. Lipoprotein lipase: from gene to obesity. Am J Physiol Endocrinol Metab 297, 271–288.

Wang, Wang, S., Wang, Z., Yun, T., Wang, C., Wang, H., 2017b. Tofacitinib ameliorates atherosclerosis and reduces foam cell formation in apoE deficient mice. Biochem. Biophys. Res. Commun. 490, 194–201.

Weiler-Normann, C., Schramm, C., 2011. Drug induced liver injury and its relationship to autoimmune hepatitis. J. Hepatol. 55, 747–749.

Wendt, M.M.N., de Oliveira, M.C., Franco-Salla, G.B., Castro, L.S., Parizotto, Â. V., Souza Silva, F.M., Natali, M.R.M., Bersani-Amado, C.A., Bracht, A., Comar, J.F., 2019. Fatty acids uptake and oxidation are increased in the liver of rats with adjuvant-induced arthritis. Biochim. Biophys. Acta - Mol. Basis Dis. 1865, 696–707.

Wijbrandts, C.A., van Leuven, S.I., Boom, H.D., Gerlag, D.M., Stroes, E.G.S., Kastelein, J.J.P., Tak, P.P., 2009. Sustained changes in lipid profile and macrophage migration inhibitory factor levels after anti-tumour necrosis factor therapy in rheumatoid arthritis. Ann. Rheum. Dis. 68, 1316–1321.

Witte, T., 2015. Methotrexate as combination partner of TNF inhibitors and tocilizumab. What is reasonable from an immunological viewpoint? Clin. Rheumatol. 34, 629–634.

Wullaert, A., van Loo, G., Heyninck, K., Beyaert, R., 2007. Hepatic Tumor Necrosis Factor Signaling and Nuclear Factor-κB: Effects on Liver Homeostasis and Beyond. Endocr. Rev. 28, 365–386.

Xiang, N., Li, X.-M., Zhang, M.-J., Zhao, D.-B., Zhu, P., Zuo, X.-X., Yang, M., Su, Y., Li, Z.-G., Chen, Z., Li, X.-P., 2015. Total glucosides of paeony can reduce the hepatotoxicity caused by Methotrexate and Leflunomide combination treatment of active rheumatoid

arthritis. Int. Immunopharmacol. 28, 802–807.

Yamaguchi, K., Yang, L., McCall, S., Huang, J., Yu, X.X., Pandey, S.K., Bhanot, S., Monia, B.P., Li, Y.-X., Diehl, A.M., 2007. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology 45, 1366–1374.

Yamaguchi, Y., Tanaka, Y., Yamada, K., Bando, Y., Kunitomo, M., 1989. Abnormal Lipid Metabolism in Adjuvant Arthritic Rats. J. Pharmacol. 50, 377.

Yang, H., Mohamed, A.S., Zhou, S., 2012. Oxidized low density lipoprotein, stem cells, and atherosclerosis. Lipids Health Dis. 11, 85.

Yazici, Y., Curtis, J., 2012. Effi cacy of tocilizumab in patients with moderate to severe active rheumatoid arthritis and a previous inadequate response to disease-modifying anti-rheumatic drugs: the ROSE study. Ann Rheum Dis 71, 198–205.

Yeh, K.-W., Lee, C.-M., Chang, C.-J., Lin, Y.-J., Huang, J.-L., 2014. Lipid Profiles Alter from Pro-Atherogenic into Less Atherogenic and Proinflammatory in Juvenile Idiopathic Arthritis Patients Responding to Anti TNF-α Treatment. PLoS One 9, e90757.

Yin, K., Liao, D., Tang, C., 2010. ATP-Binding Membrane Cassette Transporter A1 (ABCA1): A Possible Link between Inflammation and Reverse Cholesterol Transport. Mol. Med. 16, 438–449.

Youssef, G., Allam, N.T., Gaber, W., Afifi, A., Hesham, D., 2018. Increased arterial stiffness in rheumatoid arthritis and Its relation to disease activity: A cross sectional study. Egypt. Hear. J. 70, 35–40.

Yu, J.E., Han, S.Y., Wolfson, B., Zhou, Q., 2018. The role of endothelial lipase in lipid

metabolism, inflammation and cancer. Histol. Histopathol. 33, 1–10.

Yue, X., Wu, M., Jiang, H., Hao, J., Zhao, Q., Zhu, Q., Saren, G., Zhang, Y., Zhang, X., 2016. Endothelial lipase is upregulated by interleukin-6 partly via the p38 MAPK and p65 NF-κB signaling pathways. Mol. Med. Rep. 14, 1979–1985.

Yvan-Charvet, L., Wang, N., Tall, A.R., 2010. Role of HDL, ABCA1, and ABCG1 Transporters in Cholesterol Efflux and Immune Responses. Arterioscler. Thromb. Vasc. Biol. 30, 139–143.

Zannis, V.I., Su, S., Fotakis, P., 2017. Role of apolipoproteins, ABCA1 and LCAT in the biogenesis of normal and aberrant high density lipoproteins. J. Biomed. Res. 31, 471–485.

Zhang, C.-Y., Yuan, W.-G., He, P., Lei, J.-H., Wang, C.-X., 2016. Liver fibrosis and hepatic stellate cells: Etiology, pathological hallmarks and therapeutic targets. World J. Gastroenterolgy 22.

Zhang, Chen, L., Delzell, E., Muntner, P., Hillegass, W.B., Safford, M.M., Millan, I.Y.N., Crowson, C.S., Curtis, J.R., 2014a. The association between inflammatory markers, serum lipids and the risk of cardiovascular events in patients with rheumatoid arthritis. Ann. Rheum. Dis. 73, 1301–1308.

Zhang, H.H., Halbleib, M., Ahmad, F., Manganiello, V.C., Greenberg, A.S., 2002. Tumor Necrosis Factor-Stimulates Lipolysis in Differentiated Human Adipocytes Through Activation of Extracellular Signal-Related Kinase and Elevation of Intracellular cAMP. Diabetes 51, 2929–2935.

Zhang, Temel, R.E., Martel, C., 2014b. Cholesterol and Lipoprotein Metabolism. Arterioscler. Thromb. Vasc. Biol. 34, 1791–1794.

Zhang, Y., McGillicuddy, F.C., Hinkle, C.C., O'Neill, S., Glick, J.M., Rothblat, G.H., Reilly, M.P., 2010. Adipocyte Modulation of High-Density Lipoprotein Cholesterol. Circulation 121, 1347–1355.

Zhang, Z., Zhang, R., Qin, Z.-Z., Chen, J.-P., Xu, J.-Y., Qin, L.-Q., 2018. Effects of Chronic Whey Protein Supplementation on Atherosclerosis in ApoE−/− Mice. J. Nutr. Sci. Vitaminol. (Tokyo). 64, 143–150.

Zhao, S.-P., Dong, S.-Z., 2008. Effect of tumor necrosis factor α on cholesterol efflux in adipocytes. Clin. Chim. Acta 389, 67–71.

Zhou, L., Li, C., Gao, L., Wang, A., 2015. High-density lipoprotein synthesis and metabolism (Review). Mol. Med. Rep. 12, 4015–4021.

Appendix

inhibitors and Interleukin-6 inhibitors (1). However the mechanisms whereby these drugs improve cardiac function is currently unknown. These drugs have also **shown benefits in previous animal models** of rheumatoid (2) and osteoarthritis (3), with **no reported side-effects**. The drugs will be administered intraperitoneally upon the first sign of arthritis (roughly 12 days after immunization) and given as indicated (below) until the termination of the study. The other two groups will receive intraperitoneal injections of phosphate buffered saline solution. The TNFα inhibitor (Enbrel) will be given at a dosage of 10 mg/kg (2) three time a week and the IL-6 inhibitor (Actemra (tocilizumab)) will be given at a dosage of 8 mg/kg once a week as previously described (4,5). The use of these drugs will provide us with a further understanding of the effect of inflammation on vascular and cardiac function and possibly identify novel targets for the treatment of diastolic dysfunction in inflammation induced heart disease. **The protocol of inducing inflammation in the rats will not change from the previous application. The addition of these drugs will in all likelihood reduce inflammation and hence limit the discomfort of the animals**.

Use of pulse-pen to measure pulse wave velocity at termination:

At termination we would like to measure aortic pulse wave velocity (PWV) by applanation tonometry (PulsePen device, DiaTecne, Milan, Italy). The measurement of PWV is **non-invasive** and will provide a further understanding to the arterial function of the animals and the effect of inflammation on aortic stiffness. This procedure will happen during the period when the rats are anesthetized for echocardiography (as per the initial application). In order to obtain the measurement the neck and inner right thigh will be shaved and animals will be placed on a heating pad in the supine position. Briefly, the carotid and femoral pulse wave velocities will be recorded simultaneously by placing one probe on the common carotid artery and one probe on the femoral artery. The two probes will be positioned and fixed on the arteries by means of mechanical arms, equipped with micro-regulators. After that, pressure signals will be transmitted to a computer by means of an optical fiber that ensures the electromagnetic isolation for the rat undergoing the test. After the tonometric pulse wave acquisition, the sites where probes will be placed will be marked on the skin and the distance from the carotid to the femoral will be measured. PWV will be calculated as the distance between the two recording sites divided by the time delay between the two arterial waveforms at each site (6).

References

1. Baniaamam M, Paulus WJ, Blanken AB, Nurmohamed MT (2018): The effect of biological DMARDs on the risk of congestive heart failure in rheumatoid arthritis: a systematic review. Exp Opin Biol Ther 2018; DOI:10.1080/14712598.2018.1462794.

2. Totoson P, Maguin-Gate K, Prigent-Tessier A et al. Etanercept improves endothelial function via pleiotropic effects in rat adjuvant-induced arthritis. Rheumatol 2016;55:1308-17.

3. Lin Y, Liu L, Jiang H, Zhou J, Tang Y. Inhibition of interleukin-6 function attenuates the central sensitization and pain behavior induced by osteoarthritis. Eur J Pharmacol. 2017;811:260-7.

4. Kobayashi Y, Takei M, Giles JT et al. Association of tocilizumab treatment with changes in measures of regional left ventricular function in rheumatoid arthritis, as assessed by cardiac magnetic resonance imaging. Int J Rheum Dis 2016;19:1169–1174.

5. Hançerli Y, Kaplan M, Tanoğlu A et al. Efficacy of tocilizumab treatment in cerulein-induced experimental acute pancreatitis model in rats. Turk J Gastroenterol. 2017;28:485-91.

6. Salvi P, Lio G, Labat C, Ricci E, Pannier B, Benetos A. Validation of a new non-invasive portable

tonometer for determining arterial pressure wave and pulse wave velocity: the PulsePen device. J Hypertens 2004; 22:2285-2293.

Date:07/05/2018

Signature:

RECOMMENDATIONS

Date: 22/5/2018

Signature:

Chairman, AESC

ANIMAL RESEARCH ETHICS COMMITTEE
Registration number: AREC-101210-002

Date: 6 May 2019

Certificate reference: 2017/03/21C
Category: O
Applicant: Lebogang Mokotedi
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Tel: 011 717 2262; **Email**: Lebogang.Mokotedi@wits.ac.za

RE: Waiver from the Animal Ethics Research Committee of the University of the Witwatersrand

This letter is to confirm that **Mangi Denga (791586)**, Department of **Physiology** (WITS), does not require full Animal Ethics Research Committee clearance to undertake the work titled **"Liver metabolic regulation changes in chronic inflammation exposure as a risk for atherosclerotic cardiovascular disease"**.

Reason for waiver
Data from the project entitled "Cardiovascular morphology and function in a rat model of rheumatoid arthritis" has been collected from 2017 to 2018 on 125 rats. From these experiments, specimen samples were collected for future analysis. Mangi Denga will be using these specimen samples (liver and blood samples) as part of her MSC degree.

Details of the study
The aim of the study is to determine the effect of RA biologic anti-inflammatory treatment on lipid metabolism and to assess the effect of treatment on morphological changes of liver samples in Sprague Dawley rats exposed to collagen induced arthritis. Liver and blood samples collected from the previous approved ethics application will be used for this study. Liver samples will be used for histological measurements and blood samples will be used to measure circulating high density-lipoprotein, low-density lipoprotein, very-low density lipoprotein and cholesterol concentrations using ELIZA kits

Please contact me should you require further information.

Yours sincerely

Geoffrey Candy
Chair : Animal Ethics Research Committee, University of the Witwatersrand

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