From DEPARTMENT OF MEDICINE, SOLNA
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PGE2 AND OTHER LIPIDS IN RHEUMATIC DISEASES

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Stockholm 2017

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Published by Karolinska Institutet.
Printed by AJ E-print AB

ISBN 978-91-7676-574-6

PGE2 and other Lipids in Rheumatic Diseases

THESIS FOR DOCTORAL DEGREE (Ph.D.)

AKADEMISK AVHANDLING
som för avläggande av medicin doktorsexamen vid Karolinska Institutet offentligen försvaras i CMM Lecture Hall, L8:00
Friday 17th March, 2017 at 9:00 o'clock

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"Live as if you were to die tomorrow. Learn as if you were to live forever."
~ Mahatma Gandhi

To my beloved family and my dear and loving husband

ABSTRACT

Despite numerous options for treatment of rheumatic diseases, there is an unfulfilled clinical need for therapeutic strategies that can reduce inflammation and prevent tissue destruction. Lipid mediators (eicosanoids and fatty acids (FA)) are involved in the regulation of inflammatory processes and contribute to the pathogenesis of rheumatic diseases. Thus, selective targeting of the lipid mediators might enable improved anti-inflammatory treatment. Microsomal prostaglandin synthase (mPGES) -1 produces prostaglandin E_2 (PGE_2) at sites of inflammation in rheumatic diseases. Inhibitors of mPGES-1 have been proposed as a more selective anti-inflammatory treatment retaining the therapeutic potential of non-steroidal anti-inflammatory drugs (NSAIDs) but with less severe side effects associated with NSAIDs. However, the impact of mPGES-1 inhibition on different pathological and physiological processes is not completely elucidated. Moreover, chronic inflammation might cause dysregulation of lipid and FA metabolism that may contribute to skeletal muscle weakness in patients with polymyositis (PM) and dermatomyositis (DM).
The major aim of this thesis was to gain better understanding of the regulation of PGE_2 and other lipid mediators in RA, PM and in DM to improve treatment of patients.

First, we have determined the catalytic mechanism of mPGES-1 activity by site-directed mutagenesis (**Paper I**). The amino acid residues arginine (Arg) 126 and aspartate (Asp) 49 were identified as essential for the catalytic activity of mPGES-1, as when exchanged, the enzyme variants lost their enzymatic activity. Previous high-resolution structural studies predicted a role for serine (Ser) 127 in the enzymatic activity of mPGES-1. In contrast, we have demonstrated that Ser127, as well as Arg73, do not seem to be significant to the catalytic mechanism because when exchanged, their variants retained considerable activity. These results are of relevance for the development of the new generation of mPGES-1 inhibitors.

Further, we studied whether mPGES-1 deletion might be beneficial for reducing inflammation via the suppression of platelet functions (**Paper II**). Platelet activation, the formation of platelet-leukocyte aggregates, and release of platelet-derived microparticles (PMP) were significantly reduced in mPGES-1 KO mice compared to WT after lipopolysaccharide (LPS) treatment. In addition, KO mice displayed a significant decrease in platelet aggregation *ex vivo*. The reduced activation of platelets may contribute to anti-inflammatory effect and cardiovascular safety of mPGES-1 inhibitors.

In **Paper III**, we investigated effects of mPGES-1, PGIS, and cyclooxygenase (COX) -2 on vascular and renal pathways associated with asymmetric dimethylarginine (ADMA) and endothelial nitric oxide synthase (eNOS). WT mice treated with COX-2 inhibitor displayed no change in the plasma levels of cardioprotective prostacyclin (PGI_2), while mPGES-1 KO mice showed significantly higher PGI_2 levels in the plasma. In contrast to COX-2 inhibition, mPGES-1 deletion had no effect on genes responsible for the production or breakdown of ADMA in the kidney. Plasma creatinine and ADMA were elevated in mice

treated with COX-2 inhibitor or PGIS KO mice but unaltered in mPGES-1 KO mice. Furthermore, the deletion of mPGES-1 significantly improved the eNOS-driven dilator response to acetylcholine in the aorta. These data further confirmed the cardioprotective effects of mPGES-1 deletion suggesting selective inhibitors of mPGES-1 as a safer alternative to NSAIDs.

To clarify mechanisms involved in muscle weakness, we examined effects of the conventional immunosuppressive treatment on global gene expression profiles in skeletal muscle from PM and DM patients (**Paper IV**). The genes related to immune response and inflammation including the interferon and the inflammasome pathways were downregulated by treatment. The genes involved in muscle tissue remodeling and growth were negatively affected by treatment. The immunosuppressive treatment caused an induction of gene markers of fast type II fibers. Furthermore, the fiber composition of the muscle tissue from patients was switched towards type II fibers after treatment. Importantly, the expression of genes involved in lipid metabolism was altered, signifying a probable lipotoxic effect on muscles, that at least partly might explain the persistent muscle weakness and fatigue observed in PM and DM patients despite treatment.

To confirm dysregulated lipid metabolism in myositis patients, we analyzed lipid and FA profiles in serum from patients with PM and DM in comparison to healthy individuals and response to immunosuppressive treatment (**Paper V**). FA composition of total serum lipids was changed in myositis patients compared to healthy individuals. In myositis patients, the levels of palmitic 16:0 acid was significantly higher while the levels of arachidonic 20:4(n-6) acid was significantly lower. The levels of serum lipid species within phosphatidylcholine (PC), lysophosphatidylcholine (LPC) and triglycerides (TG) were also significantly changed in myositis patients compared to healthy individuals. Immunosuppressive treatment resulted in increased serum levels of C20:2(n-6) acid and C20:5(n-3) acids as well as in the changed serum PC, phosphatidylethanolamine (PE) and LPC profiles in myositis patients.

In conclusion, in this thesis, we have provided new knowledge on the catalytic mechanism and the impact of mPGES-1 on inflammation and cardiovascular safety. Furthermore, we have demonstrated that lipid metabolism is altered in PM and DM patients and might contribute to disease pathogenesis.

LIST OF SCIENTIFIC PAPERS

I. **Arg126 and Asp49 are essential for the catalytic function of microsomal prostaglandin E_2 synthase 1 and Ser127 is not**
Joan Raouf, Nazmi Rafique, Michael Christopher Goodman, Helena Idborg, Filip Bergqvist, Richard N. Armstrong, Per-Johan Jakobsson, Ralf Morgenstern, Linda Spahiu
PLoS ONE, 2016, Doi:10.1371/journal.pone.0163600

II. **mPGES-1 deletion affects platelet functions in mice**
Joan Raouf, Fariborz Mobarrez, Karin Larsson, Per-Johan Jakobsson, Marina Korotkova
Clin Sci (Lond). 2016 Oct 7. pii: CS20160463.

III. **mPGES-1 deletion increases prostacyclin and evades the elevated systemic ADMA associated with COX-2 inhibitors: relevance to cardiovascular safety of mPGES-1 inhibitors**
Nicholas S. Kirkby*, **Joan Raouf***, Blerina Ahmetaj-Shala*, Bin Liu, Sarah I. Mazi MRes, Matthew L. Edin, Marina Korotkova, Darryl C. Zeldin, Yingbi Zhou, Per-Johan Jakobsson*, Jane A. Mitchell*
Manuscript

IV. **Effects on muscle tissue remodeling and lipid metabolism in muscle tissue from adult patients with polymyositis or dermatomyositis treated with immunosuppressive agents**
Ingela Loell*, **Joan Raouf***, Yi-Wen Chen, Rongye Shi, Inger Nennesmo, Helene Alexanderson, Maryam Dastmalchi, Kanneboyina Nagaraju, Marina Korotkova, Ingrid E. Lundberg
Arthritis Research & Therapy 2016 Jun 10;18.1.:136. doi: 10.1186/s13075-016-1033-y

V. **Serum lipid and fatty acid profiles are altered in patients with polymyositis or dermatomyositis**
Joan Raouf, Helena Idborg, Petter Olsson, Helene Alexanderson, Maryam Dastmalchi, Per-Johan Jakobsson, Ingrid E Lundberg, Marina Korotkova
Submitted manuscript

*These authors contributed equally to the study.

ADDITIONAL PUBLICATIONS

The author of this thesis has also contributed to the following papers:

I. **Endurance exercise improves molecular pathways of aerobic metabolism in patients with myositis**
Munters LA, Loell I, Ossipova E, **Raouf J**, Dastmalchi M, Lindroos E, Chen YW, Esbjörnsson M, Korotkova M, Alexanderson H, Nagaraju K, Crofford LJ, Jakobsson PJ, Lundberg IE
Arthritis Rheumatol. 2016 Jul;68.7.:1738-50. doi: 10.1002/art.39624

I. **Impaired vagus-mediated immunosuppression in microsomal prostaglandin E synthase-1 deficient mice**
Le Maître E, Revathikumar P, Idborg H, **Raouf J**, Korotkova M, Jakobsson PJ, Lampa J
Prostaglandins Other Lipid Mediat. 2015 Sep;121.Pt B.:155-62. doi: 10.1016/j.prostaglandins.2015.05.006

II. **Effects of mPGES-1 deletion on eicosanoid and fatty acid profiles in mice**
Idborg H, Olsson P, Leclerc P, **Raouf J**, Jakobsson PJ, Korotkova M
Prostaglandins Other Lipid Mediat. 2013 Dec;107:18-25. doi: 10.1016/j.prostaglandins.2013.07.004

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LIST OF ABBREVIATIONS

RA	Rheumatoid Arthritis
IIM	Idiopathic Inflammatory Myopathies
FA	Fatty Acid
PGE_2	Prostaglandin E_2
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
COX	Cyclooxygenase
mPGES-1	Microsomal Prostaglandin E_2 Synthase -1
ILD	Interstitial Lung Disease
DMARDs	Disease-Modifying Anti-Rheumatic Drugs
PM	Polymyositis
DM	Dermatomyositis
IL-1β	Interleukin-1β
IFN	Type 1 Interferon
PGI_2	Prostacyclin / Prostaglandin I_2
TXA_2	Thromboxane A_2
GC	Glucocorticoids
$cPLA_2$	Cytosolic Phospholipase A_2
GR	Glucocorticoid Receptor
GRE	Glucocorticoid Response Element
NF-$_{\kappa}$B	Nuclear Factor-$_{\kappa}$B
AP-1	Activator Protein -1
nGRE	Negative Glucocorticoid Response Element
IGF-1	Insulin-Like Growth Aactor-1
MAPK	Mitogen-Activated Protein Kinase
AMP	Adenosine Monophosphate
TNF-α	Tumor Necrosis Factor –α
JAK	Janus Kinase
LPL	Lysophospholipids
PL	Phospholipid
PA	Phosphatidic Acid
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PS	Phosphatidylserine
PI	Phosphatidylinositol
mTOR	Mechanistic Target of Rapamycin
LPA	Lysophosphatidic Acid
LPC	Lysophosphatidylcholine
LPL-R	Lysophospholipids Receptor
SCFA	Short-Chain Fatty Acid
MCFA	Medium-Chain Fatty Acid
LCFA	Long-Chain Fatty Acid

VLCFA	Very Long-Chain Fatty Acid
ALA	α-Linolenic Acid
LA	Linoleic Acid
PUFA	Polyunsaturated Fatty Acid
ω-3 FA	Omega-3 Fatty Acid
ω-6 FA	Omega-6 Fatty Acid
EPA	Eicosapentaenoic Acid
DHA	Docosahexaenoic Acid
DGLA	Dihomo-γ-Linolenic Acid
AA	Arachidonic Acid
TXA_3	Thromboxane A_3
PGE_3	Prostaglandin E_3
LTB_5	Leukotriene B_5
LO	Lipoxygenase
CA^{2+}	Calcium
PGH_2	Prostaglandin Endoperoxide H_2
PGD_2	Prostaglandin D_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
GPCR	G-Protein-Coupled Cell Surface Receptors
cAMP	Cyclic Adenosine Monophosphate
MAPEG	Membrane-Associated Proteins in Eicosanoid and Glutathione
GSH	Glutathione
LPS	Lipopolysaccharide
mPGES-2	Microsomal Prostaglandin E_2 Synthase -2
cPGES	Cytosolic Prostaglandin E_2 Synthase / Prostaglandin E_2 Synthase -3
PKA	Protein Kinase A
VEGF	Vascular Endothelial Growth Factor
PGIS	Prostaglandin I_2 Synthase
PGDS	Prostaglandin D_2 Synthase
L-PGDS	Lipocalin-Type PGD_2 Synthases
H-PGDS	Hematopoietic PGD_2 Synthases
15d-PGJ_2	15-deoxy-Δ12,14-prostaglandin J_2
PPAR-γ	Peroxisome Proliferator-Activated Receptor -γ
PGFS	Prostaglandin $F_{2\alpha}$ Synthase
TXAS	Thromboxane A_2 Synthase
TXB_2	Thromboxane B_2
5-LO	Arachidonate 5-Lipoxygenase
FLAP	Arachidonate 5-Lipoxygenase Activating Protein
5-HPETE	Arachidonic Acid 5-Hydroperoxide
LTA_4	Leukotriene A_4
LTA_4H	Leukotriene A_4 Hydrolase
LTB_4	Leukotriene B_4
$BLTR_{1 \text{ and } 2}$	Leukotrienes B_4 Receptor 1 and 2
CysLT	Cysteinyl Leukotrienes
LTC_4	Leukotrienes C_4

LTD_4	Leukotrienes D_4
LTE_4	Leukotrienes E_4
LTC_4S	Leukotriene C_4 Synthase
TM	Transmembranes
Ser	Serine
Asp	Aspartate
Arg	Arginine
Egr-1	Early Growth Response Protein 1
JNK	c-Jun N-Terminal Kinase
ERKs	Extracellular Signal–Regulated Kinases
MPKA	P38 Mitogen-Activated Protein Kinases
CIA	Collagen-Induced Arthritis
WT	Wild-Type
CAIA	Collagen-Antibody-Induced
MP	Microparticles
PMP	Platelet-Derived Microparticles
CD40L	CD40 Ligand
eNOS	Endothelial Nitric Oxide Synthase
KO	Knock-Out
ADMA	Asymmetric Dimethylarginine
NO	Nitric Oxide
SLE	Systemic Lupus Erythematosus
SFA	Saturated Fatty Acid
E. coli	Escherichia Coli
TB	Terrific Broth
IPTG	Isopropyl β-D-1-Thiogalactopyranoside
SPE	Solid-Phase Extraction
HPLC-MS	High-Performance Liquid Chromatography Mass Spectrometer
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
HRP	Horseradish Peroxidase
i.p.	Intraperitoneally
PRP	Platelets Rich Plasma
FCA	Flow Cytometry-Based Platelet Aggregation Assay
ADP	Adenosine Diphosphate
IHC	Immunohistochemistry
PBS	Phosphate-Buffered Saline
H_2O_2	Hydrogen Peroxide
ABC	Avidin-Biotin-Peroxidase Complex
DAB	3,3'-Diaminobenzidine
LLE	Liquid-Liquid Extraction
LC-MS/MS	Liquid Chromatogram Tandem Mass Spectrometer
Ala	Alanine
Cys	Cysteine
GST	Glutathione Transferase
Leu	Leucine

FKBP5	FK506 Binding Protein 5
RRAD	Ras-Related Associated with Diabetes
MYBPH	Myosin Binding Protein H
MYH4	Myosin, Heavy Polypeptide 4, Skeletal Muscle
ACTN3	Alpha-Actinin-3
CERS2	Ceramide Synthase 3
GC-MS	Gas-Chromatography Mass Spectrometry
TG	Triglycerides
MUFA	Monounsaturated Fatty Acid

INTRODUCTION

The immune system is one of the most vital systems in the human body; it has evolved to protect our bodies against foreign threats such as microbes, viruses, and parasites. During normal condition, the system is in homeostasis, but during the invasion by foreign pathogens, an orchestra of cells and molecules acting via different mechanisms defends the body from the external threat. The inbuilt capacity of the system is to avoid self-damaging, but occasionally, the reason(s) being mostly unknown, the immune reactions against "self" or molecules that become auto-antigenic arise and cause chronic inflammation leading to autoimmune diseases. Approximately ~5% of the world's population suffers from autoimmune disorders, causing severe disability as well as a heavy economic burden to the society (1, 2). In chronic and destructive autoimmune disorders, such as rheumatoid arthritis (RA) and idiopathic inflammatory myopathies (IIM), inflammation and pain are common symptoms.

The anti-rheumatic treatment is often insufficient despite a wide selection of available drugs, more worrisome, many patients remain disabled. Therefore, there is a great need for selective and improved anti-inflammatory treatments. Lipid mediators such as eicosanoids and fatty acids (FA) are important regulators of inflammatory processes and contribute to the pathogenesis of rheumatic diseases. Thus, selective targeting of the lipid mediators might enable improved anti-inflammatory treatment.

Prostaglandin E_2 (PGE_2) is an essential lipid mediator of inflammation and pain that is deeply involved in the pathogenesis of RA and myositis. Non-steroidal anti-inflammatory drugs (NSAIDs), inhibiting cyclooxygenase (COX) activity, are the first line drugs for the treatment of inflammation and pain. However, due to inhibition of all downstream prostaglandins, including the ones with housekeeping functions, treatment with NSAIDs is associated with several severe side effects such as gastric ulcers and cardiovascular complications.
Hence, microsomal prostaglandin E_2 synthase (mPGES)-1 acting downstream of COX has been extensively investigated as a pharmaceutical target for anti-inflammatory treatment because of the advantage of selective inhibition of PGE_2 (3, 4).
Moreover, chronic inflammation might cause dysregulation of lipid and FA metabolism that may contribute to skeletal muscle weakness in patients with polymyositis (PM) and dermatomyositis (DM).

This Ph.D. thesis intends to provide novel knowledge on the role of PGE_2 and other lipid mediators in RA and PM/DM in order to improve treatment of patients.

Chronic inflammatory diseases

This section will focus on chronic autoimmune diseases and therapeutic strategies.

Inflammation

Every surface and every cavity of the human body is coated with a barrier, physical and chemical, to protect against foreign invaders and maintain homeostasis. In the event of the barrier breach or tissue injury, activation of danger-sensing mechanisms occurs, initiating the inflammatory and immune reactions in the attempt to fight the foreign invader and regain tissue homeostasis.

Symptoms of inflammation are classically described as pain, redness, swelling and heat. The pain alerts the host of the abnormal state of the target tissue. Redness, swelling, and heat are due to the increased blood flow and consequently plasma leakage. During normal conditions, the orchestra of molecules causing the inflammation regains the balance and a state of homeostasis restores. But, when the balance is dysregulated the inflammation amplifies causing irreversible loss of function of the afflicted organ (5).

Inflammation is defined as a response with the purpose to create a hostile environment for the invading pathogen to be overpowered as well as to repair the damaged tissue. The inflammatory response is orchestrated by cells of the targeted organ as well as the immune cells brought via the increased blood flow. The cross-talk between these cells occurs via mediators such as cytokines, chemokines, and lipid mediators; facilitating differentiation and modulation of cells of the immune system. Another very important role these mediators play is the recruitment of inflammatory cells to the afflicted organ. An essential family of mediators in this network is eicosanoids, which modulate inflammation (5, 6). This section will focus on inflammatory disorders such as rheumatoid arthritis (RA) and idiopathic inflammatory myopathies (IIM) and therapeutic strategies for these autoimmune diseases.

Rheumatoid arthritis (RA)

RA is a chronic autoimmune inflammatory disorder with an overall prevalence of 0.5-1% in western countries, with a higher prevalence (3:1) in females and an incidence of 2-50/100 000/per year (7). The disease is more predominant in North America and North Europe and is portrayed by persistent inflammation in the synovial joints causing major damage and pain. RA is characterized by joint swelling, pain, cartilage and bone erosions, ultimately leading to severe and permanent disability, although not all RA patients show signs of bone erosions. Small and medium-size joints are usually symmetrically affected (7). RA is associated with several co-morbidities such as higher risk of cancer mainly lymphomas, cardiovascular disease and interstitial lung disease (ILD) (7, 8).

The etiology of RA is still enigmatic; however, compelling evidence suggests that an interaction between the environmental and genetic factors triggers the onset of the disease (9, 10). Data from twin studies revealed a 15% increase in RA prevalence in twins, showing a clear impact of the interaction between the environmental and the genetic factors on RA risk (11, 12)

Pain is the most common symptom that patients complain about (13). Similar to the immune response, pain naturally exerts an essential protective role in the body. However, every so often patients with RA develop chronic pain. Despite treatment with NSAIDs, disease modifying anti-rheumatic drugs (DMARDs) and biologics, the pain often persists while the joint inflammation and the overall disease activity decreases as well as when the patient is in remission (14). In many cases, patients develop arthralgia before synovitis which is a predictive element in the development of RA (15).

Idiopathic inflammatory myopathies (IIM)

Clinical features of chronic inflammatory diseases such as autoimmune myopathies, including polymyositis (PM) and dermatomyositis (DM) are muscle atrophy and weakness. Common histopathological features of PM and DM are muscle fiber degeneration and regeneration, also the presence of inflammatory cells in skeletal muscle tissue (16). Both immune and non-immune mechanisms are associated with the pathogenesis of the disease (17). Immune mechanisms contributing to the disease include the presence of autoantibodies, infiltrating T cells, macrophages and dendritic cells in muscles as well as elevated expression of MHC class I molecule in muscle fibers. Furthermore, the production of pro-inflammatory cytokines *e.g.* interleukin (IL) -1β and type 1 interferons (IFNs) (18, 19) as well as the expression of enzymes involved in the production of prostaglandins and leukotrienes are elevated in muscle tissues from myositis patients (20, 21).

Non-immune mechanisms implicated in muscle weakness in patients suffering from PM or DM are endoplasmic reticulum stress and capillary-loss-induced hypoxia (16, 22). However, the precise role of the immune and non-immune mechanisms in causing muscle weakness still needs to be clarified. Moreover, the lack of correlation between inflammation and muscle weakness is not well understood (17).

Therapeutic strategies in RA and IIM

There are numerous treatment options for rheumatic patients, but despite extensive therapeutic developments none has been proven to act as a cure for the diseases. Accumulated scientific evidence suggests that the earlier the treatment begins, the earlier the activity of the disease is reduced leading to a better outcome for the patient.

Non-steroidal anti-inflammatory drugs (NSAIDs)

Rheumatic patients exhibiting inflammation and pain are traditionally given NSAIDs which target COX enzymes by inhibiting the enzyme function of COX-1 and COX-2. NSAIDs reduce pain and inflammation and further improve the joint mobility in rheumatic patients, however, in opposite to the situation in ankylosing spondylitis there is no evidence demonstrating that NSAIDs are slowing the RA disease progression. Therefore, they are prescribed as an adjuvant treatment along with more potent anti-rheumatic medications such as synthetic or biological disease-modifying drugs.

NSAIDs are associated with several side effects; common effects are nausea, vomiting, diarrhea, rash, dizziness and several others. However, there are several severe side effects associated with prolonged usage of NSAIDs including renal failure, gastrointestinal ulcers and bleedings as well as higher risk of cardiovascular events (23).

NSAIDs cause side effects by interfering with homeostatic functions of prostanoids. Traditional NSAIDs are non-selective COX-1 and COX-2 inhibitors. During long-term use NSAIDs affect the housekeeping functions of COX-1-dervied prostaglandins in the stomach causing gastric ulcers (24). Coxibs is a family of selective COX-2 selective inhibitors, initially developed to inhibit the COX-2 isoform predominantly expressed during inflammation and spare physiological functions of COX-1. Moreover, Coxibs have beneficial effects in chemoprevention of colorectal cancer but are also associated with increased cardiovascular risks (25, 26) by interfering with the prostacyclin (PGI_2)/thromboxane (TXA_2) balance. Coxibs inhibit COX-2-mediated prostacyclin formation while allowing COX-1-mediated thromboxane synthesis, leading to platelet activation, increased thrombosis and myocardial infarction.

Prostaglandins are also involved in the regulation of the water/salt balance of the body by causing vasodilation of the afferent arterioles of the glomeruli thus regulating the glomerular perfusion and glomerular filtration rate. Consequently, NSAIDs may cause afferent arterioles constriction and decreased renal perfusion pressure (27) leading to hypertonia. To dissociate the anti-inflammatory and pain killing effects of NSAIDs and Coxibs from their side effects, selective mPGES-1 inhibitors were suggested as an alternative anti-inflammatory treatment in chronic inflammatory diseases (28).

Glucocorticoids (GC)

Anti-inflammatory GC represent another important approach in treating rheumatic diseases such as RA and IIM. They are known to suppress disease activity and reduce inflammation, swelling and subsequently pain. Among other mechanisms, GC suppress cytosolic phospholipase A_2 ($cPLA_2$) and inhibits the expression of COX-2 and mPGES-1.

Oral GC such as prednisolone are frequently used as a bridging therapy in combination with a DMARD initiation to control and regulate the disease activity until the time the DMARD treatment becomes clinically effective. Moreover, low-dose GC are regularly used supplementary to DMARDs for their joint-protective effects (29), although evidence point to a negative impact on bone mineral density with increased risk for bone fractures (30) as well as diabetes. Despite the negative impact on bone mineral density and risk of developing diabetes, low-dose GC in combination with anti-rheumatic treatment provide a better clinical outcome as well as less radiographic damages (31). There are few controlled trials in PM and DM regarding treatment with GC, and treatment recommendations are thus based mainly on clinical experience. Myositis patients are usually treated with high doses of GC, ranging from 0.75-1.00mg/kg per day, for several weeks and continuously for very long time on low-dose GC. Although many patients respond with improved muscle function, few patients recover previous muscle performance, and side effects are many (22).

GC are lipid-soluble molecules and have anti-inflammatory effects mediated through several different mechanisms. Transactivation is a genomic mechanism, which is transcription dependent and activates gene transcription. GC passes freely through the cell membrane and binds to its ubiquitously expressed cytoplasmic GC-receptor (GR) that gets activated by ligand binding. The GC-receptor complexes are then translocated into the cell nucleus, where they bind to specific DNA sequences, so called GC response elements (GRE), in the promoter regions of GC-regulated genes and induce the transcription of target genes involved mainly in the resolution of inflammation, such as IL-10 and IL-1 receptor antagonist (32). The transactivation is thought to have a minor role in the anti-inflammatory effects of GC, it is believed to be associated with the many adverse effects of GC usage by augmenting the expression of genes involved in the different metabolic processes, leading to clinical manifestations like diabetes (33).

Transrepression is another genomic mechanism, which is also transcription dependent but represses gene transcription. Here the activated GR-receptor complex prevents pro-inflammatory transcription factors from binding their respective GRE (32). The GC-receptor complex suppresses, therefore, the production of adhesion molecules, pro-inflammatory cytokines, prostanoids and mediators mainly through interaction with other transcription factors such as Nuclear Factor-$_{K}$B (NF-$_{K}$B) and activator protein-1 (AP-1) (34).

There is also non-genomic mechanism described for the actions of GC which is transcription independent and involves processes that do not directly influence the gene expression. It is characterized by rapid onset, within seconds to minutes, and short duration of action, 60-90 minutes, unlike the genomic actions which could take hours, however, its effects is dose-depended, similar to the genomic effects (35). Stahn *et al*., described three categories of the non-genomic actions of GC: nonspecific interactions of GC with cellular membranes, specific interactions of membrane-bound GR either with other proteins or mRNA (36) as well as non-genomic effects mediated through binding to the cytosolic GR (37).

As with almost all drugs, long-term usage of GC is associated with several severe side effects including osteoporosis and diabetes (38). GC also have a direct catabolic effect on skeletal muscle as myopathy can be prevented by GR antagonist as has been shown in rats (39), moreover, GC interfere with insulin-like growth factor-1 (IGF-1) (40).

Disease-modifying anti-rheumatic drugs (DMARDs)

Rheumatic patients are often treated with DMARDs that affects disease progression by slowing down joint destruction and inflammation in skeletal muscle. DMARDs can be divided into two groups; synthetic molecules such as methotrexate and biological molecules such as TNF blockers.

Methotrexate is the first-line choice therapy in RA and IIM treatment (41). The use of methotrexate as an adjunct treatment to oral GC following diagnose of RA and IIM is widely accepted. Evidence point to anti-inflammatory effects of low-dose methotrexate through several mechanisms including adenosine release, the suppression of T cell proliferation, increased apoptosis as well as modulation of cytokine production (42). Methotrexate interferes with DNA synthesis by inhibiting dihydrofolate reductase that forms tetrahydrofolate. This also leads to accumulation of adenosine monophosphate (AMP) and subsequently adenosine. Via these mechanisms, methotrexate, inhibits the proliferation of lymphocytes and the production of tumor necrosis factor (TNF)-α, IL-8, IL-12 and IFN-γ (43).

TNF blockers is a therapy used to treat RA and are known to set the disease to remission (44). Many patients on TNF blockers such as infliximab have fewer symptoms of inflammation, reduced disease activity and increased joint function (45). The most beneficial effects of TNF blockers are the prevention of cartilage and bone destruction (46). Biologicals are often used in combination with DMARDs, *e.g*. the use of TNF blockers is more efficient in combination with methotrexate when treating RA patients (46). TNF blockers inhibit leukocyte trafficking in the synovial compartment caused by the reduced levels of adhesion molecules, pro-inflammatory cytokines such as IL-6 and IL-8 and chemokines (47). Moreover, TNF blockers normalize the dysfunction of regulatory T cells by increasing their number and reversing their anergic phenotype (47). A recent study consisting of 99 patients with early untreated active RA was performed to investigate whether treatment with infliximab for six months in combination with methotrexate and prednisolone treatment gave a better 2-year outcome. Indeed, the combination treatment of patients resulted in clinical remission with less joint damage development as well as delaying the radiological progression (48).

Other emerging pharmacological treatments targeting the Janus kinase (JAK) pathway include B cell depleting therapy (49), anti-IL-6, IL-1 and several others (50, 51).

Lipid mediators

Lipid mediators are a large group of biologically active molecules that are locally generated via specific biochemical pathways in response to extracellular stimuli (52). They can be transported extracellularly, act on specific receptors and regulate essential cell functions. This section will focus on the following classes of lipid mediators: lysophospholipids (LPL), fatty acids (FA) and eicosanoids.

Phospholipids (PL)

PL are amphipathic molecules, meaning they have both hydrophilic (water-loving, polar) and lipophilic (fat-loving, apolar) properties. The PL molecule consists of a negatively charged hydrophilic head, phosphate group and two uncharged, hydrophobic tails also known as FA chains (53). These components are joined by a glycerol molecule, constructing the backbone of the PL molecule, and the phosphate group of a phospholipid is modified by alcohol (54, 55). The PL family includes phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI). PL are essential components of cell membranes and critical for cell functions.

Phosphatidic acid (PA)

PA is a minor constitute of the cell membrane and influences the membrane curvature. PA can be synthetized *de novo* and is the precursor for the biosynthesis of other phospholipids. Furthermore, PA acts as a signaling lipid, recruiting cytosolic proteins to appropriate membranes. Moreover, PA is thought to induce mechanistic target of rapamycin (mTOR) signaling, a well-recognized pathway through which protein synthesis and muscle mass might be regulated by mechanical signals (56, 57). The main source of PA is soya- and egg-based derivatives. A study with athletes given PA daily for 8 weeks demonstrated that the supplementation group gained in muscle strength and increased lean body mass (58). Moreover, PA supplementation is known to increase skeletal muscle hypertrophy and strength (59).

Phosphatidylcholine (PC)

PC is a class of phospholipids that contain choline as a headgroup. PC is a ubiquitous produced, one of the most abundant phospholipids present in the cell membrane and accounts for 50% of the phospholipids. PC is the main source of choline in the body, an essential nutrient, and precursor of the neurotransmitter, acetylcholine (60). PC is vital for the composition and repair of the cell membrane.

Phosphatidylethanolamine (PE)

Another abundant phospholipid found in cell membranes is PE that account for 25% of all the phospholipids in cell membranes. PE are involved in fusion of membranes and regulation of membrane curvature as well as increasing viscosity of the membrane. PE are the main lipid component in the brain such as the white matter in the brain, nerves, spinal cord and neural tissue, where it accounts for 45% of all phospholipids (61).

Phosphatidylserine (PS)

As other phospholipids, PS is a component of the cell membrane and also play a vital role in cell signaling. During apoptosis, the PS is no longer restricted to the cytosolic side of the cellular membrane. Instead, it gets exposed on the extracellular surface, acting as signaling molecules for macrophages to engulf the dying cell (62). Moreover, PS have also a role in blood coagulation, thus platelets activation by collagen and thrombin causes an externalization of PS from their inner membrane layer (63).

Phosphatidylinositol (PI)

PI is a phospholipid which plays an important role in cell signaling and membrane trafficking (64). The *de novo* biosynthesis of PI occurs in the endoplasmic reticulum (64). PI is especially abundant in brain tissue but is present in all tissues and cell types (64). Phosphoinositides are phosphorylated derivatives of PS and are known to be involved in regulating many cellular processes (65). PI is important for intracellular signaling and anchoring of carbohydrates and proteins to outer cellular membranes (66).

Lysophospholipids (LPL)

LPL are products of the hydrolysis of phospholipids in which one or two acyl chains are lacking. The best studied LPL include lysophosphatidic acid (LPA) and lysophosphatidylcholine (LPC). They are known to be important regulators of diverse physiological and pathophysiological functions such as reproduction, angiogenesis as well as inflammation and tumorigenesis (52, 67). LPL act via binding to their specific cognate receptors called lysophospholipids receptor (LPL-R), which are members of GPCR family. The effects of LPL highly depend on the length of the acyl chain and degree of saturation. LPL are present in numerous tissues and fluids both intracellularly and extracellularly (67). The central nervous system is one of the biological systems markedly affected by LPL signaling.

Lysophosphatidic acid (LPA)

LPA is the major LPL formed and is an autocrine and/or paracrine bioactive signaling molecule, acting via different G-protein coupled LPA receptors, triggering a comprehensive series of intracellular signaling pathways (68). LPC is cleaved by the enzyme autotaxin to liberate LPA and choline (69). LPA is involved in various cellular processes including cell migration, proliferation and differentiation (69). LPA has been shown to affect fertility and reproduction, the formation of the nervous system as well as the development of the vasculature (69).

Lysophosphatidylcholine (LPC)

LPC is a product of the partial hydrolysis of PC by the enzymatic action of PLA_2, which removes one of the FA groups. LPC have a short half-life *in vivo* since it is quickly metabolized by lysophospholipase and LPC-acyltransferase. LPC accounts for <3% of the phospholipids of cell membranes and 12% of phospholipids in blood plasma (70). LPC are involved in several different processes such as induction of phagocyte recruitment when released by apoptotic cells (71). Also, LPC activates endothelial cells during atherosclerosis (72, 73) and can change the surface properties of erythrocytes (70).

LPC are known for their important functions in the development of the normal human brain (74). Furthermore, LPC induces pro-inflammatory cytokines such as IFN-γ secretion by a platelet-activating factor receptor-dependent mechanism, in peripheral blood mononuclear leukocytes from healthy blood donors (75).

Fatty Acids (FA)

FA are a carboxylic acid with a long aliphatic chain (composed of carbon and hydrogen) of an even number of carbon atoms. FA with an aliphatic tail of 4-6 carbons are called short-chain FA (SCFA), medium-chain FA (MCFA) consist of 6-12 carbons, long-chain FA (LCFA) consist of 13-21 carbons and very long-chain FA (VLCFA) consist of 22-28 carbons. FA belong to either saturated (no carbon-carbon double bonds, thus saturated with hydrogen) or unsaturated (one or more double bonds between carbon atoms) types (52). Mammals lack enzymes that introduce double bonds beyond carbon atoms C9 in the FA chain, hence, essential FA such as α-linolenic acid (ALA) and linoleic acid (LA), must be supplied in the diet. FA have several major functions in the body and are essential components of the cell membrane phospholipids and have an impact on membrane permeability, fluidity, anchoring of membrane-related proteins and thus cellular functions (76, 77).

There are two principal families of polyunsaturated FA (PUFA), namely omega-3 (ω-3) FA, and omega-6 (ω-6) FA, both are closely involved in many aspects of physiological regulation in the body (78). Both ω-6 and ω-3 PUFA compete for the same enzymes in the eicosanoid synthesis and are precursors of eicosanoids (79, 80).

ω-3 FA

ALA, as well as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) belong to the ω-3 family of FA. They are crucial for fetal growth, comprising the neuronal and the immune functions (81). They are found in green plants and fish and should be supplemented in the diet, such as by the consumption of fish oil. Moreover, the intake of specific dietary FA can modify the chemical composition of the cell membrane. For instance, the consumption of fish oil rich in EPA and DHA can lead to the partial replacement of arachidonic acid (AA) in cell membranes, altering membrane fluidity, which could affect binding of cytokines or alter the cytokine effect on the cells (80, 82).

The metabolism of ω-3 EPA results in the formation of prostaglandin E_3 (PGE_3), thromboxane A_3 (TXA_3) and leukotriene B_5 (LTB_5), which are less biologically active than their ω-6 counterparts (83). Resolvin, protectins and maresins are another class of metabolites of ω-3 EPA and DHA, which are involved in the resolution of inflammation.

ω-6 FA

The ω-6 family of FA consists of LA and its derivatives dihomo-γ-linolenic acid (DGLA) and AA (78). The rich source of ω-6 FA is vegetable oils, nuts and meat. The metabolism of ω-6 PUFA results in the formation of pro-inflammatory mediators PGE_2, TXA_2 and LTB_4.

Growing literature provides the evidence of the important role of FA in skeletal muscle inflammation, growth, and performance (54, 84-88). Structural membrane lipids composition is affected by the dietary FA, which in turns alters muscle function (88). However, the involvement of lipids and FA in the pathogenesis of IIM has not been established.

Eicosanoids

Eicosanoids are a family of lipid mediators originated from 20-carbon-atom FA and involved in the regulation of many physiological and pathological processes (6). The main classes of the potent biologically active eicosanoids are produced via the COX-pathway giving rise to prostaglandins and TXA_2, collectively known as prostanoids, or via the lipoxygenase (LO)-pathway giving rise to leukotrienes and lipoxins. At low concentrations, prostaglandins and leukotrienes have essential physiological effects and act locally due to

their short half-life. Eicosanoids are produced by most types of mammalian cells and bind to individual receptors, exhibiting a wide range of biological functions, the effects vary depending on cell and receptor type.

Biosynthesis of prostanoids

Arachidonic acid (AA), a 20-carbon polyunsaturated ω-6 FA, is released from membrane phospholipids by $cPLA_2$ in response to multiple stimuli. The stimuli leading to an increase in intracellular calcium (Ca^{2+}) levels, induce the translocation of $cPLA_2$ to the membrane and trigger hydrolysis of AA from membrane phospholipids.

The released AA is further converted by COX-1 and COX-2 to prostaglandin endoperoxide H_2 (PGH_2). The COX isoforms are membrane-bound proteins and are localized to the endoplasmic reticulum as well as the nuclear membranes (89, 90). However, their expression patterns, as well as functions, differ. COX-1 is expressed constitutively in most cells and is the dominant source of prostanoids that serves housekeeping functions such as gastric cytoprotection and homeostasis (91, 92). COX-2, however, is an inducible enzyme upon pro-inflammatory stimuli such as cytokines (IL-1β, TNF-α and IL-6), trauma, hormones and growth factors and is the most important source of prostanoids during inflammation (92) (Figure 1). PGH_2 generated by COX-1 and COX-2 then serves as a substrate for specific terminal synthases to produce the five primary prostanoids. The prostanoids family consist of PGE_2, PGI_2, prostaglandin D_2 (PGD_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and TXA_2. These lipid mediators are generated by various cells and have different important biological functions (6).

In uninflamed tissue, these lipid mediators are produced at low levels, however, upon inflammation, the production of prostanoids is dramatically increased. Prostanoids are biologically active lipid compounds with hormone-like effects and have important functions in many physiological processes such as vascular hemostasis (93-96), gastrointestinal integrity (24, 97), kidney perfusion (98), and platelet aggregation (95).

In the pathophysiological conditions, they contribute to the malignant and inflammatory processes. Prostanoids have the dual function in inflammation; they can act as pro-inflammatory mediators and also as anti-inflammatory agents (6).

They exert their biological activities in an autocrine and paracrine manner by binding to their cognate G-protein-coupled cell receptors (GPCR), located on the cell surface or nuclear membrane (Figure 1).

Prostanoids	Receptors
PGE_2	EP_1, EP_2, EP_3 and EP_4
PGI_2	IP
PGD_2	DP_1 and DP_2
$PGF_{2\alpha}$	FP
TXA_2	TP

The cellular repertoire of receptors determines the outcome of the prostanoid action, as certain GPCR activates specific signaling pathway (6, 99, 100). The signaling is achieved through the modulation of intracellular Ca^{2+} or cyclic adenosine monophosphate (cAMP) level (101).
The prostanoids are not exclusively binding to the prostanoid receptors they are assigned by the nomenclature. Since all prostanoids are very similar in structure the cognate receptor can also bind other prostanoids with lower affinities (6, 101). One example of this takes place in the cardiovascular system where a high concentration of PGE_2 can trigger the IP receptor to inhibit platelet aggregation (102) (Figure 1).

PGES and PGE_2

Several PGES converts PGH_2 to PGE_2 (Figure 1). mPGES-1 belongs to Membrane-Associated Proteins in Eicosanoid and Glutathione metabolism (MAPEG) family. mPGES-1 is glutathione (GSH)-dependent terminal synthase in the PGE_2 biosynthesis pathway and constitutes an integral membrane protein. Functionally, mPGES-1 primarily coupled with COX-2 and upon induction by proinflammatory stimuli such as IL-1β (103), TNF-α, and lipopolysaccharide (LPS) the expression of these enzymes increases and leads to an excessive PGE_2 biosynthesis. mPGES-1 is induced by pro-inflammatory stimuli in fibroblasts (104), vascular endothelial cells of the inflamed tissue (103), innate immune cells such as neutrophils (105), macrophages and dendritic cells (106, 107).

Furthermore, there are two more structurally and biologically different PGE_2 synthases acting downstream of COX-1 and COX-2 known as microsomal prostaglandin E_2 synthase (mPGES) -2 and cytosolic prostaglandin E_2 synthase (cPGES). The latter two synthases are expressed constitutively and are responsible for the production of PGE_2 with physiological functions while inducible mPGES-1 produces PGE_2 usually associated with the pathophysiological conditions. The mPGES-1 expression is low under the normal physiological condition and is found in placenta, testis, prostate, mammary glands as well as in the bladder (3). It is also constitutively expressed in the lung, kidney, spleen, and stomach (108). mPGES-1 is not detectable in normal human heart or liver; however, it is detectable after infarctions and can be strongly induced at sites of inflammation and in cancer (4, 109).

PGE_2 physiological and pathophysiological functions

PGE_2 have important physiological properties and is an essential player in inflammation and cancer. It is a relatively stable molecule *in vitro*, however, *in vivo,* it has a very rapid turnover rate. PGE_2 is involved in a wide array of physiological processes including regulation of vascular pressure, maintenance of the gastrointestinal integrity, renal function and female reproduction (110) (Figure 1). PGE_2 mediates its effect via four GPCR (6), EP_1-EP_4 and most major cell subsets of the immune system express at least one of the four EP receptors. PGE receptors couple to a range of intracellular signaling pathways that mediate the effects of receptor activation on cell functions. EP_1 activates PI metabolism via G_q leading to the formation of inositol triphosphate with the mobilization of intercellular free CA^{2+} (6). EP_2 and EP_4 receptors activate adenylyl cyclase via G_s, and therefore increasing intercellular cAMP (6). Lastly, The EP_3 receptor can couple via G_i to elevate intracellular calcium and inhibit cAMP generation (6).

In pathological conditions, PGE_2 is known as a powerful mediator of inflammation, pain and tissue destruction (111, 112). PGE_2 is a key mediator of inflammation causing heat and swelling (113, 114) by stimulating vascular smooth muscle cells, causing vasodilation and increased blood flow into the inflamed tissue. PGE_2 also mediates pain hypersensitivity by lowering the threshold of activation of nerve endings to pain mediators, both centrally and peripherally (115). PGE_2 increases levels of cAMP and enhances nociceptor sensitization by reducing the activation threshold for sodium channels via a protein kinase A (PKA) pathway (116), sensitizing primary afferent neurons to inflammatory mediator bradykinin, as well as to other mediators (117).

PGE_2 have an important role in stimulating the angiogenesis, by triggering an induction of vascular endothelial growth factor (VEGF) production in vascular endothelial cells (118).
PGE_2 has both anti-inflammatory and pro-inflammatory functions when acting on different cells. PGE_2 reduces the oxidative burst of activated neutrophils and inhibits TNF-α production in neutrophils while enhancing IL-10 production, which is an anti-inflammatory cytokine (105, 119). Also, PGE_2 is a part of an auto-regulatory loop inducing the COX-2 expression and consequently its own production at the site of inflammation (28).

Furthermore, PGE_2 modulates the adaptive immune system affecting both on B and T lymphocytes, by inhibiting the lineage development of B cells precursors and the proliferation of mature B cells while promoting IgE and IgG_1 class switch (120). In T cells, however, PGE_2 impact the T cell proliferation both in an inhibitory and stimulatory ways. These opposite effects could be explained by the fact that the T cell receptors, as well as EP_2 and EP_4 receptors, are competing for the recruitment of the same intercellular signaling molecules (121). Lastly, PGE_2 has a critical role in inducing the fever response in the brain, inducing a hostile environment for the invading pathogens (28).

Prostaglandin I_2 synthase (PGIS) and PGI_2

PGI_2 is generated by the terminal synthase PGIS via the conversion of PGH_2. PGIS is co-localized with COX in the endoplasmic reticulum in endothelial cells (6). PGI_2 is mainly produced by endothelial cells, activated monocytes and in vascular smooth muscle cells, and regulates the vascular tone and platelet function by acting as a potent vasodilator and inhibitor of platelet aggregation (96) (Figure 1).

During inflammatory conditions, PGI_2 and PGE_2 have overlapping functions. Both prostaglandins cause vasodilation when binding to respective receptors on vascular smooth muscle cells. Another overlapping function of these two lipid mediators is the induction of pain hypersensitization when acting on peripheral nerve ends (96, 101).

In the adaptive immune system, PGI_2 has both inhibitory as well as stimulatory effects; it inhibits dendritic cell activation, suppresses T cell maturation and stimulatory capacities, and induces TH_1 cell differentiation (122, 123). PGI_2 has a very short half-life *in vivo* and in biological fluids. The method of choice to analyze the PGI_2 synthesis is to measure levels of 6-keto-$PGF_{1\alpha}$, a stable hydrolyzed product of PGI_2 (96).

Prostaglandin D_2 synthase (PGDS) and PGD_2

There are two enzymes converting PGH_2 to PGD_2, namely lipocalin-type and hematopoietic PGD_2 synthases (L-PGDS and H-PGDS, respectively) (Figure 1). L-PGDS is a secretory protein (124) expressed in the central nervous system (125), male genitals organs (126) and in the human heart (124), whereas H-PGDS is a cytosolic protein belonging to the glutathione S-transferase superfamily and is mainly expressed in immune and inflammatory cells (127).

PGD_2 is mainly produced by mast cells as well as macrophages, dendritic cells, and T cells. PGD_2 acts through its cognate receptors, it mediates vasodilation, bronchodilation and inhibits platelet aggregation when bound to DP_1. Through DP_2 it mediates chemotaxis of TH_2 cells, eosinophils, and basophils (101).

In the brain, PGD_2 is involved in regulating sleep, nociception, and temperature (101, 128). Moreover, PGD_2 plays an essential role in allergy and inflammation (129). Both PGD_2 and its nonenzymatic metabolite cyclopentenone 15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ_2) are also shown to have anti-inflammatory properties in a peroxisome proliferator-activated receptor γ (PPAR-γ) depended manner by inhibiting the production of COX-2 and mPGES-1 expression (130).

Prostaglandin $F_{2\alpha}$ synthase (PGFS) and $PGF_{2\alpha}$

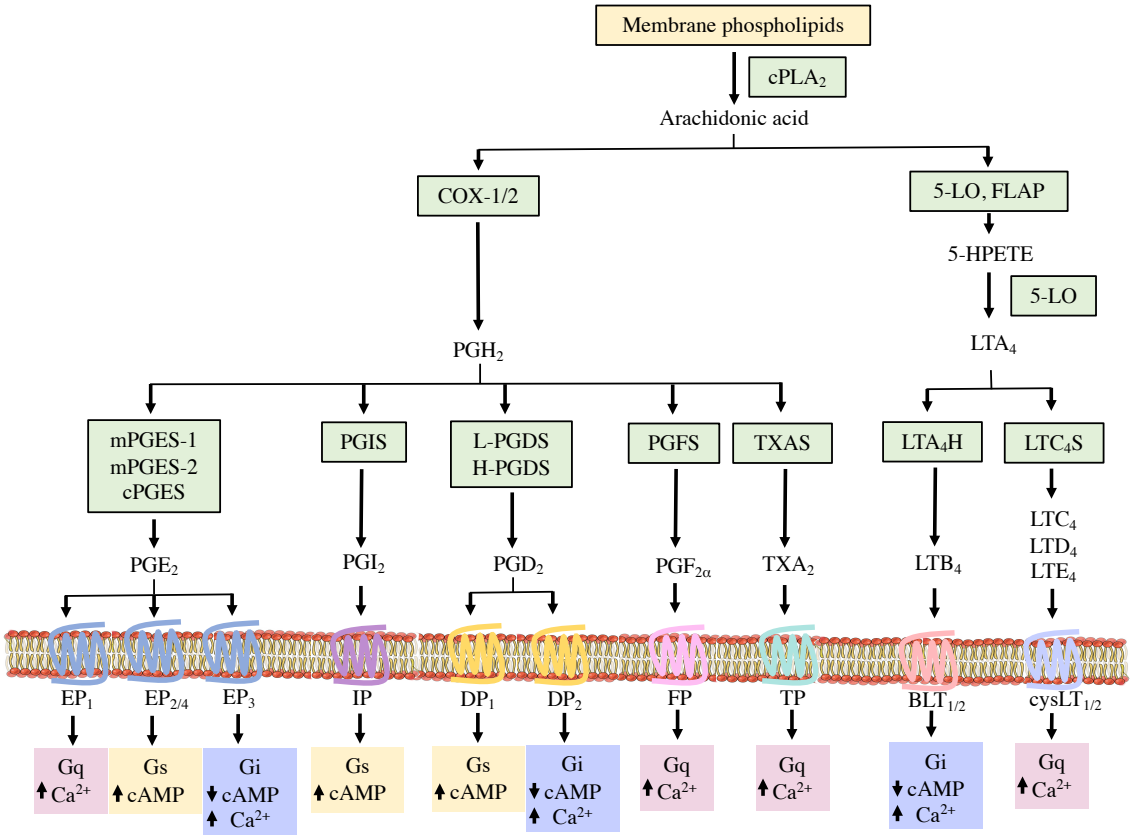
PGFS converts PGH_2 to $PGF_{2\alpha}$ which is involved in many physiologic functions including contraction of uterine and vascular smooth muscles (131) (Figure 1). $PGF_{2\alpha}$ is also involved in the female reproductive cycle and is essential in the regulation of myogenesis.

The FP receptor is not expressed on immune cells, and there is limited evidence of its involvement in inflammatory processes (6, 101). However, there is a study showing elevated biosynthesis of $PGF_{2\alpha}$ in RA, psoriatic arthritis, reactive arthritis as wells as in osteoarthritis patients (132). $PGF_{2\alpha}$ is a stable molecule in aqueous solution with a very short half-life *in vivo* (133).

Thromboxane A_2 synthase (TXAS) and TXA_2

TXA_2 is synthesized by TXAS and is a very unstable molecule, rapidly hydrolyzed to its biologically inactive metabolite thromboxane B_2 (TXB_2) *in vivo* (134) (Figure 1). TXA_2 regulates vascular tone and platelet functions by acting as a vasoconstrictor and inducer of platelet aggregation (6). It is mainly produced by platelets but can be generated by other cell types, for instance, macrophages.

TXA_2 functions through two isoforms (α and β) of the TP GPCR that differ in cellular distribution. TP stimulation leads to platelet activation, adhesion and aggregation, as well as smooth muscle contraction and proliferation (95). The TP receptor is also expressed on dendritic cells, and when bound to TXA_2 it mediates immune response by inhibiting dendritic cell-T cell interactions (135). In biological fluids, the method of choice to analyze TXA_2 synthesis is to measure TXB_2 levels.



PGE_2	PGI_2	PGD_2	$PGF_{2\alpha}$	TXA_2	LTB_4
Vascular pressure GI integrity Renal function Female reproduction	Platelet inhibition (anti-thrombotic)	Bronchodilation Sleep regulation	Parturition Myogenesis	Platelet activation (pro-thrombotic)	
					Physiological functions
Vasodilation Nociception Angiogenesis Fever	Vasodilation Nociception	Vasodilation Nociception Chemotaxis		Vasocontraction	Chemotaxis
					Inflammatory-related functions
Proliferation Fusion Differentiation	Anti-migration Fusion	Proliferation Anti-fusion Anti-differentiation	Proliferation Fusion Anti-apoptosis		Proliferation Differentiation
					Myoblasts-related functions

Figure 1 | Biosynthesis of eicosanoids.
Arachidonic acid (AA) is released from membrane phospholipids by cytosolic phospholipase A_2 ($cPLA_2$). Prostaglandins: cyclooxygenase (COX)-1/2 catalyzes the conversion of AA into prostaglandin H_2 (PGH_2). PGH_2 is then catalyzed by different terminal synthases to several lipid mediators all with biological activity. Microsomal prostaglandin E_2 synthase (mPGES)-1/2 and cytosolic prostaglandin E_2 synthase (cPGES) catalyzes the conversion of PGH_2 to prostaglandin E_2 (PGE_2). Prostacyclin (PGI_2) is formed from by prostaglandin I_2 synthase (PGIS). Lipocalin-type and hematopoietic prostaglandin D_2 synthases (L-PGDS and H-PGDS, respectively) converts PGH_2 to prostaglandin D_2 (PGD_2). PGH_2 is also converted by prostaglandin $F_{2\alpha}$ synthase (PGFS) and prostaglandin TXA_2 synthase (TXAS) into prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and thromboxane A_2 (TXA_2), respectively. Leukotrienes: Arachidonate 5-lipoxygenase (5-LO) and 5-LO activating protein (FLAP) convert AA into arachidonic acid 5-hydroperoxide (5-HPETE) and leukotriene A_4 (LTA_4). LTA_4 is further metabolized by LTA_4 hydrolase (LTA_4 H) into leukotriene B_4 (LTB_4) or by LTC_2 synthase (LTC_4S) into cysteinyl-leukotriene LTC_4, which is further metabolized to LTD_4, and LTE_4. The effects of all eicosanoids are mediated through different G-protein coupled receptors (GPCR), associated with different signal transduction pathways; acting on either the cyclic adenosine monophosphate (cAMP) or/and the calcium (CA^{2+}) release.

Biosynthesis and function of leukotrienes

Leukotrienes are powerful and potent inflammatory mediators and have pathophysiological functions in allergic reactions as well as in respiratory and inflammatory diseases *e.g.* asthma and arthritis (136) (Figure 1). The biosynthesis of leukotrienes from AA, predominately by cells of myeloid origin, is carried out by arachidonate 5-lipoxygenase (5-LO) (Figure 1).
This cellular process is dependent on the assistance of the helper protein, 5-LO activating protein (FLAP). FLAP is crucial for efficient utilization of the substrate AA (137-139). AA is converted by 5-LO interacting with FLAP to arachidonic acid 5-hydroperoxide (5-HPETE) that is an intermediate in the production of leukotriene A_4 (LTA_4). Leukotriene A_4 hydrolase (LTA_4H) converts in turn LTA_4 to leukotriene B_4 (LTB_4).
LTB_4 is synthesized predominately by neutrophils and is a powerful chemotactic mediator, binding to its cognate receptors leukotrienes B_4 receptor 1 and 2 ($BLTR_{1\ and}$ $BLTR_2$) (140) (Figure 1). There are several therapeutic targets on the market targeting the leukotriene pathway by working either as receptor antagonist or inhibiting the synthesis of leukotrienes (141).

Moreover, the regulation of vascular and smooth muscle responses takes place via the action of cysteinyl leukotrienes (CysLT), including leukotrienes C_4 (LTC_4), leukotrienes D_4 (LTD_4) and leukotrienes E_4 (LTE_4). LTA_4 is conjugated with glutathione and forms LTC_4 via the action of leukotriene C_4 synthase (LTC_4S). LTC_4 can be converted to form LTD_4 and LTE_4, which retain biological activity (137, 138). LTC_4S deficient mice have been shown to be resistant to asthmatic challenges (142) (Figure 1).

mPGES-1

This section will focus on mPGES-1 structure and function in rheumatic diseases as well as in cardiovascular safety.

mPGES-1 structure, regulation and function

The human mPGES-1 gene is situated on chromosome 9p34.3 and contains three exons and two introns (143, 144). The mPGES-1 protein is a homotrimer, and each monomer contains four transmembranes (TM) α –helixes, in which the hydrophobic helix transverses the membrane. The charge distribution of mPGES-1 suggests that the N-terminus and C-terminus face the lumen of the endoplasmic reticulum (28). The cleft between TM helix I of one monomer and TM IV of a neighboring monomer constitutes the PGH_2 entry point to the active site where glutathione resides. The mPGES-1 harbors three distinct active site cavities inside the membrane-spanning region in each monomer interface of the trimeric structure. In a molecular dynamics simulation, it was indicated that only one substrate molecule could bind to one of the pockets and form the active complex. This suggests that mPGES-1 trimer has only one pocket active at any given time *i.e.* third of the sites reactivity (145).

There is an extra domain reported in the crystal structure of mPGES-1 which is not found in other members of the MAPEG super family of proteins represented by a small cytosolic domain inserted between transmembrane helix I and II (146). Furthermore, it has been shown that the mPGES-1 has a cone-shaped cavity extending from the cytosolic side of the membrane-spanning region, which is suggested to be a substrate PGH_2 access cavity (146). Moreover, the cofactor GSH adopts a horse-shoe shaped conformation in the active site of mPGES-1 and enters via the cytosol (146).

Several catalytic mechanisms have been proposed for mPGES-1 (146-148) based on predictions and structural studies, and initially Serine (Ser) 127 was thought to be important for the catalytically activity of mPGES-1. In contrast, a recent study investigating the significance of several amino acid residues, all close to the GSH molecule, demonstrated that arginine (Arg) 126 and aspartate (Asp) 49 are important for catalytic activity of mPGES-1, whereas Ser127 is not (147, 149).

The expression of mPGES-1 is primarily regulated at the transcriptional level (150) and also post-transcriptional mechanisms are involved, *e.g.* via mRNA stability (151). However, whether mPGES-1 might be regulated by alternative splicing has not been elucidated. There are several signal transduction pathways impacting mPGES-1 expression. Thus, transcriptional factor early growth response protein 1 (Egr-1) binds to the proximal GC-box in the promotor region and induces transcription of the mouse and human mPGES-1 gene (150). c-Jun N-terminal kinase (JNK) and $NF\text{-}_{\kappa}B$ are two signal transduction pathways for mPGES-1 regulation by TNF-α in gingival fibroblasts (152). Inhibition of

these two TNF-α-activated signal pathways reduces the expression level of mPGES-1 (152). Furthermore, extracellular signal–regulated kinases 1 and 2 (ERK 1/2*)* and *P38* mitogen-activated protein kinases (MPKA) are two signal transduction pathways involved in mPGES-1 regulation by IL-1β in osteoarthritic human cartilage (153). Moreover, PGE_2 might increase the expression of mPGES-1 via positive feedback in certain cells. Hence activation of the PGE_2/EP_2/PKA signaling pathway induces the phosphorylation of the cAMP-response elements binding protein, resulting in transcriptional activation of mPGES-1 in macrophages stimulated with LPS (154). It has also been shown JNK can participate in the regulation of mPGES-1 protein synthesis in cardiomyocytes (155).

mPGES-1 in rheumatic diseases

The AA cascade and the production of eicosanoids are activated in rheumatic patients; during inflammation, an induction of the Ca^{2+}-signaling pathways and cytokine-dependent induction of related enzymes occurs. The expression of COX-2 and mPGES-1, as well as the expression of 5-LO and 15-LO, are upregulated during inflammation, resulting in the activation of eicosanoid production (20, 21).

mPGES-1 in the pathogenesis of RA

In vitro studies of synovial fluid mononuclear cells from RA joint have shown upregulated expression of mPGES-1 and COX-2 upon pro-inflammatory stimuli such as IL-1β, TNF-α or LPS, leading to elevated PGE_2 production (156, 157). Consequently, synovial fluid from untreated RA patients demonstrates prominent levels of prostaglandins such as PGE_2, $PGF_{2\alpha}$, TXB_2, PGD_2 and 6-keto-$PGF_{1\alpha}$ (non-enzymatically hydrolyzed PGI_2) (158).

mPGES-1 and PGE_2 are markedly upregulated due to the inflammatory milieu in the joint, contributing to heat, pain as well as recruitment and activation of inflammatory cells (6). RA patients have high expression of mPGES-1 and therefore elevated levels of PGE_2 in the inflamed synovial tissue (159). mPGES-1 gene polymorphism has been shown to be associated with earlier disease onset as well as higher baseline disease activity score (160). T lymphocytes do not express mPGES-1 (159), however, there is a study demonstrating mPGES-1 expression in B lymphocytes (161).

mPGES-1 is known to be functionally coupled to COX-2 (112, 162) and has been demonstrated to be co-localized in the RA synovial fluid mononuclear cells as well as in cells of the RA synovial lining layer(157). Their expression is also upregulated in synovial fibroblasts and macrophages of the sub-lining layer, as well as in vascular endothelial cells (161). At the sub-cellular level, the co-localization of these enzymes has been reported in

the endoplasmic reticulum as well as in the perinuclear membrane (112). Thus, these enzymes show an efficient coupling function.

The induced expression of mPGES-1 and COX-2 in the synovium of RA patients remained unaffected by TNF blockade (161, 163) and B cell depletion therapy (161), indicating a need for complementary therapy with mPGES-1 inhibitors for optimal anti-inflammatory treatment. However, the expression of mPGES-1 in primary human chondrocytes have been shown to be inhibited in a dose-dependent manner with a certain DMARD aurothiomalate (164).

mPGES-1 in the pathogenesis of IIM

The expression and localization of mPGES-1, as well as other enzymes related to the PGE_2 biosynthesis, have been shown to be enhanced in muscle tissue from PM and DM patients compared to healthy individuals, implicating a role of the PGE_2 pathway in the pathogenesis of inflammatory myositis.

Analysis by double immunofluorescence confirmed a most prevalent expression of mPGES-1 in macrophages in muscle tissue from myositis patients (20). Furthermore, immunosuppressive treatment with GC in combination with DMARDs affected the expression of COX-2 while mPGES-1 expression in inflamed muscle was not changed (20). An explanation could be that in order to suppress mPGES-1 expression, a higher dosage of GC is needed (157).

Importantly, mPGES-1 is induced in rheumatic diseases by a wide range of different stimuli such as hypoxia (165), mechanical stress (166), adipokines (167, 168), smoking (169) and cytokines (170, 171), causing persistent expression in RA synovium and inflamed muscle in PM and DM patients. Therefore, the inhibition of the PGE_2 biosynthesis, by targeting mPGES-1, might complement different anti-rheumatic therapies to gain an ideal anti-inflammatory control of the disease.

mPGES-1 as a therapeutic target in rheumatic diseases

mPGES-1 has been proven to be strongly upregulated at sites of inflammation in RA and in myositis (20, 157, 159) and is not properly targeted by current anti-rheumatic treatments (20, 157, 161, 172). Therefore, mPGES-1 might contribute to the subclinical inflammation and later relapses of the diseases.

The role of mPGES-1 as an alternative therapeutic target for anti-inflammatory treatment of rheumatic diseases has been emphasized by studies in experimental models. Multiple studies evidence that genetic deletion or pharmacological inhibition of mPGES-1 is protective in several experimental models of inflammatory disorders (4, 20). The severity

and incidence of collagen-induced arthritis (CIA) are reduced in mPGES-1 deficient mice. These mice exhibit less pain, joint destruction and reduced levels of anti-collagen type II antibodies compared with wild-type (WT) mice (114).
Similarly, in a collagen-antibody-induced (CAIA) model of human RA, mPGES-1 deficient mice exhibited less severity of the disease (173). In addition, reduced synovial hyperplasia, less infiltration of inflammatory cells as well a reduction of osteoclast number has been reported (114, 173, 174). Moreover, the inhibition or deletion of mPGES-1 does not alter blood pressure or predispose to thrombosis in mouse models (175).

These data together illustrate that the deletion of mPGES-1 reduces inflammatory response, could potentially be cardioprotective and is unlikely to cause side effects seen with selective COX-2 inhibitors.

While COX-1 is considered to be a housekeeping gene and is expressed constitutively in most cells including platelets and endothelial cells (6, 176), the COX-2 expression is highly restricted to the vasculature (177) and the kidney (178), and is up-regulated by pro-inflammatory stimuli. Studies in a mouse model of thrombosis have demonstrated that deletion of COX-2 in the vasculature reduces PGI-M in urine and predisposes the animal to both hypertension and thrombosis (179). Furthermore, selective deletion of COX-2 in mouse cardiomyocytes causes decreased cardiac output, and enhanced susceptibility to induce arrhythmogenesis (180). Because of the side effects related to COX-2 inhibitors, the developments of new drugs targeting prostaglandins in inflammation as well as in cancer (181, 182) has been prevented. Inhibitors of mPGES-1 have been proposed as an alternative treatment that comprises the therapeutic potential of NSAIDs but lacks severe side effects associated with NSAIDs.

The essential characteristics of mPGES-1 inhibitors should be improved efficacy and cardiovascular safety as compared to COX inhibitors. However, knowledge on the effects of mPGES-1 inhibition on the cardiovascular system, especially on platelet and endothelial functions is limited. Platelets express PGE_2 receptors, and their functions might be affected by PGE_2. Studies of the role of mPGES-1 in platelet activation and aggregation will clarify the cardiovascular safety of mPGES-1 inhibitors. Moreover, comparative studies of the effects of the mPGES-1 and COX-2 inhibitors on cellular and molecular processes will identify novel biomarkers and pathways related to anti-inflammatory or side effects of the drugs and eventually lead to improved anti-inflammatory treatment.

mPGES-1 and cardiovascular safety

Prostaglandins are important in the cardiovascular system where they have both homeostasis and pathological functions. PGE_2 is involved in the regulation of blood pressure whereas the TXA_2 and PGI_2 balance is essential for platelet activation. PGE_2 is present in atherosclerotic plaque (183). It has been shown that macrophages produce high

nanomolar concentrations of PGE_2, which are implicated in cardiovascular pathologies such as plaque rupture and abdominal aortic aneurysm formation (184). There is evidence supporting that PGE_2 derived from atherosclerotic plaque can exit the plaque and act directly on platelets (185).

In endothelial cells COX-1 and COX-2 produce PGI_2, which is a cardioprotective hormone, whilst platelet produce TXA_2 which could contribute to thrombosis (6, 94). An dysbalance between these two metabolites, as the results from treatments with Coxibs, can lead to severe cardiovascular side effects. Therefore, mPGES-1 inhibition is thought to be a novel mechanism of a NSAID with no or diminished cardiovascular side effects.

There are several studies implicating that mPGES-1 inhibitors might be more cardioprotective compared to COX-2 inhibitors (186, 187). In a mouse model of thrombotic carotid artery occlusion, genetic deletion of mPGES-1 did not accelerate thrombogenesis (175). Furthermore, suppression of PGE_2 accounts for the protective effect of mPGES-1 deletion in a model of atherosclerosis, leading to a redirection of PGH_2 to PGI_2 production which is thought to be the dominant contributor to a favorable thrombogenic profile (187). Additional studies have demonstrated that global deletion of mPGES-1 has a favorable atheroprotective impact and delays aneurysm formation in hyperlipidemic mice (188, 189).

mPGES-1/PGE_2 pathway in platelet functions

One of the major functions of platelets is to stop bleeding at the site of interrupted endothelium 190, and they are only found in mammals. Platelets lack a cell nucleus and are fragments of the megakaryocytes of the bone marrow. Beyond their role in hemostasis, platelets have been shown to promote inflammation by releasing cytokines, chemokines and other lipid mediators (190, 191) and contribute to the pathophysiology of RA and the cardiovascular system (191, 192). The activation platelets markers P-selectin, CD40L as wells as platelet-derived microparticles (PMP) are frequently found accumulated in blood and synovial fluid of RA patients (193-195).

The release of microparticles (MP) by platelets modulate some inflammatory processes (196). MP are small membrane-coated vesicles produced by budding and fission of the plasma membrane of activated or apoptotic cells (197). MP display surface proteins from their parental cells and are considered to be biological messengers by exhibiting surface phospholipids, cellular origin antigens, and cytokines. In some cases, they could contain mRNA and microRNA (198).

MP have also shown to be involved in the pathogenesis of RA (193, 194). Activated platelets can generate PMP which may play a role in the pathogenesis of RA and the maintenance of the chronic inflammation. PMP have been identified in joint fluid from RA as well as in joint fluid from patients with other inflammatory diseases.

During platelet activation, PMP are produced by the shedding of the platelet membrane (194). PMP containing IL-1β have been shown to have pro-inflammatory properties by initiating cytokine response of synovial fibroblasts (193, 194). Furthermore, enhanced platelet activation and accumulation of PMP in the synovial fluid of inflamed joints from RA patients have been demonstrated (199).

Soluble P-selectin is an indicator of platelet activation and has been shown elevated in plasma from patients with RA compared to healthy individuals. A correlation between disease activity and the levels of detectable platelet-derived proteins such as soluble P-selectin and CD40 ligand (CD40L) in plasma (195) has also been reported. Interestingly, MPs derived from leukocytes strongly induce COX-2 and mPGES-1 expression in RA synovial fibroblasts and shown to stimulate the production of PGE_2 (200).

Furthermore, platelet-depleted mice display a decrease in inflammatory arthritis as evaluated by clinical scoring and by histological analysis. Therefore, platelets are involved in the pathogenesis of inflammatory arthritis in mice (194).

Human and murine platelets do not express mPGES-1 and as a consequence cannot produce inducible PGE_2 (186, 201). However, platelets do express PGE_2 receptors, so their functions might be modulated by the mPGES-1/PGE_2 pathway, induced in inflammatory conditions. The most abundant receptors for PGE_2 are EP_3 and EP_4, both of them expressed on human platelets (202). In addition, EP_2 is also expressed on human platelets, while EP_1 has not been detected on platelets thus far (202-204).

Together, these data suggest that platelets and PMP contribute to the pathogenesis of RA and the maintenance of the chronic inflammation and platelets have been suggested as a candidate to link systemic inflammation, cardiovascular risk and active synovitis in RA patients. Studies of the role of mPGES-1 in platelet activation and aggregation intend to further elucidate the anti-inflammatory actions and cardiovascular safety of mPGES-1 inhibitors.

mPGES-1 and the endothelial function

Endothelial cells play a vital role in a wide range of biological processes including haemostasis, wound healing, angiogenesis and inflammatory disorders (205). They produce AA metabolites, hence, regulating coagulation, vascular tone and immune response. It has been shown that macro vessel-derived endothelial cells from fetal and adults do not produce PGE_2 enzymatically (206). The same authors also demonstrated that macro vessel-derived endothelial cells do not express mPGES-1 (207, 208). However, other studies have demonstrated the expression of mPGES-1 in human microvascular endothelial cells (209).

Tumor cells induce the expression of COX-2 and mPGES-1 expression in microvascular endothelial cells mainly via IL-1 receptor activation (208). In a study with COX-2 knock-

out (KO) mice, the authors showed that COX-2 profoundly limits atherosclerosis and the protection is independent of local PGI_2 release (210). Therefore, more studies on new targets and pathways to define the COX/mPGES-1/NSAID/cardiovascular risk axis are required. In line, a link between the inhibition of COX-2 and the endothelial nitric oxide synthase (eNOS) pathway has recently been revealed (211). This finding sheds new light on the mechanisms how COX-2 and PGI_2 protect the cardiovascular system. In fact, the naturally occurring eNOS inhibitor, asymmetric dimethylarginine (ADMA) (212) is elevated in patients taking Coxibs, which may lead to vascular dysfunction (211, 213).

Lipid mediators in IIM

This section will focus on prostaglandins and FA function in skeletal muscle.

Prostaglandins and their function in skeletal muscle

Skeletal muscle tissue is composed of several different cells types including myocytes, fibroblasts, endothelial cells and smooth muscle cells; all these cells could be a source of prostaglandins. Inflammatory cells such as neutrophils, macrophages, and mast cells are also recruited to the muscle tissue during inflammation or injury and able to producing prostaglandins. Prostaglandins have been appointed essential functions in muscle growth by controlling different steps of myogenesis (214-217). These include survival (218), proliferation (215, 216), differentiation (215), as well as migration and fusion of myoblast (214) (Figure 1).

PGE_2 is also produced by human and mouse myoblasts during proliferation and differentiation (216). PGE_2 regulates muscle growth by promoting proliferation, differentiation and fusion of myoblasts (215, 216).

PGI_2 affects myogenesis via negative regulation of the myoblast migration. It also promotes cell-cell contact and induces cell fusion without affecting myoblast differentiation (214). PGI_2 has been identified in primary mouse myoblasts at different stages of myogenesis. PGIS and the PGI_2 receptor are expressed by mouse primary myoblasts (214).

PGD_2 enhances cell proliferation, reduces myoblast fusion and inhibits myotube formation (219). Both PGD_2 and its metabolite 15d-PGJ_2 are associated with inhibition of myogenesis (219).

$PGF_{2\alpha}$ is produced by human and mouse myoblasts during proliferation and differentiation in to muscle cells (216). $PGF_{2\alpha}$ has been shown to be essential for the induction of myoblast proliferation and promotes the fusion of myoblast to myotubes (215, 216), as well as increases myotube size by preventing myoblast apoptosis (218). FP receptors have been shown to be expressed in differentiated muscle cells (218).

In muscle from healthy individuals, the levels of prostaglandins can become elevated by several different factors such as physical exercise, that induce the release of PGE_2, $PGF_{2\alpha,}$ and 6-keto- $PGF_{1\alpha}$ which promote vasodilation in this organ. Another factor that contributes to the release of prostaglandins by muscle cells is hypoxia that leads to activation of $cPLA_2$ and subsequently increased levels of AA (220).

Since prostaglandins are implicated in muscle cell differentiation and muscle growth, they are also likely to be important in muscle repair. Anti-inflammatory treatment with NSAIDs or GC may in this context has negative effects in restoring muscle tissue after damage.

Prostaglandin production in muscle tissue might be enhanced in response to inflammatory stimuli and further promotes pain, inflammation and muscle wasting.

COX and 5-LO pathways in IIM

The expressions of mPGES-1, COX-1 and COX-2 are increased in muscle tissue from myositis patients in comparison to healthy individuals (159). Conventional immunosuppressive treatment of myositis patients including GC and immunosuppressive drugs downregulates the expression of COX-2 in muscle tissue from patients. Interestingly, the expression of mPGES-1 and COX-1 were not changed after treatment. This data suggests a potential role of these enzymes in persistent inflammation in myositis (20).

It has also been demonstrated that the LTB_4 pathway is upregulated in muscle tissue from patients with myositis. There is a negative correlation between the LTB_4 production and the muscle performance in these patients, suggesting a possible role for LTB_4 in muscle weakness (21).

FA and their functions in skeletal muscle

It is well established that lipid mediators such as prostaglandins and leukotrienes are involved in myogenesis, muscular pain, and inflammation, and thus may contribute to the pathogenesis of myositis. However, modifications in membrane FA composition of inflammatory cells can change the production of inflammatory mediators such as eicosanoids, cytokines, and adhesion molecules, and accordingly, modulate the inflammatory processes in the skeletal muscle.

Patients with a chronic autoimmune disease such as RA, systemic lupus erythematosus (SLE) and juvenile arthritis have altered FA composition in plasma or blood cells compared to healthy individuals (53, 221-223). There are several reasons for these alterations of FA profiles, such as changes in the dietary intake, dysregulation of FA metabolism (55), lifestyle changes leading to less exercising (224, 225), inflammatory processes and the GC treatment (223). It has been shown that conventional immunosuppressive treatment has substantial consequences on the expression of genes related to lipid and FA metabolism in skeletal muscle tissue from myositis patients (226). This suggests that changes in the lipid and FA profiles could contribute to the persistent muscle weakness often seen in patients with myositis despite treatment.

It is well recognized that FA have a great impact on skeletal muscle growth, functions and inflammation (54, 78, 84, 85, 227). Treatment with different FA affects the proliferation

and differentiation of skeletal muscle cells *in vitro* (85-87), while dietary supplementation with certain PUFA affect skeletal muscle performance in experimental animal models and humans (88, 228-230). Saturated FA (SFA) dramatically increased the expression of COX-2 and consequently the production of PGE_2 in mouse skeletal muscle cells. In contrast, PUFA did not have this effect, instead, they abolished the overexpression of COX-2 caused by SFA (84). Lee and cowers have shown that SFA but not PUFA induced the expression of COX-2 mediated through the activation of Toll-like receptor 4 and 2 (231, 232).

Interestingly, an association of specific and distinct FA profiles with different disease markers and muscle strength has been determined in a mouse model of Duchenne muscular dystrophy. These results suggest that profiling the FA composition of tissue lipids could be a beneficial strategy for finding predictive biomarkers as well as potential therapeutic targets in muscle diseases (86). Lipid dysregulation might lead to the generation of lipotoxic mediators which could contribute to cell dysfunction or death. However, whether dysregulation of lipid and FA metabolism is involved in the pathogenesis of myositis and impaired muscle performance has not been elucidated.

Dietary fats and exercise alters FA composition

FA influence skeletal muscle growth and functions as well as participate in regulating inflammation. There are several factors affecting the composition of FA in skeletal muscle, which is at least partly influenced by the diet. Serum FA concentration reflects to some degree the composition of dietary fat (55). Although, the correlation between the estimated intake of specific FA and the proportions in the tissue varies for different FA (55, 78), there is a significant relationship between the dietary intake of PUFA and the equivalent proportions of the same FA in the plasma (78, 80).

Dietary FA have direct influence on skeletal muscle contractile performance and could possibly contribute to the muscle fatigue (229). Substantial changes in the FA composition arise throughout myogenic differentiation, whereas supplementation with diverse FA alters the proliferation and differentiation of skeletal muscle cells (85, 87, 233). Indeed, it has been demonstrated, that by enriching certain type of PUFA in the diet, a positive effect on running endurance, contractile recovery after exercise and skeletal muscle fatigue can be achieved in animal models (88, 228, 229). Furthermore, PUFA supplementation was associated with the improved rate of muscle protein synthesis (234) in humans as well as higher muscle mass and strength in (230, 235).

A randomized, double-blind controlled study has demonstrated anti-inflammatory effects of fish oil in early RA patients. Moreover, there are results suggesting that marine-derived ω-3 PUFA have beneficial effects on reducing the levels of LTB_4 in RA patients (236).

Moreover, studies clearly shows that physical exercise could change the FA composition in skeletal muscle (224) and could consequently affect the muscle function by affecting the FA composition and membrane fluidity.

AIMS

The overall aim of this thesis is to gain a new and better understanding of the involvement of lipid mediators in rheumatic diseases to improve treatment of patients. The author of this thesis has performed studies on catalytic activity and cardiovascular safety of mPGES-1 in the hope of adding more support for the development of mPGES-1 inhibitors. The author has also investigated dysregulated lipid metabolism in IIM patients to provide more knowledge on disease pathogenesis and to explore potential biomarkers of disease activity and treatment.

Specific aims

- To further determine the catalytic mechanism of mPGES-1 activity by site-directed mutagenesis.
- To examine effects of mPGES-1 deletion on platelet functions during inflammation.
- To investigate how mPGES-1, PGIS, and COX-2 influence vascular and renal pathways associated with ADMA and eNOS.
- To examine effects of the conventional immunosuppressive treatment on global gene expression profiles in skeletal muscle from myositis patients.
- To analyze lipid/FA-profiles in serum from patients with PM/DM in comparison to healthy individuals (HI) and response to conventional immunosuppressive treatment.

EXPERIMENTAL PROCEDURES

In this following section, a brief description of the methods used in Paper I to Paper V will be presented and discussed. Only methods directly employed by the author of this thesis were selected to be reviewed. This section will primarily provide some general aspects of the methods including advantages and disadvantages. Detailed methods descriptions can be found in the respective paper and the references therein.

Membrane protein over-expression

mPGES-1 is a membrane protein that naturally occurs at low levels in membrane from different cells and tissues. In rat liver, up to 5% of the total protein isolated from membrane fractions after stimuli with pro-inflammatory cytokines could be accounted for the mPGES-1 protein (237, 238).

To overexpress protein of interest, bacterial over-expression was applied. The recombinant DNA technology was first described by Cohen and Boyer, where they introduced foreign DNA into the host cell *Escherichia coli (E. coli)* (239, 240), allowing for rapid genetic manipulation (241) in a short time.

In short, the first step was to identify and isolate the gene of interest. Restriction enzymes were used to cut DNA at or near the specific recognition nucleotide sequences known as restriction sites, giving specific short fragments. The DNA was then inserted into the plasmid by DNA ligase, an enzyme that facilitates the join of DNA strands together by catalyzing the formation of phosphodiester bonds. The manipulated plasmid is called a vector and carries the gene of interest.

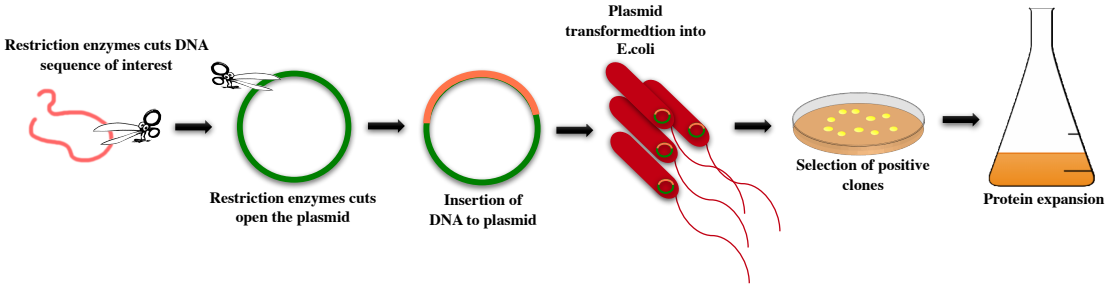


Figure 2 | The illustration of bacterial expression procedure.
Restriction enzymes were used to cut DNA and plasmid of interest. DNA was inserted by DNA ligation to the plasmid. The plasmid was then transformed into the desired host followed by a positive clone selection and big-scale protein expansion.

The construction of recombinant DNA was followed by transforming the recombinant DNA into the host cell, *E. coli,* a gram-negative rod-shaped bacterium (242). Screening by selecting positive clones was done after transformation of the host cells (243). Followed by a big-scale protein expansion in terrific broth (TB) media induced by isopropyl β-D-1-thiogalactopyranoside (IPTG), an inducer that triggers transcription of the lac operon to induce protein expression, the mPGES-1 gene is under the control of lac operon (Figure 2).

The main advantage of this method includes large quantities of desired recombinant protein expressed in a relatively short period of time due to the rapid proliferation of *E. coli*. One disadvantage of this system is the lack of many enzymes that eukaryotic organisms use for post-translational modifications of proteins. Also, this system can only produce a few functional proteins. Another thing to bear in mind is the risk of contaminations with endotoxins, particular if the protein will be used in, *in vivo* or *in vitro*.

This method was applied in **Paper I** where the catalytic function of mPGES-1 was studied. The protocol used for the expression, subcellular fractionation, and purification to homogeneity has previously been established and described (244).

PGE_2 activity assay

To measure the mPGES-1 enzyme activity *in vitro*, the established method was used (162). Briefly, mPGES-1 catalyzes the isomerization of PGH_2 to PGE_2 (3). The substrate PGH_2 is unstable in aqueous solution, and at room temperature, for these reasons, it is necessary to aliquot the acetone dissolved substrate on dry ice prior to the experiment. These steps are crucial in avoiding background noise as well as non-enzymatic degradation of PGH_2 into PGE_2 and PGD_2.
Each sample was diluted in the activity assay buffer containing potassium buffer to a final volume of 100µl. Each sample was then incubated with PGH_2 for 60 seconds at room temperature, in duplicates. As controls, denatured by boiling samples were included. The reaction was then terminated by the addition of the $FeCl_2$ solution (245) and the samples were put on wet ice before the lipid isolation by solid-phase extraction (SPE), to isolate lipids.
Into each sample, the internal standard was added to estimate the extraction efficiency. A standard curve was also included in the experiment in order to quantify the products in the sample. Samples were then separated by high-performance liquid chromatography coupled to a mass spectrometer (HPLC-MS). The chromatograms were integrated, and the area under the curve of each peak was then quantified.

This assay is an excellent versatile assay to use when analyzing the enzymatic activity of mPGES-1. The condition such as incubation time, temperature as well as the concentration of protein can easily be modified. The assay is sensitive and can detect levels in the low picomole range. One disadvantage is that the method is time-consuming since there is a limitation to how many samples that could be analyzed during a single day.

This method was used in **Paper I** to measure the activity of native mPGES-1 as well as mPGES-1 variants expressed in *E. coli*.

Western blot

To identify specific proteins, western blot also known as immunoblot analysis was applied. western blot is used as an analytic technique to identify and semi-quantify specific proteins in complex protein mixtures. The principles behind the technique are protein separation by electrophoresis (246).

In short, samples, either isolated cells or tissue were homogenized mechanically by a homogenizer or by sonication after the addition of lysis buffer. To avoid protein denaturing and degradation, samples were prepared on wet ice, and anti-proteases were added to the lysis buffer. Proteins were then separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).
Strong reducing agents was used to prevent secondary and tertiary structure and thus allowing separation of proteins by their masses. Sampled proteins get negatively charged by the SDS and move to the positively charged electrode through the acrylamide gel. Smaller proteins migrate faster in the gel whereas the larger proteins get trapped, thus leading to a protein separation according to size.
A visual protein ladder was used to estimate the size of the target protein. Proteins within the gel were transferred to a non-specific binding membrane, to make the proteins accessible for antibody detection. This binding is due to hydrophobic interactions as well as charged interactions between the membrane and proteins.

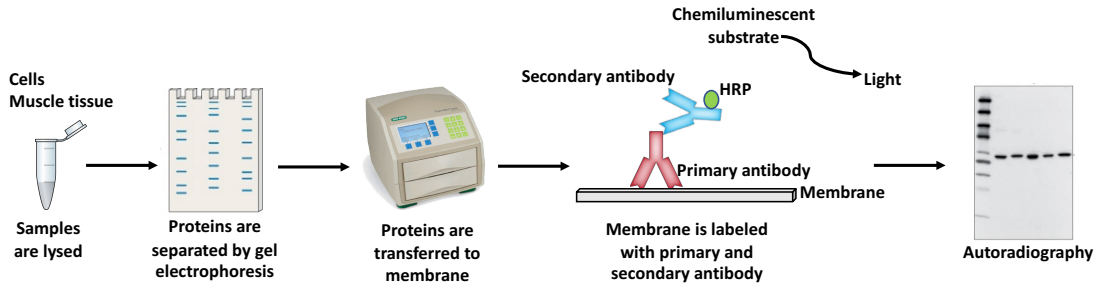


Figure 3 | Schematic overview of western blot procedure.
Samples either cells or tissue were lysed in lysis buffer supplemented with anti-proteases, followed by reduction and denaturation of the samples before gel electrophoresis. Proteins were transferred to a membrane and incubated with the primary antibody overnight. Before and after the secondary antibody incubation, the membrane was washed and subsequently developed.

Blocking of non-specific binding by diluted protein solution was done to avoid the antibody binding to the membrane which has protein binding properties. This step is crucial to avoid the antibody binding to the membrane other than on the binding site of the specific target protein, thus to avoid false positive binding and reduce the background. The membrane was incubated with the primary antibody with gentle agitation overnight at 4ºC, followed by rinsing of the membrane to remove unbound primary antibody.
The secondary antibody linked to horseradish peroxidase (HRP) which is a reporter enzyme, was added to record the signal. The chemiluminescent agent was cleaved to get the production of luminescence which is in proportion to the amount of the protein. A sensitive photographic film was placed on the membrane, and the image of the antibodies bound to the blot was visualized (247) (Figure 3).

The biggest advantage of using Western blot analysis is the high sensitivity of the method, allowing detecting as little as 0.1 nanogram protein in the sample. Although great advantages, Western blot is a delicate process which requires precision in every step for proper identification of proteins.

This method was applied in **Paper I, Paper II** and **Paper IV**.
In **Paper I** the method was used to investigate the protein expression of WT mPGES-1 and variants. In **Paper II** the method was applied to identify whether platelets express mPGES-1 or not. In **Paper IV** the method was used to validate the gene expression profiling data.

In vivo model of inflammation

To investigate whether mPGES-1 deletion might have beneficial effects on platelet-mediated function as well as if it influences pathways associated with ADMA and eNOS during inflammation, a mouse *in vivo* model was applied. Mice with a deletion of the Ptges gene, which encodes mPGES-1 and on a DBA/1lacJ genetic background, were generated by breeding heterozygous littermates as previously described (114).

In studies of pathways associated with ADMA and eNOS, mPGES-$1^{+/+}$ and mPGES-$1^{-/-}$ treated with 100mg/kg parecoxib in drinking water for 5 days. After sacrifice of mice with carbon dioxide, blood from the inferior vena cava, and urine was collected, as well as the aortic ring and kidney tissue were collected from mice.

In studies of platelet-mediated function, WT ($^{+/+}$) and mPGES-1 KO ($^{-/-}$) mice were injected with 2µg of LPS or saline, intraperitoneally (i.p.) for 24 hours. After anesthetization of mice with isoflurane, blood was collected by cardiac puncture, furthermore, mouse liver and spleen tissue were collected (Figure 4).

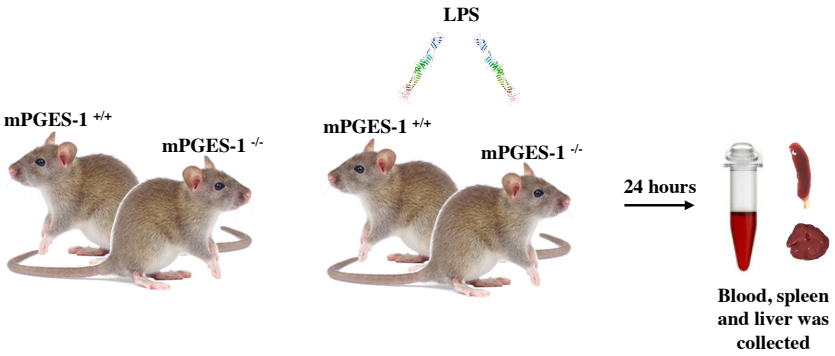


Figure 4 | Schematic overview of the *in vivo* mouse model. mPGES-1$^{+/+}$ and mPGES-1$^{-/-}$ were injected with 2µg LPS. After 24 hours' blood, spleen and liver were collected.

Animal experiment *in vivo* remains a crucial step for the validation of *in vitro* experimental findings. It is an important tool to use when validating a drug target and ensuring the safety of the drug to improve the human health. One disadvantage with this method is that although the animals used have similar anatomy as the humans, the reaction of the drug tested in animals could be quite different from the reaction in humans.

This method was used in **Paper II** and **Paper III**.
In **Paper II** the method was used in order to investigate effects of mPGES-1 deletion on platelet-mediated function during inflammation. In **Paper III** the method was used to investigate beneficial effects of mPGES-1 deletion compared to COX-2 inhibitors.

Flow cytometry

Flow cytometry is a laser-based technology employed predominately in cell sorting, cell counting, as well as biomarker detection. The principle of the method is an analysis of stained cells in suspension passing by an electron detection apparatus in a stream of fluid (248).

To investigate the platelet function in whole blood and PMPs from platelets rich plasma (PRP), flow cytometry was used. Briefly, the samples, either whole blood or PMP were labeled with antibodies recognizing platelets and platelet activation markers (P-selectin and CD40L).

Platelets and PMPs were defined by their size characteristics and antibody recognition. In addition, platelet aggregation was measured by flow cytometry-based platelet aggregation assay (FCA) using a modified protocol (249). In short, whole blood was divided into two

equal portions; each portion was labeled with different antibodies. After the incubation for 15 minutes', the portions were combined and incubated additionally for 15 minutes. Platelets were then activated by incubation with adenosine diphosphate (ADP) by shaking for 10 minutes. Cell fixation was performed before the analysis by flow cytometry (Figure 5).

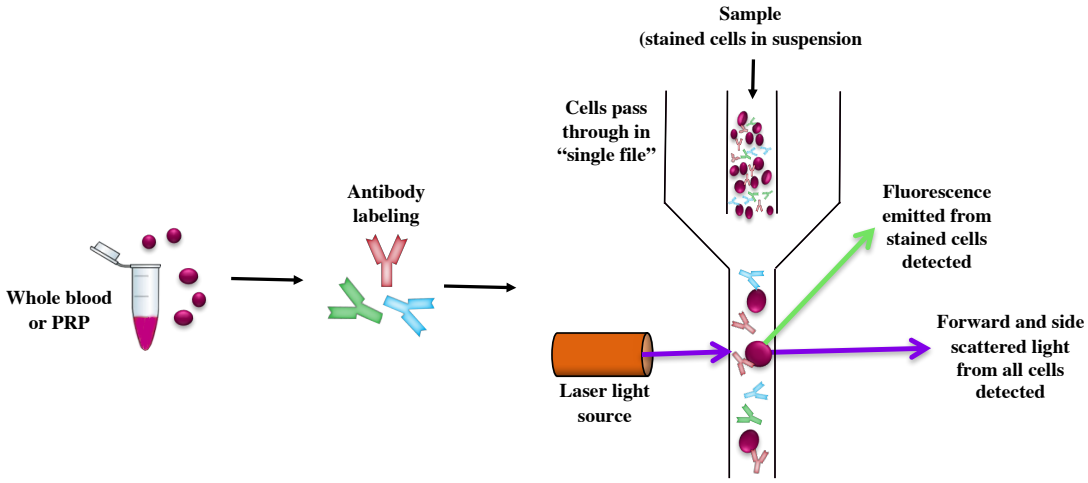


Figure 5 | The illustration of the flow cytometry procedure.
Whole blood or platelet rich plasma (PRP) was labeled with fluorescent antibodies. The suspended sample was run using flow cytometry. The cell suspension gets focused by fluid and causing cells to pass through a laser beam, one cell at the time. Fluorescence emitted from stained cells as well as forward and side scattered light is detected.

The main advantage utilizing this method is the ability to characterize antigen expression on a cell-by-cell basis in the large heterogeneous population of cells.

This method was used in **Paper II** to measure the platelets PMPs levels, the platelet-leukocyte aggregates and platelet aggregation.

Immunohistochemistry (IHC)

IHC was used to detect the presence of certain cells as well as to study any alterations after treatment with GC at cellular and protein levels in cross-sectional muscle tissue from patients (250, 251).

Briefly, sections were rehydrated with phosphate-buffered saline (PBS). As a permeabilizing detergent, the saponin was used; the addition of the saponin is important when staining of intracellular markers. Before the application of primary antibody, the endogenous peroxidase activity was blocked by incubation with hydrogen peroxide (H_2O_2) in the dark. The blocking is important in order to avoid non-specific background since many cells and tissues contain endogenous peroxidases.

The tissue was then blocked with the serum from the same species as the studied tissue, before and during the application of primary antibody. The avidin-biotin blocking solution was applied to reduce the non-specific background since many cells contain endogenous biotin. Avidin, a large glycoprotein, has a very high affinity for biotin, a low molecular weight vitamin, which can be conjugated to antibodies and other biological molecules. Also, before incubation with the biotinylated secondary antibody, the tissue was blocked with serum from the same species origin as the secondary antibody.

Incubation with avidin-biotin-peroxidase complex (ABC) was done. The ABC technique comprises of three steps, at the first step, an unlabeled primary antibody is added, at the second step, the biotinylated secondary antibody is added and at the third step, the complex of avidin-biotin-peroxidase is applied. The peroxidase then converts a substrate to different colored end products. In our experiments, the 3,3'-diaminobenzidine (DAB) was used as the substrate to develop the reaction. Mayer's Hematoxylin was used to counterstain the tissue and to make the nuclei of cells visible. As the final step slides were mounted in glycerol for observation by microscope (Figure 6).

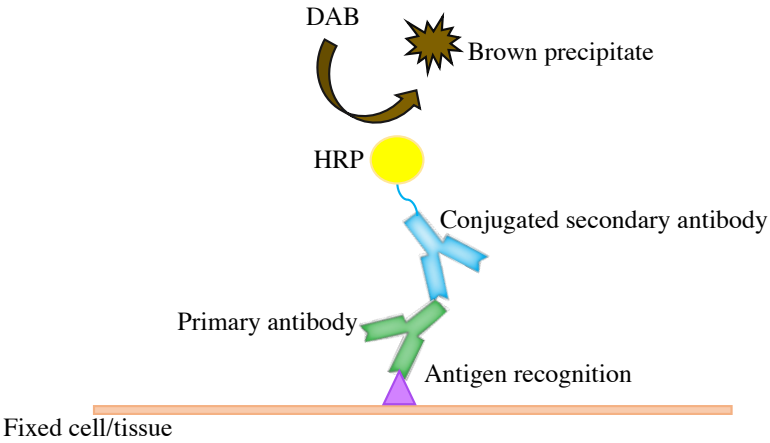


Figure 6 | Schematic overview of the IHC procedure.
An antigen on the cell surface was recognized by the primary antibody. The HRP conjugated secondary antibody binds to the primary antibody. DAB was used as a substrate to visualize the binding of the antibodies.

The main advantage of this method is that it provides the qualitative description and semi-quantitative scoring of the protein pattern in the tissue. Although incorrect freezing, storing and sectioning of biopsies could cause artifacts.

This method was used in **Paper IV** to detect the presence of immune cells as well as to study alteration in the protein expression after treatment with GC at the cellular level in muscle tissue from PM and DM patients.

Solid-phase extraction (SPE)

SPE is suitable to separate analytes of importance (252) from a wide selection of material, such as urine, water, blood, soil and animal tissue homogenate (253). In brief, the filter cartridge was equilibrated with methanol, which is a slightly polar solvent, although a more non-polar solvent can also be used. As a result, the surface gets wet, and the solvent

penetrates the polymer in the column making it assessable to the samples. The samples were spiked with internal standards and were loaded onto the column and washed trough the stationary phase.
The analytes that have polar properties interact and maintain on the polar sorbent, other non-polar impurities pass through the column. A washing solution containing slightly polar or non-polar solvent, was added to remove further impurities. The molecules bound to the column were then eluted by adding the polar solvent with the proper pH. With this technique, the hydrophobic material in hydrophilic solvents sticks to the column and the hydrophilic material runs through.

The main advantages of this method are the high accuracy and no risk for cross-contamination, also the sample handling is easy due to fewer process steps (254).

This method was used in **Paper I** to measure the prostanoid production by WT mPGES-1 and variants incubated with PGH_2.

Liquid-liquid extraction (LLE)

LLE is a technique used to disperse compounds based on their solubility in two different liquids such as water and an organic solvent (255, 256). Briefly, samples were thawed in the refrigerator and centrifuged. Internal standards were added to each sample together with a mixture of methanol and chloroform (2:1). The samples were then vortexed and sonicated. The organic phase, containing lipids, was removed and saved in Eppendorf tubes. The samples were then re-extracted by the same procedures as described above. The samples containing lipids were combined and evaporated to increase the concentration of lipids in the extraction solvent.

LLE extractions offer many analytical benefits; the primary advantage is the ability to operate in a continuous, multistage countercurrent mode, giving rise to a very efficient separation. One disadvantage is that the method is time-consuming when extract needs to be enriched, and multiple extractions are necessary.

This method was used in **Paper V** to measure lipids and FA in serum from myositis patients.

Statistical analyses

In **Paper I**, unpaired t-test was used to analyze data. $P<0.05$ was considered to be statistically significant Data are expressed as the mean ± SD.

In **Paper II**, to obtain a normal distribution data were log transformed before statistical analysis. WT and KO mice were compared using the unpaired Student's t-test. P values less than 0.05 were considered to be statistically significant. Prism 6.0 software (GraphPad software, USA) was used for statistical analysis. Data are expressed as the mean ± S.E.M.

In **Paper III**, where duplicate measurements of tissue from the same animals were made the values were averaged and considered as n=1. Data were compared using Student's unpaired t-test, one sample t-test, one-way or two-way ANOVA with Dunnett's posthoc test as indicated in individual figure legends. The level of significance was set at a $P \leq 0.05$. Data were analyzed using Prism 6.0 software (GraphPad software, USA). Data are expressed as the mean ± standard error.

In **Paper IV**, Wilcoxon signed rank test was used for analysis of clinical and experimental values. P values less than 0.05 were considered to be statistically significant. Data are expressed as the mean ± SD.

In **Paper V**, Mann-Whitney's U test and Wilcoxon's signed rank test was used to analyze data. The Spearman rank correlation test was used to evaluate the correlation between the clinical parameters and the levels of specific lipid species or FA in serum. Data are expressed as the mean ± SD, differences were considered significant if $P<0.05$.

Ethics

The Local Regional Ethical Review Board, Stockholm, Sweden approved all studies included in this thesis. Data collection, as well as the publication of results, have been conveyed so that the anonymity and integrity of the patients were guaranteed. Mice used for the experiments included in this thesis were handled with respect and were not subject to unnecessary pain. The number of mice used in this thesis were limited so that statistically significant results could be obtained.

Ethical approval numbers:
Paper I: None.

Paper II: N86/13 and N364/11, Karolinska Institutet.

Paper III: N86/13, N364/11, Karolinska Institutet and SUMC2013-043, SUMC2014-095, Guangdong province, China.

Paper IV: Dnr 98-045, 2005/792-31/4, 2008/449-32 and 2011/1374-32, Karolinska Institutet.

Paper V: Dnr 98-045, 2005/792-31/4, 2008/449-32 and 2011/1374-32, Karolinska Institutet.

RESULTS AND DISCUSSION

This thesis comprises five different studies regarding the functions and effects of PGE_2 as well as other lipids in rheumatic diseases. The results of three studies have been published, and the publications can be found at the end of this thesis as **paper I**, **paper II** and **paper IV**. Two studies including **paper III** and **paper V** have not yet been published and are available in the form of manuscripts. In this section the main findings are presented and discussed in the context of the current literature.

Essential residues for the catalytic function of mPGES-1

This section will focus on the catalytic function of mPGES-1.

Determination of the catalytic mechanism of mPGES-1 activity by site-directed mutagenesis (Paper I)

In Paper I, we extended our studies of mPGES-1 activity to examine and clarify which residues are important for the catalytic function of mPGES-1. There are two main reasons why it is important to study the catalytic function of mPGES-1. Firstly, it would provide new information for characterization and mapping of the catalytic mechanisms of MAPEG superfamily members. Secondly and more importantly, knowing the exact catalytic function of the enzyme would help in drug development of mPGES-1 inhibitors that will be used as future anti-inflammatory drugs.

Several catalytic mechanisms have been proposed for mPGES-1 (146-148). Based on these findings regarding predictions and structural studies, we aimed to investigate the role of the amino acid residues Asp49, Arg73, Arg126 and Ser127 in the catalytic function of mPGES-1, all in close vicinity of the GSH molecule. The variants of mPGES-1 with substituted amino acids were obtained by site-directed mutagenesis. The mutated enzyme variants were then cloned and expressed in both the *E. coli* and the *Baculovirus* expression systems. Their catalytic significance was evaluated by activity measurements with prostanoid profiling by liquid-chromatography tandem mass spectrometer LC-MS.

We have found that all constructs expressed mPGES-1 to varying degrees and that only WT mPGES-1, Arg73 and Ser127 variants catalyzed the formation of PGE_2 at a significant level compared to their negative controls consisting of denatured by boiling protein. All other quantified prostanoids were produced at background level in our *E. coli* system. Our measurements are in the range with the initially observed specific activity of purified mPGES-1 (257).

Previous structural data suggested that Ser127 might be important residue for the catalytic function of mPGES-1 (146). However, our results demonstrate that mutation of Ser127 to

alanine (Ala) only lowers the activity of the enzyme, measured in *E. coli* membranes. It has been some inconsistency with obtaining similar data when using different expression systems that might influence the activity of the enzyme. Therefore, we decided to use a second expression system, *Baculovirus* expression system. In addition, the activity of Ser127 cysteine (Cys) variant from *SF9 cells* was measured by glutathione transferase (GST) activity assay and quantified by PGH_2 isomerization and showed only lowered activity compared to WT mPGES-1. Thus, regardless of the assay employed, the Ser127Ala variant retained activity. We concluded that Ser127 residue is not important for the catalytic activity, which are in line with recent findings by Brock *et al*. (147).

Furthermore, we have also demonstrated that mutations of Asp49 to Ala and Arg126 to either Ala or leucine (Leu) in membrane fractions from the *E. coli* expression system resulted in complete loss of activity confirming a central role of these residues in the catalytic function of mPGES-1. Interestingly, no formation of $PGF_{2\alpha}$ was detected, which is contradictory to previous observations (147, 148).

In summary, we propose two alternative chemical reactions for the mPGES-1 catalyzed PGE_2 synthesis mechanism (Figure 7A). The first proposition is that an attack of the GSH thiolate on C9 oxygen occurs, forming a sulfenic acid ester, followed by a proton receiving at C11 and proton abstraction at C9 by Asp49. Arg126 is thought to stabilize the leaving GSH thiolate, leading to the PGE_2 formation. The second proposition is that Asp49 initiates the reaction by abstracting a proton at C9, splitting the protonated GSH and donating a proton instead, leading to the formation of PGE_2 (Figure 7B).

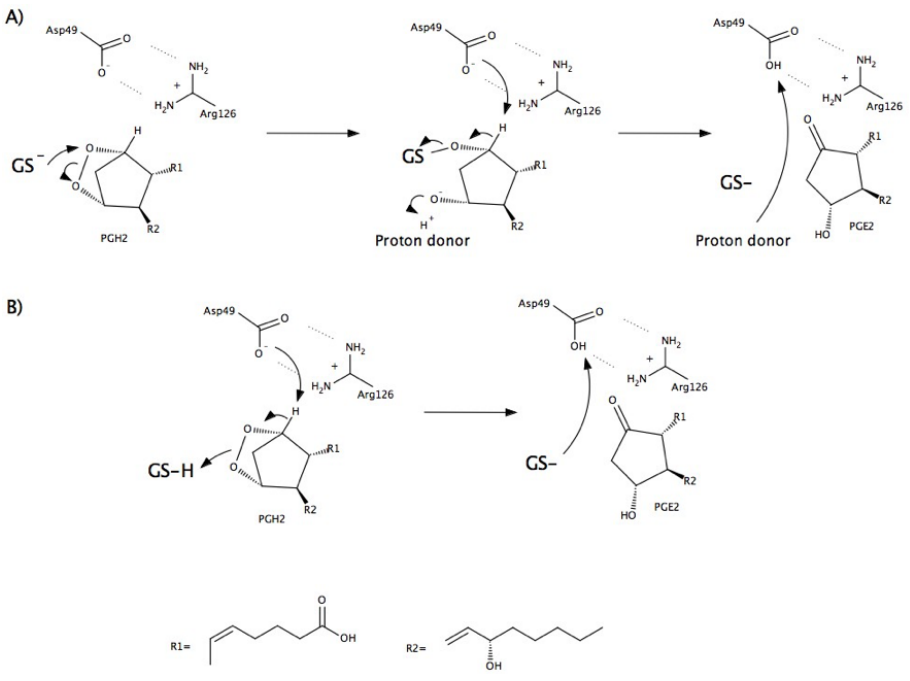


Figure 7 | Suggested mechanism of PGH_2 isomerization to PGE_2 by the active site of mPGES-1.
A) The thiol of glutathione (GSH) gets activated followed by a proton donation from PGH_2 via Asp49. Asp49 abstracts then the proton from where a carbonyl forms as the oxygen sulfur bond is broken. The leaving GSH thiolate is stabilized by Arg126. B) The thiol of GSH gets activated followed by proton donation via the GSH thiol to Asp49 that forms a complex with Arg126. Both suggested reactions result in the formation of the product PGE_2 (149).

To conclude, in **Paper I** we showed that Arg126 and Asp49 are absolutely required for the catalytic activity of mPGES-1 while Ser127 and Arg73 do not seem to be central to the catalytic mechanism of the enzyme. These findings have important implications with regard to the development of novel mPGES-1 inhibitors. Our results are important since clarifying the catalytic mechanisms of mPGES-1 will help to structure-based design and development of mPGES-1 inhibitors.

Role of mPGES-1 inhibition in inflammation and cardiovascular safety

This section will focus on the beneficial role of mPGES-1 inhibition in cardiovascular safety.

mPGES-1 deletion affects platelet functions (Paper II)

In Paper II, we aimed to examine the effect of genetic deletion of mPGES-1 regarding platelet number and activation in normal and inflammatory conditions. mPGES-1 is involved in the pathogenesis of RA promoting inflammatory processes via multiple mechanisms, however, its role in platelet functions has not been studied so far. Recently, activated platelets were recognized as one of the major players in RA (258, 259) linking active synovitis, systemic inflammation and cardiovascular manifestations in RA patients.

Dovizio and coworkers (201) demonstrated that mPGES-1 is not expressed in human platelets. In line with their observation, we have shown that murine platelets do not either express mPGES-1 (186), but they are known to express EP receptors (183, 202). Consequently, genetic deletion of mPGES-1 via attenuating the induced PGE_2 production may potentially regulate functions of platelets. Indeed, we showed a reduced platelet number in whole blood from WT mice treated with LPS compared to that from KO mice. This reduction in platelets number could be due to the LPS-induced activation of platelets followed by consumption of the platelets, most likely by attaching to cells/endothelium or by accumulation in the liver or lungs (186, 260, 261).

Furthermore, the expression of platelet activation markers, P-selectin and CD40L, were stronger in WT mice treated with LPS compared to KO mice (Figure 8). CD40L modulates inflammatory immune responses by binding to its cognate receptor CD40 expressed on neutrophils (262). Reducing platelet activation will subsequently lead to impaired activation of neutrophils and reduced inflammation. Further, the levels of soluble P-selectin and CD40L in plasma of RA patients is found to correlate with disease activity (195). Thus, lower levels of P-selectin and CD40L in KO mice might indicate less inflammation.

Moreover, mPGES-1 deletion significantly reduced the formation of platelet–leukocyte aggregates and platelet aggregation in mice after LPS stimulation (Figure 8). This finding is in line with the results from another study demonstrating that a low concentration of PGE_2 enhances platelet aggregation in mice and that activation of EP_3 is sufficient to mediate the pro-aggregatory actions of low levels of PGE_2 (102). The release of PMP was also significantly lower in response to LPS stimulation in KO mice compared with WT mice. Activated platelets are known to release PMPs, an important player in cell communications (196) and an essential pro-inflammatory factor in the pathogenesis of RA (193-195). The decreased PMP levels would reflect reduced activation of platelets and consequently the lower degree of inflammation.

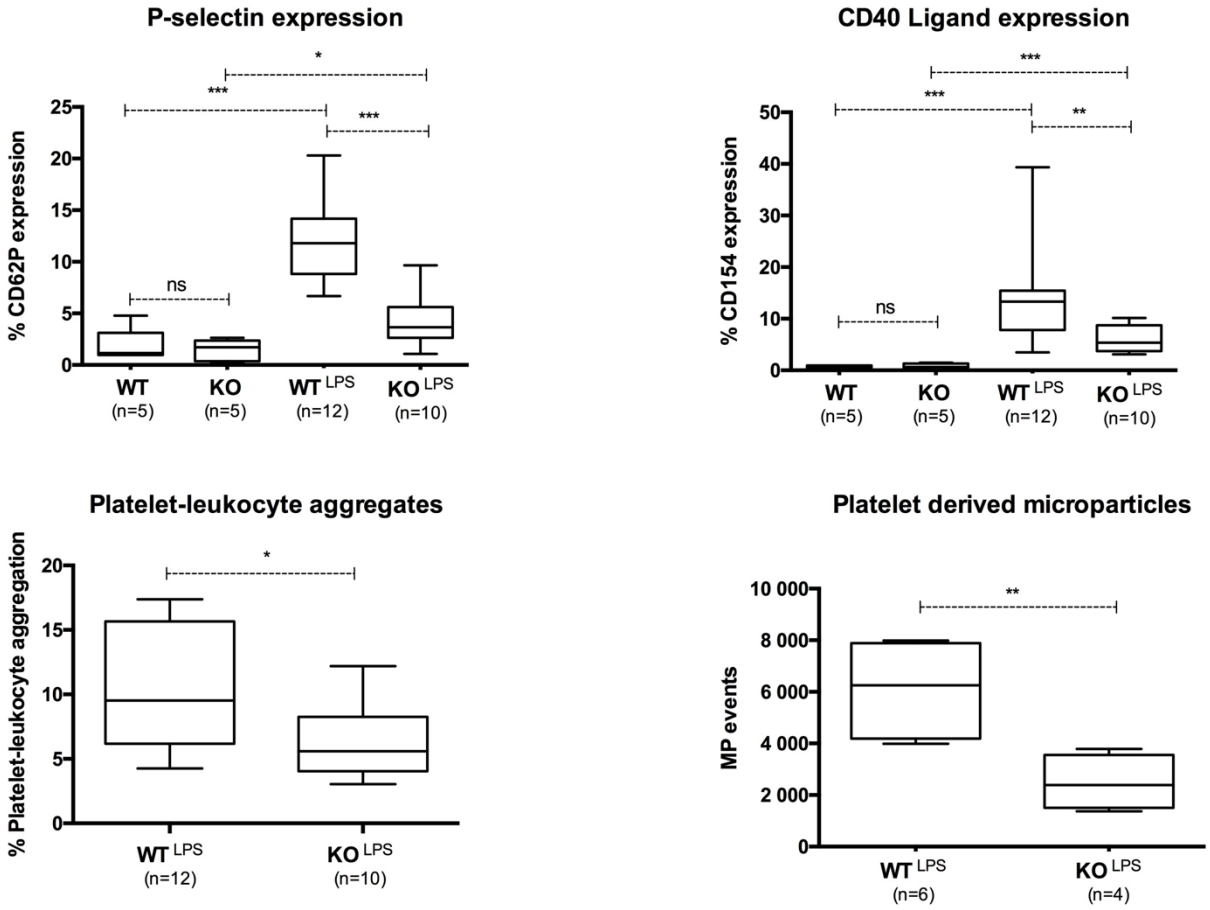


Figure 8 | Platelet activation in WT and mPGES-1 KO mice.
Platelet activation was assessed by (A) CD62P expression, (B) CD154 expression, (C) platelet–leukocyte aggregates and (D) PMP counts. Samples were obtained from WT and mPGES-1 KO mice treated with or without LPS for 24 hours. The horizontal line in the boxes represents the median. *p<0.05, **p<0.01, ***p<0.00, ns, not significant (186).

In addition, platelet accumulation and tendency to aggregate using immunofluorescent CD41 and fibrinogen staining were examined in livers from WT and KO mice stimulated with LPS for 24 hours. No changes were seen in the CD41 expression in WT mice compared to KO. Fibrinogen staining was detected in vessels, moreover, a trend towards less fibrinogen staining in the vessels of KO mice was observed. The percentage of total vessel area was significantly lower in KO mice compared to WT mice, indicating less inflammation.

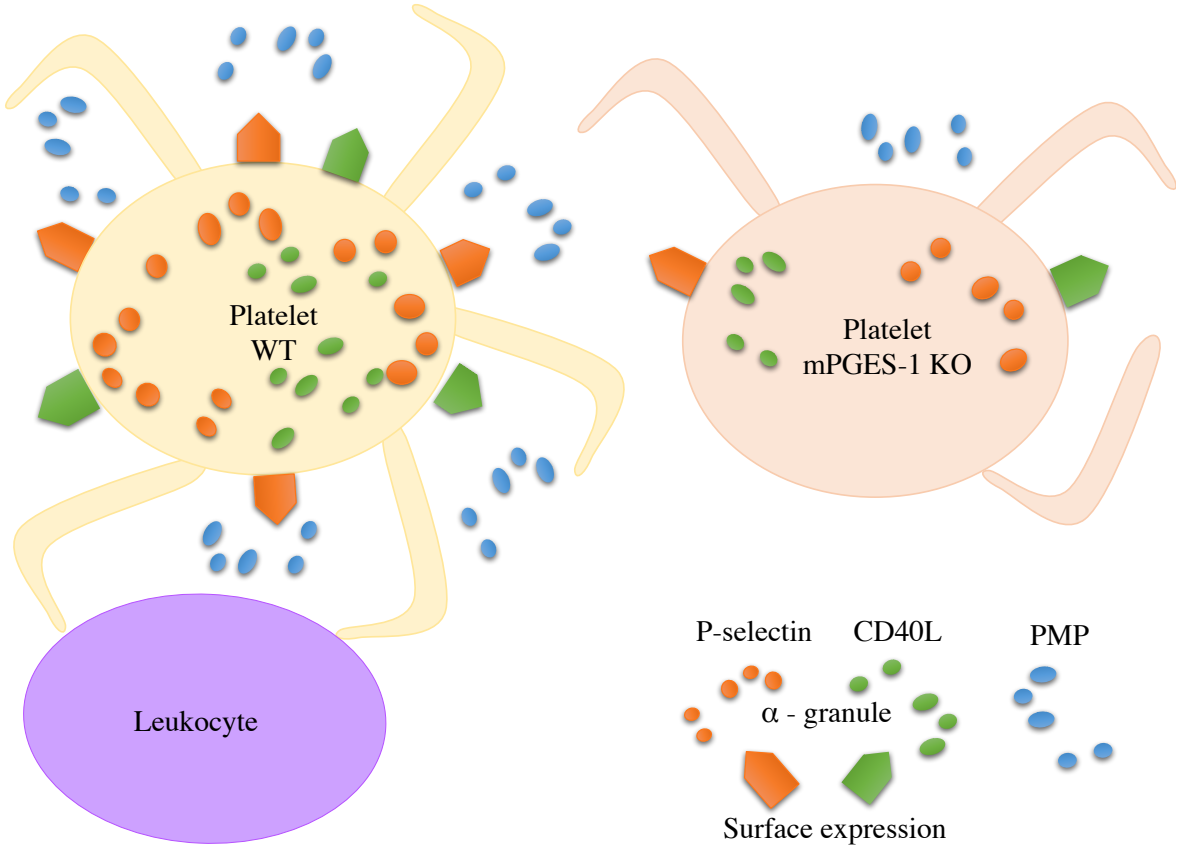


Figure 9 | Schematic overview of platelet activation in WT and mPGES-1 KO mice.
The expression of platelet activation markers CD62P and CD154, the release of platelet-derived microparticles (PMP), as well as the platelet binding to leukocytes were significantly reduced in mPGES-1 KO mice compared to WT in inflammatory condition.

In summary, we demonstrated that systemic lack of mPGES-1 decreased platelet activation, PMP formation, platelet binding to leukocytes and reduced platelet aggregation in settings of inflammation. These results imply that pharmacological inhibition of mPGES-1 might be beneficial regarding both inflammation and the cardiovascular safety which is essential for the development of novel mPGES-1 inhibitors (Figure 9).

Beneficial effects on the cardiovascular and renal system by mPGES-1 deletion compared to COX-2 inhibitors (Paper III)

In order to estimate the relative cardiovascular safety of mPGES-1 as a therapeutic target, in Paper III we aimed to investigate how mPGES-1 and PGIS in comparison with COX-2 influence the vascular as well as the renal pathway associated with ADMA and eNOS. For this purpose, we have utilized mPGES-1 and PGIS deleted mice.

First, we measured prostaglandins generation in circulation, in the plasma, and in isolated blood vessel *ex vivo*. In the freshly isolated aorta from WT mice, ten times higher formation of PGI_2 was detected compared to PGE_2. mPGES-1 deletion led to reduced release of PGE_2 in the freshly isolated aorta, suggesting that mPGES-1 is constitutively expressed in large vessels where it contributes to physiological PGE_2 production. In plasma, the levels of PGE_2 were five times higher than the levels of PGI_2. Interestingly, the levels of plasma PGI_2 were elevated in KO mice, suggesting that at some sites of the body, the shunting of PGH_2 to PGIS occurs.

We also demonstrated that COX-2 is highly expressed in the renal medulla where it acts as a key regulator of the genes controlling the formation of the endogenous eNOS inhibitor ADMA. (211). mPGES-1, on the other hand was mostly expressed in renal cortex.

COX-2 inhibition with parecoxib increased the expression of genes responsible for ADMA synthesis while reducing expression of genes responsible for the degradation. Consequently, the ADMA levels were elevated in plasma of mice treated with parecoxib. In contracts, mPGES-1 deletion did not affect these genes related to ADMA production and degradation and subsequently did not affect the ADMA levels in plasma. These observations suggest that targeting mPGES-1 instead of COX-2 would spare this renal function. These data are also in line with the results of another study suggesting that mPGES-1 is not the major regulator of blood pressure and salt/water handling in the kidney (263).

In order to address whether PGI_2 signaling contributes to the renal pathway, the levels of ADMA and creatinine were measured in PGIS deleted mice. Both parameters were increased to the similar degree as seen in mice treated with parecoxib, signifying that PGI_2 and not PGE_2 is the major prostaglandin regulating renal functions including ADMA metabolism.

Finally, we examined the effect of mPGES-1 deletion on eNOS depended vasodilator response induced by acetylcholine and found that mPGES-1 deletion significantly enhanced the eNOS driven dilator response to acetylcholine in the aorta.

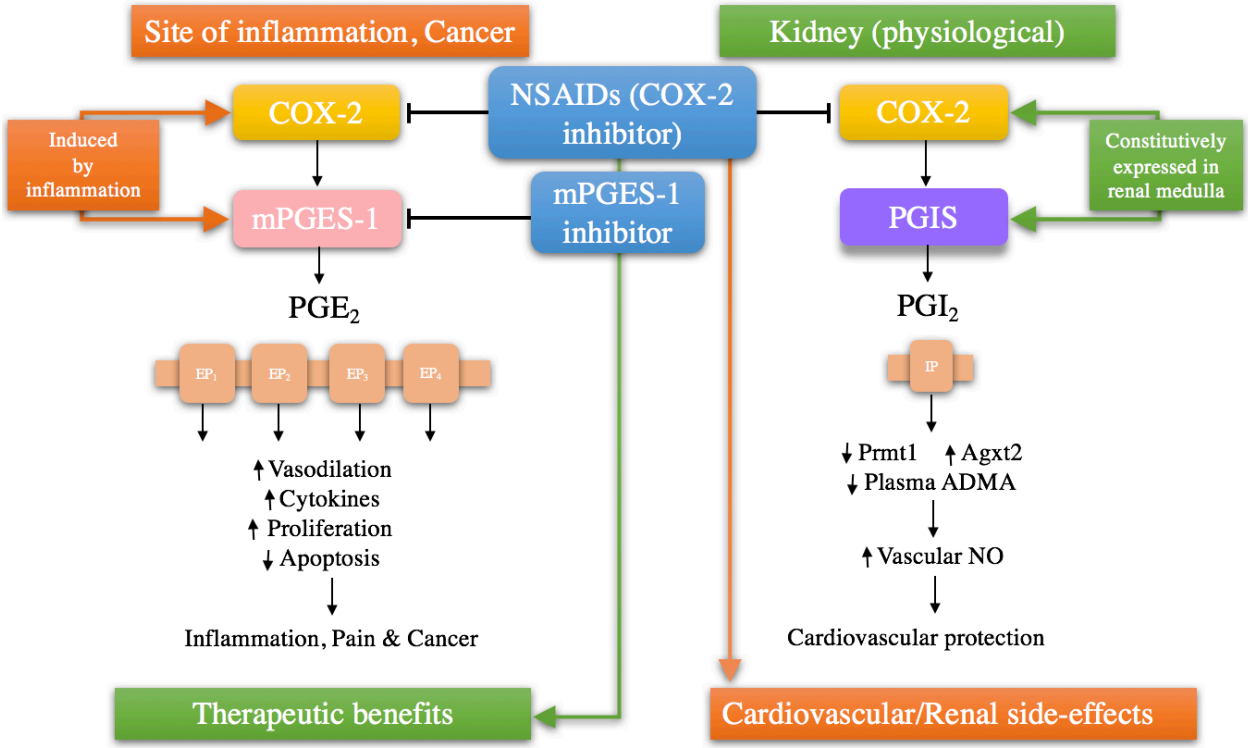


Figure 10 | Schematic overview of findings and hypothesis.
The inhibition of mPGES-1 could potentially be cardioprotective and these properties are hypothesized to be due to the retained anti-inflammatory and anti-cancer benefits of NSAIDs (COX-2 inhibitor) while avoiding the renal and cardiovascular PGI_2/ADMA axis.

In summary, the PGIS but not mPGES-1 retains the cardiovascular protective functions of COX-2 on the renal ADMA pathway. These data provide interesting evidence for the development of selective inhibitors of mPGES-1 as safer alternatives to NSAIDs for treating inflammation, pain, and cancer (Figure 10).

The role of lipid mediators in the pathogenesis of IIM

This section will focus on lipid mediators involved in the pathogenesis of myositis.

Effects of conventional immunosuppressive treatment on molecular mechanisms in muscle from adult patients with PM or DM (Paper IV)

In paper IV, we aimed to elucidate further the molecular events contributing to the unrelenting compromised muscle function despite conventional immunosuppressive treatment in myositis patients by utilizing gene expression profiling. This approach gives us the possibility to identify new molecular mechanisms involved in the pathogenesis of myositis and treatment response in patients. We have studied global gene expression profiles in muscle biopsies from six patients before and after immunosuppressive treatment.

We observed a significant downregulation of genes related to immune response and inflammation such as MHC class I and II, co-stimulatory molecules, chemokines and cytokines, after treatment with GC. Furthermore, we showed the suppression of genes involved in the IFN pathway after treatment (226). Previous studies have shown the overexpression of the IFN-inducible genes in whole blood from myositis patients which correlated with disease activity and was reduced after immunomodulatory therapies (264, 265). Type I IFN signature genes are expressed at high levels at baseline in the skeletal muscle of myositis patients, this is thought to be a predictor of the response to rituximab in PM and DM, where higher expression of the IFN-inducible genes is associated with a greater response (266). Our novel finding demonstrates that the IFN-inducible genes are expressed in the muscle tissue from patients and are downregulated by immunosuppressive treatment, most likely reducing inflammation in the muscle tissue. Thus, our findings have provided further insight into the mechanisms of the beneficial outcomes of conventional immunosuppressive treatment in patients with PM or DM.

Our group has previously demonstrated a persisting expression of mPGES-1, COX-2, and 5-LO in muscle tissue from PM or DM patients despite treatment leading to the production of pro-inflammatory lipids PGE_2 and LTB_4 (20, 21). In line with our previous findings, we could not detect any changes in the expression of these enzymes or alterations at the protein level of EP_3, EP_4, and $CysLTR_1$ receptors in response to treatment. This persisting expression of pro-inflammatory enzymes and lipid mediators might contribute to the chronic inflammation and muscle weakness associated with myositis.

Moreover, genes associated with muscle tissue remodeling were altered after treatment, suggesting protein breakdown and delayed muscle regeneration, consequently affecting muscle growth. The expression of FK506 binding protein 5 (FKBP5) gene, implicated in muscle tissue remodeling, was upregulated after the immunosuppressive treatment. The expression of FKBP5 was further validated by western blot where an increase of the expression of the protein was detectable after treatment, suggesting a negative effect on muscle tissue remodeling and growth. Moreover, we detected increased expression levels of myostatin, suggesting inhibition of myogenesis and a negative effect on muscle growth.

Also, the expression of Ras-related associated with diabetes (RRAD) gene and myosin binding protein H (MYBPH) gene were both downregulated after treatment; this could be a sign of reduced muscle regeneration.

In addition, myosin, heavy polypeptide 4 (MYH4) and alpha-actinin-3 (ACTN3) genes, related to fast type II muscle fibers, were both upregulated after treatment. Furthermore, this finding was verified by the analysis of the fiber-type composition where a switch towards type II muscle fibers in response to treatment was observed (Figure 11). Our results are in agreement with previous studies where they have reported a shift towards the fast-twitch type II muscle fibers in myositis patients, which might be restored by exercise (267, 268).

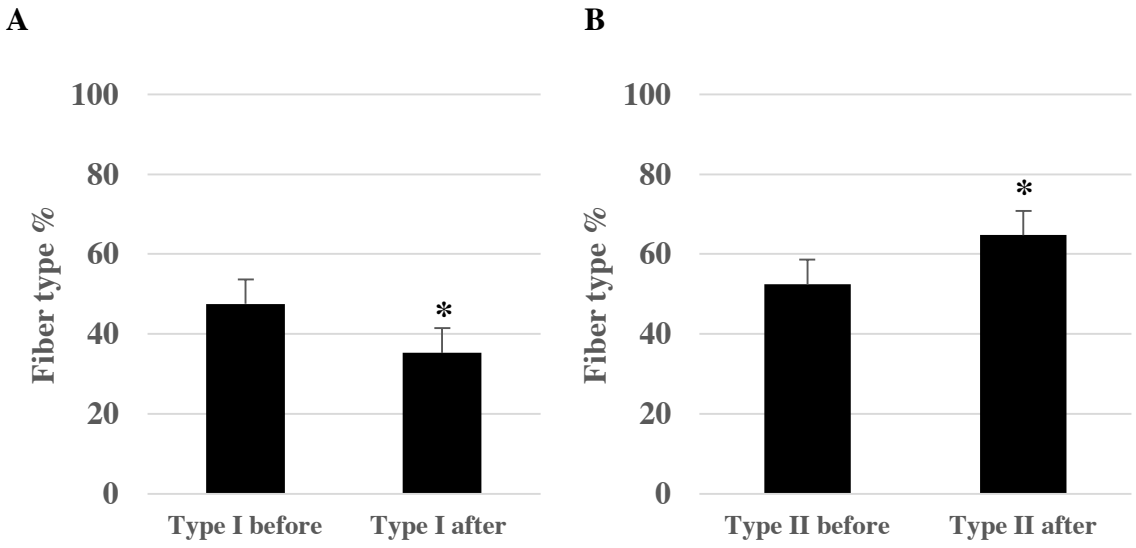


Figure 11 | The fiber type composition before and after treatment in PM and DM patients.
(A) The percentage of type I muscle fibers was significantly decreased after glucocorticoid treatment from median 52 % to median 43 % (*p <0.05). (B) The proportion of type II fibers was significantly increased after treatment from median 48 % to median 57 % (*p <0.05) (226).

The most striking finding was that the expression of a number of genes involved in lipid and FA metabolism, lipid uptake, and lipid accumulation was changed after immunosuppressive treatment, signifying a probable lipotoxic effect on muscles. We detected the upregulated expression of ceramide synthase 3 (CERS3) gene, suggesting an increased accumulation of ceramide which has previously been linked to insulin resistance (269). Additionally, ceramides have been associated with skeletal dysfunction and fatigue in chronic inflammatory conditions (270). The altered expression of the genes related to lipid metabolism suggests deregulated intramuscular lipid storage that might lead to skeletal muscle dysfunction.

In conclusion, we have revealed that the anti-inflammatory effect of conventional immunosuppressive treatment was combined with the negative impact on genes involved in muscle tissue remodeling and lipid metabolism, at least partly underlying the persistent muscle weakness and fatigue often observed in patients despite treatment.

Serum lipids and FA profiles are altered in patients with PM or DM (Paper V)

Changes in the expression of genes related to lipid and FA metabolism that we detected in the patients with PM or DM after treatment (Paper IV) might lead to altered lipid and FA profiles. Multiple studies demonstrated that skeletal muscle FA profiles could affect muscle growth, functions and inflammation (54, 84, 85, 87). To clarify lipid/FA involvement in the pathogenesis of IIM, we aimed to evaluate the lipid and FA-profiles in serum from patients with PM or DM in comparison to healthy individuals and in response to immunosuppressive treatment utilizing gas chromatography (GC-MS) and LC-MS/MS.

We detected, that the FA composition of total serum lipids was altered in PM and DM patients compared to healthy individuals. The levels of palmitic (16:0) acid in serum were significantly higher in myositis patients compared to healthy individuals. Several studies have suggested that palmitate has pro-inflammatory properties inducing inflammation and atrophy in myotube *in vitro* (271). Moreover, the levels of AA were significantly lower in myositis patients in comparison to healthy individuals. The low AA levels are most likely due to the conversion of AA to pro-inflammatory eicosanoids by COX and 5-LO; both enzymes are upregulated in PM and DM patients (20, 21). Furthermore, the FA composition of serum lipid classes confirmed that the levels of the PC species comprising PUFA were lower while the levels of the PC and triglycerides (TG) species containing SFA and monounsaturated FA (MUFA) were higher in myositis patients compared to healthy individuals. Our results are in line with the previous reports that RA patients have lower levels of PUFAs and higher levels of SFA in the serum PC, erythrocytes, and adipose tissue compared with healthy controls (53, 221).

The role of GC in the dysregulation of lipid metabolism in skeletal muscle has recently been described (226, 272). In myotubes from mice *in vitro* and skeletal muscle *ex vivo*, GC usage impacted the expression of genes involved in lipid storage, mobilization, and utilization (272). Moreover, our study revealed that in myositis patients, GC treatment affected the expression of genes involved in lipid and FA metabolism in skeletal muscle (226) which possibly could lead to changed lipid and FA profiles in those patients. In line with these findings, we could detect increased levels of EDA and EPA in myositis patients after immunosuppressive treatment. EDA have been shown to exert anti-inflammatory effects, affecting PGE_2 and nitric oxide (NO) pathways (273). EPA, on the other hand, gives rise to metabolites that are less inflammatory than those produced from AA as well as reduces production of cytokines (82). This data indicates that increased serum EPA and EDA levels after treatment might have a beneficial anti-inflammatory effect.

In addition, changes in serum PC, PE and LPC profiles in myositis patients after treatment was observed. Remarkably, the same lipid species such as (32:1), (34:3), (36:6) and (36:5) were upregulated within both PC and PE, whereas (38:4) and (40:6) were lower after the immunosuppressive treatment. Furthermore, we observed increased levels of LPC(16:1) and LPC(20:5) after treatment. Nevertheless, we did not detect a clearly defined pattern in

the changes of PUFA or SFA-enriched lipid species in response to the immunosuppressive treatment.

Also, a significant positive correlation (rs=0.821) was observed between a muscle performance index and the levels of PC(38:4) in serum from patients after immunosuppressive treatment, that might reflect lipid and FA metabolism deregulation in PM and DM patients contributing to muscle fatigue and impairment.

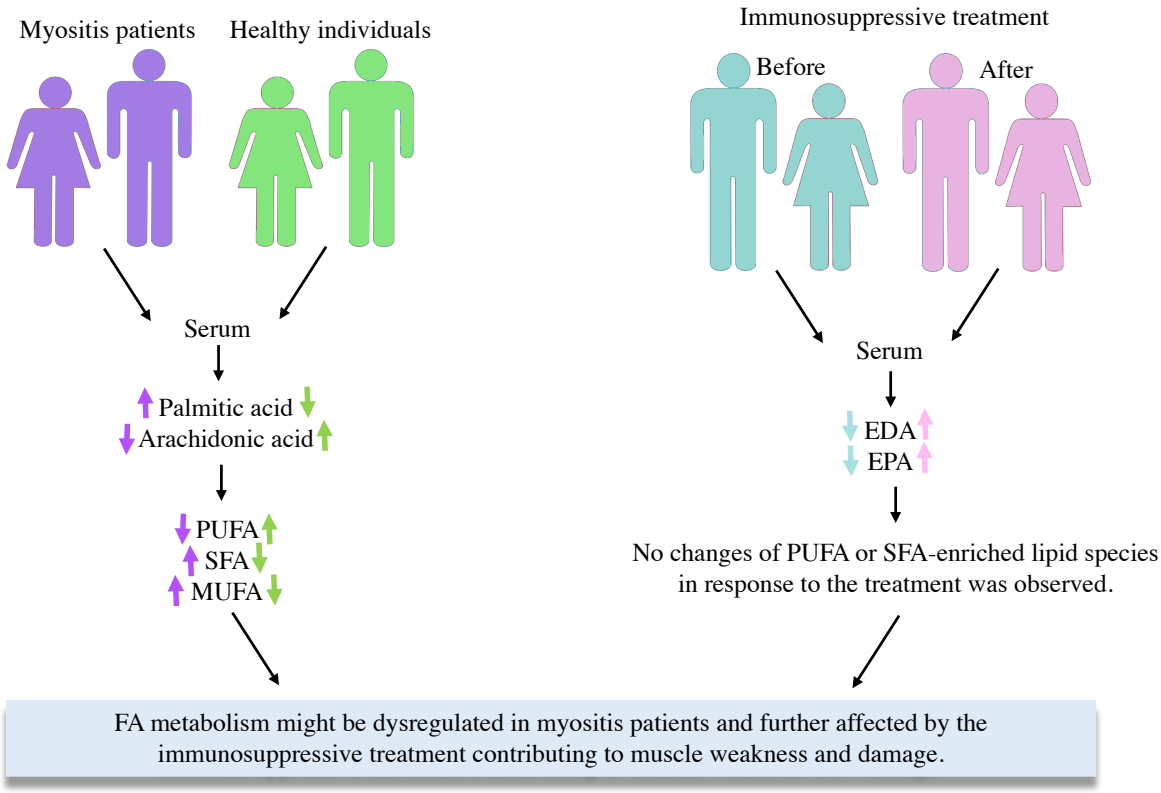


Figure 12 | Schematic overview of findings.
FA composition of total serum lipids and lipid classes are altered in myositis patients compared to healthy individuals and in relation to immunosuppressive treatment. However, no clearly defined pattern in the changes of PUFA or SFA-enriched lipid species in response to the immunosuppressive treatment was detected.

In conclusion, we have found that FA composition of total serum lipids and lipid classes are significantly altered in PM and DM patients compared to healthy individuals and in relation to immunosuppressive treatment (Figure 12). These results imply that FA metabolism might be dysregulated in PM and DM patients and further affected by the treatment contributing to muscle weakness and damage.

CONCLUSIONS AND FUTURE PERSPECTIVES

This thesis has contributed to a better understanding of the involvement of lipid mediators in chronic autoimmune diseases such as RA and IIM. We have provided new knowledge on the catalytic mechanism and the impact of mPGES-1 on inflammation and cardiovascular safety. Furthermore, we have demonstrated that lipid metabolism is altered in myositis patients and might contribute to disease pathogenesis.

There have been many attempts to clarify the catalytic mechanism of the mPGES-1 enzyme, however, with somewhat controversial results. To understand the mechanism and identify the critical residues for the mPGES-1 function is of great value when it comes to the development of a new generation of selective NSAIDs. In **Paper I**, we have shown that the residues Arg126 and Asp49 are essential for the catalytic activity of mPGES-1. We also demonstrated that residues Ser127 and Arg73 are not central to the catalytic mechanism. Further investigations facilitating site-directed mutagenesis and steady state kinetics are important to clarify the most important residues for the catalytic function of mPGES-1. This will lead to better understanding of the exact reaction mechanism of mPGES-1 and will be beneficial for the development of new mPGES-1 inhibitors, for instance by synthesizing transition state inhibitors. Future experiments including co-crystallization of the protein with the inhibitor would provide important information regards to the location of the inhibitor binding site as well as the orientation of the inhibitor within the enzyme.

Although deletion or pharmacological suppression of mPGES-1 leads to suppression of inflammation via multiple mechanisms, more studies are required to elucidate the impact of mPGES-1 inhibition on different pathological and physiological processes. In **Paper II**, we have investigated whether mPGES-1 deletion might be beneficial for reducing inflammation via the suppression of platelet functions. We have demonstrated that systemic deletion of mPGES-1 prevented platelet activation, PMP release, the formation of platelet-leukocyte aggregates and reduced platelet aggregation in inflammatory conditions. These effects can also contribute to the cardiovascular safety of mPGES-1 inhibitors. In line, the results presented in **Paper III** further confirmed the cardioprotective effects of mPGES-1 deletion. We have demonstrated that the cardiovascular protective functions of COX-2 on the plasma ADMA and vascular eNOS are not mediated by mPGES-1. These data provide better confidence in the development of selective inhibitors of mPGES-1 as a safer alternative to NSAIDs for anti-inflammatory treatment of chronic autoimmune diseases.
Further investigations will clarify the cardiovascular safety properties of mPGES-1 inhibitors. Several mPGES-1 inhibitors have become available for research use; this provides with an exceptional opportunity to characterize the beneficial and potential side effects of the inhibitors both *in vitro* studies as well as *in vivo* animal studies utilizing different disease models. The characterization of mPGES-1 inhibitors could provide information regarding the importance of mPGES-1 derived PGE_2 in acute and chronic inflammation as well as in cancer. The challenge by inhibiting mPGES-1 activity is to keep the balance between damaging and protective PGE_2 effects. Moreover, it would be of great therapeutic value to elucidate whether mPGES-1 inhibitors in combination with anti-

rheumatic drugs such as GC and DMARDs would provide the benefits in controlling synovial inflammation.

In **Paper IV**, we have studied effects of GC treatment on gene expression profiles in muscle tissue from myositis patients. We have found that the beneficial anti-inflammatory effect of treatment was combined with the negative impact on genes involved in muscle tissue remodeling and lipid metabolism. These results suggest that deregulation of muscle lipid and FA metabolism may contribute to sustained muscle impairment seen in myositis patients despite immunosuppressive therapy. Future studies will elucidate the molecular mechanisms involved in disease pathogenesis. Deeper analysis by validating the global gene expression data will help to determine important genes affected by the GC treatment that could potentially be used as biomarkers for treatment response in myositis patients.

To validate the observed changes in lipid metabolism in myositis patients, in **Paper V**, we have analyzed the serum FA and lipid profiles. We have found that FA composition of total serum lipids and lipid classes are significantly altered in myositis patients compared to healthy individuals and in response to immunosuppressive treatment. These results imply that indeed, FA/lipid metabolism is dysregulated in myositis patients and further affected by the treatment possibly contributing to muscle weakness and damage.
Future investigations will clarify if the changes in serum FA composition indeed reflect the FA composition in the muscle tissue and if they may be used as predictive markers for patients with myositis. It would also be interesting to investigate whether FA and lipid profiles might be improved in myositis patients in response to exercise or by dietary supplements.

ACKNOWLEDGEMENTS

Postgraduate education is a journey of personal growth and development. The only way to truly appreciate and love science is the ups and downs of it. There have been times when the struggle of science has been too heavy to bear when I almost decided to quit and to give up. My mom often spoke of a silver lining after hard times and she was right! Suddenly after many failures when I was about to give up I caught the wind in my sails carrying me across the scientific vast ocean, cheerful and successful. During my travelers, I had the great fortune of having great leadership and guidance, great fellowship and lab partners in crime. But the essence of my accomplishments grow deep as the roots of a very old three, it comes from my devoted family and my cherished husband, whom always have been there for me, in sickness and in health, always loving me unconditionally. I have also been blessed with amazing friends that have made my life richer and more joyful.

There are many people that I would like to express my gratitude to. I would like to thank my supervisor and co-supervisors for allowing me to complete my studies in such open learning environment. I am very grateful for all the opportunities that they have bestowed open me. I wish to thank you all for sharing your knowledge with me and for great guidance.

Thank you, **Marina Korotkova**, my main supervisor, for having faith in me, for always been there and encouraging me, for always making me feel important and for making be a better scientist. In my opinion great leadership is to give freedom and space to grow, to allow mistakes and encourage success, that you have done and more.

My co-supervisor, **Per-Johan Jakobsson**, thank you for giving me the opportunity to do my Ph.D. in your research group. I am thankful for all the opportunities to travel abroad. My co-supervisor, **Ingrid E Lundberg**, thank you for always answering an email no matter what. For always being kind, sharing knowledge and making me feel welcome. Your kindness has been a source of motivation for me.

Lars Klareskog, thank you for giving me the opportunity to join the Rheumatology Unit and for being the inspiring person that you are.

I would like to thank my dear research group, **Cátia C**, **Helena I**, **Elena O**, **Karin L**, **Johan L**, **Fari M**, **Julia S**, **Filip B**, **Louise B**, **Yvonne S**, **Peter R** and **Mingmei S.** It has been wonderful working with you all. **Cátia C**, thank you for always being so considerate and gentle. You have many good personal qualities, one of my favorites is your need to always be helpful. Thank you for the companionship during my years as a Ph.D. student, I appreciate all the help. **Helena I**, thank you for always running my samples on the HPLC. For always been so kind and helpful. Foremost, thank you for getting emotional and happy when I told you about Mohammed and me. **Elena O**, thank you for all your support and patience with my A549 proteomics and mPGES-1 isoform-1 project. More importantly, thank you for all amazing nail polishes, my nails look fabulous thanks to you. **Karin L**, thank you for always giving me advice about antibodies and for sharing your knowledge about protein expression and Game of Thrones of course. **Johan L**, you have a kind heart, thank you for been so generous. **Fari M**, my mouse platelets partner in crime, thank you for

all the laughs and the sarcasm in the lab. Thank you for standing up for me and for being so ironic. How you can make everything fun is a mystery to me, never stop making people laugh. **Julia S**, thank you for being such a quick learner and for having a kind soul. **Filip B**, I would like to thank you for being so fabulous. Never stop being the great person that you are. **Louise B** and **Yvonne S**, thank you for making the lab a great working place and thank you for being kind-hearted.

I would like to thank **Patrick L** and **Ganna O**, past members of the Jakobssons group. **Patrick L**, thank you for introducing me to research and for your guidance and patience. I very much enjoyed sharing home-made dolma and biryani with you. **Ganna O**, thank you for being the kind and generous person that you are, and for all the amazingly cool nail polishes, some changing colors with temperature others with UV light, I still use them frequently.

My dear members of the myositis group, **Cátia C**, **Eva L**, **Quan T**, **Maryam D**, **Antonella N**, **John S**, **Mei Z**, **Helene A**, **Malin R**, **Louise E**, **Anna T**, **Angeles G**, **Cecilia W**, **Li A.M**, **Paulius V**, **Karina G**, **Valérie L**, **Christina O** and **Lara D**. It has been fantastic working with you all, you have been very welcoming and helpful. Special thanks, **Eva L**, for all your help and for being one of the gentlest person I know.

I would like to thank my nom nom curry squad, without you guy's life at floor 4 would be very dull. **Brinda D**, thank you for being the fantastic person that you are, professionally and personally. You have a gentle heart, thank you for being caretaking and very much involved. In you, I found true friendship. You deserve the very best in life. **Priya R**, thank you for your hospitality and generosity and for being so kind-hearted and really considerate. My mPGES-1 KO mice partner in crime, you have become more than a colleague, I consider you as a true and loyal friend. I will never forget the shock in your face the first time we talked as friends, it was priceless. My girls I would like to thank you both for all the laughs, dinners, parties, trips and all the long (very long) talks about work and life. **Vijay B**, thank you for all the laughs after me shocking you (which happens quite frequently) and all the fun at conferences and during lunch. **Akilan K**, thank you for helping me at the lab and all the fun at conferences and lunch.

Heidi W, thank you for your great spirit and your need for justice, it is highly contagious! The lab is not the same without your laughs. **Gunnel B**, thank you for your sympathy and competence doing the administrative work. **Stina N**, thank you for being a person of greatness. Thank you for being kind and fair. You always made time for me after the administrative tasks I had, listening and giving advice. In my opinion, you are extraordinary.

I would like to thank my office colleagues, **Karin C**, **Angeles G**, **Lina DG**, **Cátia C**, **Daniel R**, **Klementy S**, thank you for answering all my questions and thank you for all the laughs.

I would like to thank my collaborators: **Ralf Morgenstern** and **Linda Spahiu**, from Karolinska Institutet (IMM) for great teamwork. Thank you, **Jane Mitchell** and **Nicholas**

S. Kirkby, from the Imperial College London, for excellent collaboration. Thank you, **Kanneboyina Nagaraju**, from the Children's National Medical Center, USA, for your patience and for sharing your competence. Thank you, **Dieter Steinhilber, Beatrix Süß** and **Meike Saul** from the Goethe University, respectively Universität, Darmstadt, Germany for the nice collaboration.

I would like to thank, **Helena EH and group**, for the fun events we had and for being humble when crushing our group in game activities. I would like to thank everybody at floor 4 at CMM for always being kind and helpful and of course for the Christmas parties and the nice fika every week! Thank you all for those precious moments. A special thanks to **Maria S**, **Roham P, Jorge R**, **Agnes S**, **Hanna A**, **Sabrina R**, **Christina G**, **Rita I**, **Nastya K**, **Raya S**, **Susanne N**, **Eliane P**, **Venus A** and **Meng S** for all the help and laughs.

My beloved and cherished mamsen, **Delaram R**, the reason why I stand here today and the reason I am the person I am today, is all thanks to you. You gave up on your life to give me and my sister opportunities you never got. For that, I will forever be grateful and in debt to you. You have very humbly taught me the importance of love, kindness, and knowledge. Raising two daughters by yourself in a country far away from home with foreign language and lifestyle is difficult, easy to say you succeed and more. Thank you for being my mom, my dad, my uncle and my aunt, thank you for being one person but many at the same time. I love you most in the world and will always be by your side, regardless of what. To my absent and beloved father, **Azad K**, whom I never met. I know you are proud of me and what I have accomplished, wherever you are. See you soon again dearest.

My beloved sister, Shosha, **Shirin R**, life without you would lose its meaning. You have always stood by my side, even if I did mischief (although you caused most of it). I feel privileged being your sister, your creativity, style and your humor can't be found anywhere else. Thank you for all the laughs, the fights and the love. Thank you for totally destroying my hair cut more than once, I still love you. I am really proud of all of your accomplishments, getting admitted to Konstfack has been your lifelong dream and you did it all by yourself. Foremost, your biggest accomplishment is giving us the most precious gift of all, **Ardalia**, being her aunt has been the most important thing in my life. I love her so much and I'm so proud of her, no words can describe my feelings. I promise that I will always be by her side, guiding her, indulging her and loving her. She is my first love. Jag älskar dig Arda, bebej pla.

Dada Hadar K, they say blood is thicker than water, if so, then you're more blood to me than anyone else. What should I have done without you, dear aunt? I was very young when I meet you, although big age difference I found my soulmate in you. You are my best friend, always being there, giving me advice, making me laugh, supporting me and being a shoulder to cry on. My partner in crime. Your presence in my life have made me a better person, you inspire me to be kind, thoughtful and sharing. Thank you for showing me what's important in life. Thank you for being my voice of reason. No recognition is enough for the uncountable knowledge and advice you have been given me since I was a little girl.

I couldn't image my life without you and your beautiful kids. **Peri K**, precious little sister, I feel proud calling myself your older sister. I will always be there, regardless of what. I adore and love you. My dear boys, **Haval K**, **Aram K** and **Dara K**, what would life be without you troublemakers? I love you all.

My dear friends, new and old, **Marta B**, thank you for always putting a smile on my face and for being so clumsy. Thank you for being you. **Yalda M**, thank you for always being on my side, always listening and caring. And of course, thank you for being one of the most sarcastic/real persons I know! Thank you for everything, most of all thank you for being my sister. **Sonia I** aka Sonii Ponii, **Soran Z** aka Zoran den maskerade kurden, **Aseel A** aka **Asoole**, **Aleksandra** aka **Alex** and **Perihan** aka **Peri**, thank you all for making my life more fun, thank you for all the laughs and support, thank you for being my friends.

Just when I thought that life couldn't get any better, a new (not really new, but old) person enters my life, making it so bright with joy that I almost get blinded, making it so rich with love that my hearts feels like exploding and filling it with so much laughter that I literally get six packs. Imagine to fall in love with your absolutely best friend and then he turns out to be your soulmate, your better half. All this time standing in front of me and I was blind not to see. How foolish I have been, but never again. **Mohammed A**, my one and only true love, my guiding star, my best friend, the sun of my life, my cherished husband and my everything, thank you for making every day count, thank you for making me a better person. But foremost, thank you for loving me unconditionally and for making me feel so precious. You once told be a tale of two souls, bonded together by the universe since its inception, except their bound came undone. The souls belong to each other, they are one. When reunited at last neither time nor space could keep them apart. For they are finally where they belong, moving through eternity together as one. I realize now that our souls are one and that my soul has always been in love with yours and I will love you forever. To Andromeda and Infinity, my love. *Ever thine. Ever mine. Ever ours.*

Last but not least, I would like to thank my new wonderful family **Al Abassi´s**, my dear **Amo** and **Khale**, thank you for welcoming me to your beautiful family. This sense of belonging means the world to me. To my dear sister in laws, **Zeineb A**, **Isra A** and **Baraa A**, thank you for making me feel loved and for inviting me into your lovely family with open arms. Thank you for loving Mohammed and for sharing some of that love with me. I love you, girls!

REFERENCES

1. Jacobson, DL, Gange SJ, Rose NR, Graham NM. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. Clinical immunology and immunopathology. 1997;84(3):223-43.
2. Zeissig, Y, Petersen BS, Franke A, Blumberg RS, Zeissig S. Rare phenotypes in the understanding of autoimmunity. Immunology and cell biology. 2016;94(10):943-8.
3. Jakobsson, PJ, Thoren S, Morgenstern R, Samuelsson B. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc Natl Acad Sci U S A. 1999;96(13):7220-5.
4. Korotkova, M, Jakobsson PJ. Microsomal prostaglandin E synthase-1 in rheumatic diseases. Front Pharmacol. 2010;1(146):1-8.
5. Nathan, C. Points of control in inflammation. Nature. 2002;420(6917):846-52.
6. Ricciotti, E, FitzGerald GA. Prostaglandins and inflammation. Arteriosclerosis, thrombosis, and vascular biology. 2011;31(5):986-1000.
7. Scott, DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet (London, England). 2010;376(9746):1094-108.
8. Feldmann, M, Brennan FM, Maini RN. Rheumatoid arthritis. Cell. 1996;85(3):307-10.
9. Klareskog, L, Stolt P, Lundberg K, Kallberg H, Bengtsson C, Grunewald J, Ronnelid J, Harris HE, Ulfgren AK, Rantapaa-Dahlqvist S, Eklund A, Padyukov L, Alfredsson L. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis and rheumatism. 2006;54(1):38-46.
10. Svendsen, AJ, Junker P, Houen G, Kyvik KO, Nielsen C, Skytthe A, Holst R. Incidence of chronic persistent rheumatoid arthritis and the impact of smoking. Arthritis care & research. 2016.
11. Hensvold, AH, Magnusson PK, Joshua V, Hansson M, Israelsson L, Ferreira R, Jakobsson P-J, Holmdahl R, Hammarström L, Malmström V. Environmental and genetic factors in the development of anticitrullinated protein antibodies (ACPAs) and ACPA-positive rheumatoid arthritis: an epidemiological investigation in twins. Annals of the rheumatic diseases. 2013:annrheumdis-2013-203947.
12. Svendsen, AJ, Kyvik KO, Houen G, Junker P, Christensen K, Christiansen L, Nielsen C, Skytthe A, Hjelmborg JV. On the origin of rheumatoid arthritis: the impact of environment and genes—a population based twin study. PLoS One. 2013;8(2):e57304.
13. Walsh, DA, McWilliams DF. Mechanisms, impact and management of pain in rheumatoid arthritis. Nature reviews Rheumatology. 2014;10(10):581-92.
14. Report of the American College of Rheumatology Pain Management Task Force. Arthritis Care Res (Hoboken). 2010;62(5):590-9.
15. McWilliams, DF, Zhang W, Mansell JS, Kiely PD, Young A, Walsh DA. Predictors of change in bodily pain in early rheumatoid arthritis: an inception cohort study. Arthritis Care Res (Hoboken). 2012;64(10):1505-13.
16. Mastaglia, FL, Garlepp MJ, Phillips BA, Zilko PJ. Inflammatory myopathies: clinical, diagnostic and therapeutic aspects. Muscle & nerve. 2003;27(4):407-25.
17. Tieu, J, Lundberg IE, Limaye V. Idiopathic inflammatory myositis. Best practice & research Clinical rheumatology. 2016;30(1):149-68.

18. Lundberg, I, Ulfgren AK, Nyberg P, Andersson U, Klareskog L. Cytokine production in muscle tissue of patients with idiopathic inflammatory myopathies. Arthritis & Rheumatism. 1997;40(5):865-74.
19. Nyberg, P, Wikman A, Nennesmo I, Lundberg I. Increased expression of interleukin 1alpha and MHC class I in muscle tissue of patients with chronic, inactive polymyositis and dermatomyositis. The Journal of rheumatology. 2000;27(4):940-8.
20. Korotkova, M, Helmers SB, Loell I, Alexanderson H, Grundtman C, Dorph C, Lundberg IE, Jakobsson P-J. Effects of immunosuppressive treatment on microsomal prostaglandin E synthase 1 and cyclooxygenases expression in muscle tissue of patients with polymyositis or dermatomyositis. Annals of the rheumatic diseases. 2008;67(11):1596-602.
21. Loell, I, Alemo Munters L, Pandya J, Zong M, Alexanderson H, Fasth AE, Stahl Hallengren C, Radmark O, Lundberg IE, Jakobsson PJ, Korotkova M. Activated LTB4 pathway in muscle tissue of patients with polymyositis or dermatomyositis. Annals of the rheumatic diseases. 2012.
22. Zong, M, Lundberg IE. Pathogenesis, classification and treatment of inflammatory myopathies. Nature Reviews Rheumatology. 2011;7(5):297-306.
23. Brune, K, Patrignani P. New insights into the use of currently available non-steroidal anti-inflammatory drugs. J Pain Res. 2015;8:105-18.
24. Takeuchi, K. Pathogenesis of NSAID-induced gastric damage: importance of cyclooxygenase inhibition and gastric hypermotility. World journal of gastroenterology. 2012;18(18):2147-60.
25. Bertagnolli, MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K, Tang J, Rosenstein RB, Wittes J, Corle D, Hess TM, Woloj GM, Boisserie F, Anderson WF, Viner JL, Bagheri D, Burn J, Chung DC, Dewar T, Foley TR, Hoffman N, Macrae F, Pruitt RE, Saltzman JR, Salzberg B, Sylwestrowicz T, Gordon GB, Hawk ET. Celecoxib for the prevention of sporadic colorectal adenomas. The New England journal of medicine. 2006;355(9):873-84.
26. Bresalier, RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, Lines C, Riddell R, Morton D, Lanas A, Konstam MA, Baron JA. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. The New England journal of medicine. 2005;352(11):1092-102.
27. Rahman, S, Malcoun A. Nonsteroidal antiinflammatory drugs, cyclooxygenase-2, and the kidneys. Primary Care: Clinics in Office Practice. 2014;41(4):803-21.
28. Samuelsson, B, Morgenstern R, Jakobsson P-J. Membrane prostaglandin E synthase-1: a novel therapeutic target. Pharmacological reviews. 2007;59(3):207-24.
29. Singh, JA, Saag KG, Bridges SL, Akl EA, Bannuru RR, Sullivan MC, Vaysbrot E, McNaughton C, Osani M, Shmerling RH. 2015 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. Arthritis & Rheumatology. 2016;68(1):1-26.
30. Schett, G, Saag KG, Bijlsma JW. From bone biology to clinical outcome: state of the art and future perspectives. Ann Rheum Dis. 2010;69(8):1415-9.
31. Zhou, H, Cooper MS, Seibel MJ. Endogenous glucocorticoids and bone. Bone research. 2013;1(2):107.
32. Ratman, D, Vanden Berghe W, Dejager L, Libert C, Tavernier J, Beck IM, De Bosscher K. How glucocorticoid receptors modulate the activity of other transcription factors: a scope beyond tethering. Molecular and cellular endocrinology. 2013;380(1-2):41-54.
33. Schacke, H, Schottelius A, Docke WD, Strehlke P, Jaroch S, Schmees N, Rehwinkel H, Hennekes H, Asadullah K. Dissociation of transactivation from

transrepression by a selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(1):227-32.
34. Barnes, P. Glucocorticoids. History of Allergy. 100: Karger Publishers; 2014. p. 311-6.
35. Alangari, AA. Genomic and non-genomic actions of glucocorticoids in asthma. Annals of thoracic medicine. 2010;5(3):133-9.
36. Jiang, CL, Liu L, Tasker JG. Why do we need nongenomic glucocorticoid mechanisms? Frontiers in neuroendocrinology. 2014;35(1):72-5.
37. Stahn, C, Buttgereit F. Genomic and nongenomic effects of glucocorticoids. Nature clinical practice Rheumatology. 2008;4(10):525-33.
38. Schacke, H, Docke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. Pharmacology & therapeutics. 2002;96(1):23-43.
39. Konagaya, M, Bernard PA, Max SR. Blockade of glucocorticoid receptor binding and inhibition of dexamethasone-induced muscle atrophy in the rat by RU38486, a potent glucocorticoid antagonist. Endocrinology. 1986;119(1):375-80.
40. Singleton, JR, Baker BL, Thorburn A. Dexamethasone inhibits insulin-like growth factor signaling and potentiates myoblast apoptosis. Endocrinology. 2000;141(8):2945-50.
41. Rexhepi, S, Rexhepi M, Sahatçiu-Meka V, Mahmutaj V, Boshnjaku S. The Impact of Low-Dose Disease-modifying Anti-rheumatics Drugs (DMARDs) on Bone Mineral Density of Premenopausal Women in Early Rheumatoid Arthritis. Medical Archives. 2016;70(2):101.
42. Romão, VC, Lima A, Bernardes M, Canhão H, Fonseca JE. Three decades of low-dose methotrexate in rheumatoid arthritis: Can we predict toxicity? Immunologic research. 2014;60(2-3):289-310.
43. Brown, PM, Pratt AG, Isaacs JD. Mechanism of action of methotrexate in rheumatoid arthritis, and the search for biomarkers. Nature reviews Rheumatology. 2016;12(12):731-42.
44. Feldmann, M. Development of anti-TNF therapy for rheumatoid arthritis. Nature reviews Immunology. 2002;2(5):364-71.
45. Neovius, M, Arkema E, Olsson H, Eriksson J, Kristensen LE, Simard J, Askling J, Bäcklund E, Cöster L, Forsblad-d'Elia H. Drug survival on TNF inhibitors in patients with rheumatoid arthritis comparison of adalimumab, etanercept and infliximab. Annals of the rheumatic diseases. 2015;74(2):354-60.
46. Klareskog, L, van der Heijde D, de Jager JP, Gough A, Kalden J, Malaise M, Martin Mola E, Pavelka K, Sany J, Settas L, Wajdula J, Pedersen R, Fatenejad S, Sanda M. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. Lancet (London, England). 2004;363(9410):675-81.
47. Monaco, C, Nanchahal J, Taylor P, Feldmann M. Anti-TNF therapy: past, present and future. International immunology. 2015;27(1):55-62.
48. Leirisalo-Repo, M, Kautiainen H, Laasonen L, Korpela M, Kauppi MJ, Kaipiainen-Seppänen O, Luosujärvi R, Luukkainen R, Karjalainen A, Blåfield H. Infliximab for 6 months added on combination therapy in early rheumatoid arthritis: 2-year results from an investigator-initiated, randomised, double-blind, placebo-controlled study (the NEO-RACo Study). Annals of the rheumatic diseases. 2013;72(6):851-7.
49. Emery, P, Fleischmann R, Filipowicz-Sosnowska A, Schechtman J, Szczepanski L, Kavanaugh A, Racewicz AJ, van Vollenhoven RF, Li NF, Agarwal S, Hessey EW, Shaw TM. The efficacy and safety of rituximab in patients with active

rheumatoid arthritis despite methotrexate treatment: results of a phase IIB randomized, double-blind, placebo-controlled, dose-ranging trial. Arthritis and rheumatism. 2006;54(5):1390-400.
50. Nakayamada, S, Kubo S, Iwata S, Tanaka Y. Recent Progress in JAK Inhibitors for the Treatment of Rheumatoid Arthritis. BioDrugs : clinical immunotherapeutics, biopharmaceuticals and gene therapy. 2016;30(5):407-19.
51. Damsky, W, King BA. JAK inhibitors in dermatology: The promise of a new drug class. Journal of the American Academy of Dermatology. 2017.
52. Murakami, M. Lipid mediators in life science. Exp Anim. 2011;60(1):7-20.
53. Jacobsson, L, Lindgärde F, Manthorpe R, Akesson B. Correlation of fatty acid composition of adipose tissue lipids and serum phosphatidylcholine and serum concentrations of micronutrients with disease duration in rheumatoid arthritis. Annals of the rheumatic diseases. 1990;49(11):901-5.
54. Frayn, KN, Arner P, Yki-Jarvinen H. Fatty acid metabolism in adipose tissue, muscle and liver in health and disease. Essays in biochemistry. 2006;42:89-103.
55. Vessby, B. Dietary fat, fatty acid composition in plasma and the metabolic syndrome. Current opinion in lipidology. 2003;14(1):15-9.
56. You, JS, Frey JW, Hornberger TA. Mechanical stimulation induces mTOR signaling via an ERK-independent mechanism: implications for a direct activation of mTOR by phosphatidic acid. PLoS One. 2012;7(10):e47258.
57. Hornberger, TA, Chu WK, Mak YW, Hsiung JW, Huang SA, Chien S. The role of phospholipase D and phosphatidic acid in the mechanical activation of mTOR signaling in skeletal muscle. Proceedings of the National Academy of Sciences of the United States of America. 2006;103(12):4741-6.
58. Hoffman, JR, Stout JR, Williams DR, Wells AJ, Fragala MS, Mangine GT, Gonzalez AM, Emerson NS, McCormack WP, Scanlon TC, Purpura M, Jager R. Efficacy of phosphatidic acid ingestion on lean body mass, muscle thickness and strength gains in resistance-trained men. Journal of the International Society of Sports Nutrition. 2012;9(1):47.
59. Joy, JM, Lowery RP, Dudeck JE, De Souza EO, Jäger R, McCleary SA, Wilson SM, Purpura M, Wilson JM. Phosphatidic acid supplementation increases skeletal muscle hypertrophy and strength. Journal of the International Society of Sports Nutrition. 2013;10(S1):P13.
60. Billah, MM, Anthes JC. The regulation and cellular functions of phosphatidylcholine hydrolysis. Biochemical Journal. 1990;269(2):281.
61. Vance, JE, Tasseva G. Formation and function of phosphatidylserine and phosphatidylethanolamine in mammalian cells. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2013;1831(3):543-54.
62. Verhoven, B, Schlegel RA, Williamson P. Mechanisms of phosphatidylserine exposure, a phagocyte recognition signal, on apoptotic T lymphocytes. J Exp Med. 1995;182(5):1597-601.
63. Lentz, BR. Exposure of platelet membrane phosphatidylserine regulates blood coagulation. Progress in lipid research. 2003;42(5):423-38.
64. D'Souza, K, Epand RM. Enrichment of phosphatidylinositols with specific acyl chains. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2014;1838(6):1501-8.
65. Mayinger, P. Phosphoinositides and vesicular membrane traffic. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2012;1821(8):1104-13.
66. Dufrisne, MLB, Clarke OB, Tomasek D, Jorge CD, Kim M, Banerjee S, Rajashankar KR, Shapiro L, Hendrickson WA, Santos H. Structural Basis for

Phosphatidylinositol-Phosphate Biosynthesis. Biophysical Journal. 2016;110(3):61a.
67. Choi, JW, Chun J. Lysophospholipids and their receptors in the central nervous system. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2013;1831(1):20-32.
68. Yung, YC, Stoddard NC, Chun J. LPA receptor signaling: pharmacology, physiology, and pathophysiology. Journal of lipid research. 2014;55(7):1192-214.
69. Sheng, X, Yung YC, Chen A, Chun J. Lysophosphatidic acid signalling in development. Development. 2015;142(8):1390-5.
70. Munder, P, Modolell M, Andreesen R, Weltzien H, Westphal O, editors. Lysophosphatidylcholine (lysolecithin) and its synthetic analogues. Immunemodulating and other biologic effects. Springer Seminars in Immunopathology; 1979: Springer.
71. Lauber, K, Bohn E, Kröber SM, Xiao Y-j, Blumenthal SG, Lindemann RK, Marini P, Wiedig C, Zobywalski A, Baksh S. Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. Cell. 2003;113(6):717-30.
72. Matsumoto, T, Kobayashi T, Kamata K. Role of lysophosphatidylcholine (LPC) in atherosclerosis. Current medicinal chemistry. 2007;14(30):3209-20.
73. Li, X, Fang P, Li Y, Kuo YM, Andrews AJ, Nanayakkara G, Johnson C, Fu H, Shan H, Du F, Hoffman NE, Yu D, Eguchi S, Madesh M, Koch WJ, Sun J, Jiang X, Wang H, Yang X. Mitochondrial Reactive Oxygen Species Mediate Lysophosphatidylcholine-Induced Endothelial Cell Activation. Arterioscler Thromb Vasc Biol. 2016;36(6):1090-100.
74. Guemez-Gamboa, A, Nguyen LN, Yang H, Zaki MS, Kara M, Ben-Omran T, Akizu N, Rosti RO, Rosti B, Scott E. Inactivating mutations in MFSD2A, required for omega-3 fatty acid transport in brain, cause a lethal microcephaly syndrome. Nature genetics. 2015;47(7):809-13.
75. Huang, Y, Schafer-Elinder L, Wu R, Claesson H, Frostegard J. Lysophosphatidylcholine (LPC) induces proinflammatory cytokines by a platelet-activating factor (PAF) receptor-dependent mechanism. Clinical and experimental immunology. 1999;116(2):326-31.
76. Husted, KS, Bouzinova EV. The importance of n-6/n-3 fatty acids ratio in the major depressive disorder. Medicina. 2016.
77. Calder, PC. Functional roles of fatty acids and their effects on human health. Journal of Parenteral and Enteral Nutrition. 2015;39(1 suppl):18S-32S.
78. Calder, PC. n− 3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. The American journal of clinical nutrition. 2006;83(6):S1505-19S.
79. Kinsella, JE, Broughton KS, Whelan JW. Dietary unsaturated fatty acids: interactions and possible needs in relation to eicosanoid synthesis. The Journal of nutritional biochemistry. 1990;1(3):123-41.
80. James, MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. The American journal of clinical nutrition. 2000;71(1):343s-8s.
81. Ergas, D, Eilat E, Mendlovic S, Sthoeger ZM. n-3 fatty acids and the immune system in autoimmunity. The Israel Medical Association journal : IMAJ. 2002;4(1):34-8.
82. Miles, EA, Calder PC. Influence of marine n-3 polyunsaturated fatty acids on immune function and a systematic review of their effects on clinical outcomes in rheumatoid arthritis. British Journal of Nutrition. 2012;107(S2):S171-S84.

83. Calder, PC. Immunoregulatory and anti-inflammatory effects of n-3 polyunsaturated fatty acids. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas. 1998;31(4):467-90.
84. Kadotani, A, Tsuchiya Y, Hatakeyama H, Katagiri H, Kanzaki M. Different impacts of saturated and unsaturated free fatty acids on COX-2 expression in C(2)C(12) myotubes. American journal of physiology Endocrinology and metabolism. 2009;297(6):E1291-303.
85. Hurley, MS, Flux C, Salter AM, Brameld JM. Effects of fatty acids on skeletal muscle cell differentiation in vitro. British journal of nutrition. 2006;95(03):623-30.
86. Tuazon, MA, Henderson GC. Fatty acid profile of skeletal muscle phospholipid is altered in mdx mice and is predictive of disease markers. Metabolism. 2012;61(6):801-11.
87. Lee, J-H, Tachibana H, Morinaga Y, Fujimura Y, Yamada K. Modulation of proliferation and differentiation of C2C12 skeletal muscle cells by fatty acids. Life sciences. 2009;84(13):415-20.
88. Henry, R, Peoples GE, McLennan PL. Muscle fatigue resistance in the rat hindlimb in vivo from low dietary intakes of tuna fish oil that selectively increase phospholipid n-3 docosahexaenoic acid according to muscle fibre type. The British journal of nutrition. 2015;114(6):873-84.
89. Spencer, AG, Woods JW, Arakawa T, Singer, II, Smith WL. Subcellular localization of prostaglandin endoperoxide H synthases-1 and -2 by immunoelectron microscopy. The Journal of biological chemistry. 1998;273(16):9886-93.
90. Morita, I, Schindler M, Regier MK, Otto JC, Hori T, DeWitt DL, Smith WL. Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. The Journal of biological chemistry. 1995;270(18):10902-8.
91. Dubois, RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, Lipsky PE. Cyclooxygenase in biology and disease. The FASEB journal. 1998;12(12):1063-73.
92. Crofford, LJ. COX-1 and COX-2 tissue expression: implications and predictions. The Journal of Rheumatology Supplement. 1997;49:15-9.
93. Avendano, MS, Martinez-Revelles S, Aguado A, Simoes MR, Gonzalez-Amor M, Palacios R, Guillem-Llobat P, Vassallo DV, Vila L, Garcia-Puig J, Beltran LM, Alonso MJ, Cachofeiro MV, Salaices M, Briones AM. Role of COX-2-derived PGE2 on vascular stiffness and function in hypertension. Br J Pharmacol. 2016;173(9):1541-55.
94. Mitchell, JA, Warner TD. COX isoforms in the cardiovascular system: understanding the activities of non-steroidal anti-inflammatory drugs. Nature reviews Drug discovery. 2006;5(1):75-86.
95. Smyth, EM. Thromboxane and the thromboxane receptor in cardiovascular disease. Clinical lipidology. 2010;5(2):209-19.
96. Vane, J, Corin RE. Prostacyclin: a vascular mediator. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2003;26(6):571-8.
97. Lichtenberger, LM, Romero JJ, Dial EJ, Moore JE. Naproxen-PC: a GI safe and highly effective anti-inflammatory. Inflammopharmacology. 2009;17(1):1-5.
98. Harris, RC, Zhang MZ. Cyclooxygenase metabolites in the kidney. Comprehensive Physiology. 2011.
99. Korotkova, M, Jakobsson P-J. Persisting eicosanoid pathways in rheumatic diseases. Nature Reviews Rheumatology. 2014;10(4):229-41.

100. Funk, CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 2001;294(5548):1871-5.
101. Hirata, T, Narumiya S. Prostanoid receptors. Chemical reviews. 2011;111(10):6209-30.
102. Fabre, J-E, Nguyen M, Athirakul K, Coggins K, McNeish JD, Austin S, Parise LK, FitzGerald GA, Coffman TM, Koller BH. Activation of the murine EP3 receptor for PGE 2 inhibits cAMP production and promotes platelet aggregation. The Journal of clinical investigation. 2001;107(5):603-10.
103. Uracz, W, Uracz D, Olszanecki R, Gryglewski RJ. Interleukin 1beta induces functional prostaglandin E synthase in cultured human umbilical vein endothelial cells. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 2002;53(4 Pt 1):643-54.
104. Stichtenoth, DO, Thoren S, Bian H, Peters-Golden M, Jakobsson PJ, Crofford LJ. Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. Journal of immunology (Baltimore, Md : 1950). 2001;167(1):469-74.
105. Sottile, A, Venza M, Venza I, Teti D. Prostaglandins affect the respiratory burst of human neutrophils. Immunopharmacology and immunotoxicology. 1995;17(2):311-21.
106. Vassiliou, E, Jing H, Ganea D. Prostaglandin E2 inhibits TNF production in murine bone marrow-derived dendritic cells. Cellular immunology. 2003;223(2):120-32.
107. Harizi, H, Grosset C, Gualde N. Prostaglandin E2 modulates dendritic cell function via EP2 and EP4 receptor subtypes. Journal of leukocyte biology. 2003;73(6):756-63.
108. Sampey, AV, Monrad S, Crofford LJ. Microsomal prostaglandin E synthase-1: the inducible synthase for prostaglandin E2. Arthritis Res Ther. 2005;7(3):114-7.
109. Larsson, K, Kock A, Idborg H, Henriksson MA, Martinsson T, Johnsen JI, Korotkova M, Kogner P, Jakobsson P-J. COX/mPGES-1/PGE2 pathway depicts an inflammatory-dependent high-risk neuroblastoma subset. Proceedings of the National Academy of Sciences. 2015;112(26):8070-5.
110. Murakami, M, Kudo I. Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway. Progress in lipid research. 2004;43(1):3-35.
111. Jakobsson, P-J, Thorén S, Morgenstern R, Samuelsson B. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proceedings of the National Academy of Sciences. 1999;96(13):7220-5.
112. Murakami, M, Naraba H, Tanioka T, Semmyo N, Nakatani Y, Kojima F, Ikeda T, Fueki M, Ueno A, Oh-ishi S. Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. Journal of Biological Chemistry. 2000;275(42):32783-92.
113. Engblom, D, Saha S, Engström L, Westman M, Audoly LP, Jakobsson P-J, Blomqvist A. Microsomal prostaglandin E synthase-1 is the central switch during immune-induced pyresis. Nature neuroscience. 2003;6(11):1137-8.
114. Trebino, CE, Stock JL, Gibbons CP, Naiman BM, Wachtmann TS, Umland JP, Pandher K, Lapointe J-M, Saha S, Roach ML. Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. Proceedings of the National Academy of Sciences. 2003;100(15):9044-9.
115. Ito, S, Okuda-Ashitaka E, Minami T. Central and peripheral roles of prostaglandins in pain and their interactions with novel neuropeptides nociceptin and nocistatin. Neuroscience research. 2001;41(4):299-332.

116. England, S, Bevan S, Docherty RJ. PGE2 modulates the tetrodotoxin-resistant sodium current in neonatal rat dorsal root ganglion neurones via the cyclic AMP-protein kinase A cascade. The Journal of physiology. 1996;495 (Pt 2):429-40.
117. Neugebauer, V, Schaible HG, Schmidt RF. Sensitization of articular afferents to mechanical stimuli by bradykinin. Pflugers Archiv : European journal of physiology. 1989;415(3):330-5.
118. Tamura, K, Sakurai T, Kogo H. Relationship between prostaglandin E2 and vascular endothelial growth factor (VEGF) in angiogenesis in human vascular endothelial cells. Vascular pharmacology. 2006;44(6):411-6.
119. Shinomiya, S, Naraba H, Ueno A, Utsunomiya I, Maruyama T, Ohuchida S, Ushikubi F, Yuki K, Narumiya S, Sugimoto Y, Ichikawa A, Oh-ishi S. Regulation of TNFalpha and interleukin-10 production by prostaglandins I(2) and E(2): studies with prostaglandin receptor-deficient mice and prostaglandin E-receptor subtype-selective synthetic agonists. Biochem Pharmacol. 2001;61(9):1153-60.
120. Roper, RL, Phipps RP. Prostaglandin E2 and cAMP inhibit B lymphocyte activation and simultaneously promote IgE and IgG1 synthesis. Journal of immunology (Baltimore, Md : 1950). 1992;149(9):2984-91.
121. Sakata, D, Yao C, Narumiya S. Prostaglandin E2, an immunoactivator. Journal of pharmacological sciences. 2010;112(1):1-5.
122. Zhou, W, Hashimoto K, Goleniewska K, O'Neal JF, Ji S, Blackwell TS, Fitzgerald GA, Egan KM, Geraci MW, Peebles RS, Jr. Prostaglandin I2 analogs inhibit proinflammatory cytokine production and T cell stimulatory function of dendritic cells. Journal of immunology (Baltimore, Md : 1950). 2007;178(2):702-10.
123. Nakajima, S, Honda T, Sakata D, Egawa G, Tanizaki H, Otsuka A, Moniaga CS, Watanabe T, Miyachi Y, Narumiya S, Kabashima K. Prostaglandin I2-IP signaling promotes Th1 differentiation in a mouse model of contact hypersensitivity. Journal of immunology (Baltimore, Md : 1950). 2010;184(10):5595-603.
124. Urade, Y, Eguchi Y, Eguchi N, Kijima Y, Matsuura Y, Oda H, Seiki K, Hayaishi O. Secretion of lipocalin-type prostaglandin D synthase (beta-trace) from human heart to plasma during coronary circulation. Advances in experimental medicine and biology. 1999;469:49-54.
125. Beuckmann, CT, Lazarus M, Gerashchenko D, Mizoguchi A, Nomura S, Mohri I, Uesugi A, Kaneko T, Mizuno N, Hayaishi O, Urade Y. Cellular localization of lipocalin-type prostaglandin D synthase (beta-trace) in the central nervous system of the adult rat. The Journal of comparative neurology. 2000;428(1):62-78.
126. Fouchecourt, S, Dacheux F, Dacheux JL. Glutathione-independent prostaglandin D2 synthase in ram and stallion epididymal fluids: origin and regulation. Biology of reproduction. 1999;60(3):558-66.
127. Jowsey, IR, Thomson AM, Flanagan JU, Murdock PR, Moore GB, Meyer DJ, Murphy GJ, Smith SA, Hayes JD. Mammalian class Sigma glutathione S-transferases: catalytic properties and tissue-specific expression of human and rat GSH-dependent prostaglandin D2 synthases. The Biochemical journal. 2001;359(Pt 3):507-16.
128. Huang, ZL, Urade Y, Hayaishi O. Prostaglandins and adenosine in the regulation of sleep and wakefulness. Current opinion in pharmacology. 2007;7(1):33-8.
129. Hirai, H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, Ichimasa M, Sugamura K, Nakamura M, Takano S, Nagata K. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. J Exp Med. 2001;193(2):255-61.
130. Mendez, M, LaPointe MC. PPARgamma inhibition of cyclooxygenase-2, PGE2 synthase, and inducible nitric oxide synthase in cardiac myocytes. Hypertension (Dallas, Tex : 1979). 2003;42(4):844-50.

131. Smith, WL, Urade Y, Jakobsson P-J. Enzymes of the cyclooxygenase pathways of prostanoid biosynthesis. Chemical reviews. 2011;111(10):5821-65.
132. Basu, S, Whiteman M, Mattey DL, Halliwell B. Raised levels of F(2)-isoprostanes and prostaglandin F(2alpha) in different rheumatic diseases. Ann Rheum Dis. 2001;60(6):627-31.
133. Granstrom, E. On the metabolism of prostaglandin F 2 in female subjects. Structures of two metabolites in blood. Eur J Biochem. 1972;27(3):462-9.
134. Needleman, P, Moncada S, Bunting S, Vane JR, Hamberg M, Samuelsson B. Identification of an enzyme in platelet microsomes which generates thromboxane A2 from prostaglandin endoperoxides. Nature. 1976;261(5561):558-60.
135. Kabashima, K, Murata T, Tanaka H, Matsuoka T, Sakata D, Yoshida N, Katagiri K, Kinashi T, Tanaka T, Miyasaka M, Nagai H, Ushikubi F, Narumiya S. Thromboxane A2 modulates interaction of dendritic cells and T cells and regulates acquired immunity. Nature immunology. 2003;4(7):694-701.
136. Samuelsson, B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science. 1983;220(4597):568-75.
137. Byrum, RS, Goulet JL, Griffiths RJ, Koller BH. Role of the 5-lipoxygenase–activating protein (FLAP) in murine acute inflammatory responses. The Journal of experimental medicine. 1997;185(6):1065-76.
138. Dixon, R, Diehl R, Opas E, Rands E, Vickers P, Evans J, Gillard J, Miller D. Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. 1990.
139. Vickers, PJ. 5-Lipoxygenase-activating protein (FLAP). Journal of lipid mediators and cell signalling. 1995;12(2):185-94.
140. Funk, CD, Chen XS, Johnson EN, Zhao L. Lipoxygenase genes and their targeted disruption. Prostaglandins Other Lipid Mediat. 2002;68-69:303-12.
141. Holgate, ST, Bradding P, Sampson AP. Leukotriene antagonists and synthesis inhibitors: new directions in asthma therapy. The Journal of allergy and clinical immunology. 1996;98(1):1-13.
142. Kim, DC, Hsu FI, Barrett NA, Friend DS, Grenningloh R, Ho IC, Al-Garawi A, Lora JM, Lam BK, Austen KF, Kanaoka Y. Cysteinyl leukotrienes regulate Th2 cell-dependent pulmonary inflammation. Journal of immunology (Baltimore, Md : 1950). 2006;176(7):4440-8.
143. Forsberg, L, Leeb L, Thoren S, Morgenstern R, Jakobsson P. Human glutathione dependent prostaglandin E synthase: gene structure and regulation. FEBS letters. 2000;471(1):78-82.
144. Ekstrom, L, Lyrenas L, Jakobsson PJ, Morgenstern R, Kelner MJ. Basal expression of the human MAPEG members microsomal glutathione transferase 1 and prostaglandin E synthase genes is mediated by Sp1 and Sp3. Biochimica et biophysica acta. 2003;1627(2-3):79-84.
145. He, S, Wu Y, Yu D, Lai L. Microsomal prostaglandin E synthase-1 exhibits one-third-of-the-sites reactivity. Biochemical Journal. 2011;440(1):13-21.
146. Sjögren, T, Nord J, Ek M, Johansson P, Liu G, Geschwindner S. Crystal structure of microsomal prostaglandin E2 synthase provides insight into diversity in the MAPEG superfamily. Proceedings of the National Academy of Sciences. 2013;110(10):3806-11.
147. Brock, JS, Hamberg M, Balagunaseelan N, Goodman M, Morgenstern R, Strandback E, Samuelsson B, Rinaldo-Matthis A, Haeggström JZ. A dynamic Asp–Arg interaction is essential for catalysis in microsomal prostaglandin E2 synthase. Proceedings of the National Academy of Sciences. 2016;113(4):972-7.
148. Hammarberg, T, Hamberg M, Wetterholm A, Hansson H, Samuelsson B, Haeggström JZ. Mutation of a critical arginine in microsomal prostaglandin E

synthase-1 shifts the isomerase activity to a reductase activity that converts prostaglandin H2 into prostaglandin F2α. Journal of Biological Chemistry. 2009;284(1):301-5.
149. Raouf, J, Rafique N, Goodman MC, Idborg H, Bergqvist F, Armstrong RN, Jakobsson PJ, Morgenstern R, Spahiu L. Arg126 and Asp49 Are Essential for the Catalytic Function of Microsomal Prostaglandin E2 Synthase 1 and Ser127 Is Not. PLoS One. 2016;11(9):e0163600.
150. Naraba, H, Yokoyama C, Tago N, Murakami M, Kudo I, Fueki M, Oh-ishi S, Tanabe T. Transcriptional regulation of the membrane-associated prostaglandin E2 synthase gene Essential role of the transcription factor Egr-1. Journal of Biological Chemistry. 2002;277(32):28601-8.
151. Moore, AE, Young LE, Dixon DA. MicroRNA and AU-rich element regulation of prostaglandin synthesis. Cancer metastasis reviews. 2011;30(3-4):419-35.
152. Bage, T, Lindberg J, Lundeberg J, Modeer T, Yucel-Lindberg T. Signal pathways JNK and NF-kappaB, identified by global gene expression profiling, are involved in regulation of TNFalpha-induced mPGES-1 and COX-2 expression in gingival fibroblasts. BMC genomics. 2010;11:241.
153. Masuko-Hongo, K, Berenbaum F, Humbert L, Salvat C, Goldring MB, Thirion S. Up-regulation of microsomal prostaglandin E synthase 1 in osteoarthritic human cartilage: critical roles of the ERK-1/2 and p38 signaling pathways. Arthritis and rheumatism. 2004;50(9):2829-38.
154. Diaz-Munoz, MD, Osma-Garcia IC, Fresno M, Iniguez MA. Involvement of PGE2 and the cAMP signalling pathway in the up-regulation of COX-2 and mPGES-1 expression in LPS-activated macrophages. The Biochemical journal. 2012;443(2):451-61.
155. Degousee, N, Angoulvant D, Fazel S, Stefanski E, Saha S, Iliescu K, Lindsay TF, Fish JE, Marsden PA, Li RK, Audoly LP, Jakobsson PJ, Rubin BB. c-Jun N-terminal kinase-mediated stabilization of microsomal prostaglandin E2 synthase-1 mRNA regulates delayed microsomal prostaglandin E2 synthase-1 expression and prostaglandin E2 biosynthesis by cardiomyocytes. The Journal of biological chemistry. 2006;281(24):16443-52.
156. Kojima, F, Naraba H, Sasaki Y, Okamoto R, Koshino T, Kawai S. Coexpression of microsomal prostaglandin E synthase with cyclooxygenase-2 in human rheumatoid synovial cells. The Journal of rheumatology. 2002;29(9):1836-42.
157. Korotkova, M, Westman M, Gheorghe KR, af Klint E, Trollmo C, Ulfgren AK, Klareskog L, Jakobsson PJ. Effects of antirheumatic treatments on the prostaglandin E2 biosynthetic pathway. Arthritis & Rheumatism. 2005;52(11):3439-47.
158. Bombardieri, S, Cattani P, Ciabattoni G, Munno O, Pasero G, Patrono C, Pinca E, Pugliese F. THE SYNOVIAL PROSTAGLANDIN SYSTEM IN CHRONIC INFLAMMATORY ARTHRITIS: DIFFERENTIAL EFFECTS OF STEROIDAL AND NONSTEROIDAL ANTI-INFLAMMATORY DRUGS. British journal of pharmacology. 1981;73(4):893-901.
159. Westman, M, Korotkova M, af Klint E, Stark A, Audoly LP, Klareskog L, Ulfgren AK, Jakobsson PJ. Expression of microsomal prostaglandin E synthase 1 in rheumatoid arthritis synovium. Arthritis Rheum. 2004;50(6):1774-80.
160. Korotkova, M, Daha NA, Seddighzadeh M, Ding B, Catrina AI, Lindblad S, Huizinga TW, Toes RE, Alfredsson L, Klareskog L, Jakobsson PJ, Padyukov L. Variants of gene for microsomal prostaglandin E2 synthase show association with disease and severe inflammation in rheumatoid arthritis. European journal of human genetics : EJHG. 2011;19(8):908-14.

161. Gheorghe, KR, Thurlings RM, Westman M, Boumans MJ, Malmström V, Trollmo C, Korotkova M, Jakobsson P-J, Tak P-P. Prostaglandin E2 synthesizing enzymes in rheumatoid arthritis B cells and the effects of B cell depleting therapy on enzyme expression. PloS one. 2011;6(1):e16378.
162. Thoren, S, Jakobsson PJ. Coordinate up-and down-regulation of glutathione-dependent prostaglandin E synthase and cyclooxygenase-2 in A549 cells. European journal of biochemistry. 2000;267(21):6428-34.
163. Keystone, EC, Kavanaugh AF, Sharp JT, Tannenbaum H, Hua Y, Teoh LS, Fischkoff SA, Chartash EK. Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. Arthritis and rheumatism. 2004;50(5):1400-11.
164. Tuure, L, Hämäläinen M, Moilanen T, Moilanen E. Aurothiomalate inhibits the expression of mPGES-1 in primary human chondrocytes. Scandinavian journal of rheumatology. 2015;44(1):74-9.
165. Xue, X, Shah YM. Hypoxia-inducible factor-2α is essential in activating the COX2/mPGES-1/PGE2 signaling axis in colon cancer. Carcinogenesis. 2012:bgs313.
166. Gosset, M, Berenbaum F, Levy A, Pigenet A, Thirion S, Cavadias S, Jacques C. Mechanical stress and prostaglandin E2 synthesis in cartilage. Biorheology. 2008;45(3-4):301-20.
167. Kusunoki, N, Kitahara K, Kojima F, Tanaka N, Kaneko K, Endo H, Suguro T, Kawai S. Adiponectin stimulates prostaglandin E(2) production in rheumatoid arthritis synovial fibroblasts. Arthritis and rheumatism. 2010;62(6):1641-9.
168. Del Prete, A, Salvi V, Sozzani S. Adipokines as potential biomarkers in rheumatoid arthritis. Mediators Inflamm. 2014;2014:425068.
169. Amadio, P, Baldassarre D, Tarantino E, Zacchi E, Gianellini S, Squellerio I, Amato M, Weksler BB, Tremoli E, Barbieri SS. Production of prostaglandin E2 induced by cigarette smoke modulates tissue factor expression and activity in endothelial cells. The FASEB Journal. 2015;29(9):4001-10.
170. Tawara, T, Shingu M, Nobunaga M, Naono T. Effects of recombinant human IL-1 beta on production of prostaglandin E2, leukotriene B4, NAG, and superoxide by human synovial cells and chondrocytes. Inflammation. 1991;15(2):145-57.
171. Kojima, F, Naraba H, Miyamoto S, Beppu M, Aoki H, Kawai S. Membrane-associated prostaglandin E synthase-1 is upregulated by proinflammatory cytokines in chondrocytes from patients with osteoarthritis. Arthritis Res Ther. 2004;6(4):R355.
172. Gheorghe, KR, Sadique S, Leclerc P, Idborg H, Wobst I, Catrina AI, Jakobsson PJ, Korotkova M. Limited effect of anti-rheumatic treatment on 15-prostaglandin dehydrogenase in rheumatoid arthritis synovial tissue. Arthritis Res Ther. 2012;14(3):R121.
173. Kamei, D, Yamakawa K, Takegoshi Y, Mikami-Nakanishi M, Nakatani Y, Oh-ishi S, Yasui H, Azuma Y, Hirasawa N, Ohuchi K. Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin e synthase-1. Journal of Biological Chemistry. 2004;279(32):33684-95.
174. Kojima, F, Kapoor M, Yang L, Fleishaker EL, Ward MR, Monrad SU, Kottangada PC, Pace CQ, Clark JA, Woodward JG. Defective generation of a humoral immune response is associated with a reduced incidence and severity of collagen-induced arthritis in microsomal prostaglandin E synthase-1 null mice. The Journal of Immunology. 2008;180(12):8361-8.

175. Cheng, Y, Wang M, Yu Y, Lawson J, Funk CD, FitzGerald GA. Cyclooxygenases, microsomal prostaglandin E synthase-1, and cardiovascular function. The Journal of clinical investigation. 2006;116(5):1391-9.
176. Kirkby, NS, Lundberg MH, Harrington LS, Leadbeater PD, Milne GL, Potter CM, Al-Yamani M, Adeyemi O, Warner TD, Mitchell JA. Cyclooxygenase-1, not cyclooxygenase-2, is responsible for physiological production of prostacyclin in the cardiovascular system. Proceedings of the National Academy of Sciences. 2012;109(43):17597-602.
177. Kirkby, NS, Zaiss AK, Urquhart P, Jiao J, Austin PJ, Al-Yamani M, Lundberg MH, MacKenzie LS, Warner TD, Nicolaou A. LC-MS/MS confirms that COX-1 drives vascular prostacyclin whilst gene expression pattern reveals non-vascular sites of COX-2 expression. PloS one. 2013;8(7):e69524.
178. Komhoff, M, Grone H-J, Klein T, Seyberth HW, Nusing R. Localization of cyclooxygenase-1 and-2 in adult and fetal human kidney: implication for renal function. American Journal of Physiology-Renal Physiology. 1997;272(4):F460-F8.
179. Yu, Y, Ricciotti E, Scalia R, Tang SY, Grant G, Yu Z, Landesberg G, Crichton I, Wu W, Pure E, Funk CD, FitzGerald GA. Vascular COX-2 modulates blood pressure and thrombosis in mice. Science translational medicine. 2012;4(132):132ra54.
180. Wang, D, Patel VV, Ricciotti E, Zhou R, Levin MD, Gao E, Yu Z, Ferrari VA, Lu MM, Xu J, Zhang H, Hui Y, Cheng Y, Petrenko N, Yu Y, FitzGerald GA. Cardiomyocyte cyclooxygenase-2 influences cardiac rhythm and function. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(18):7548-52.
181. Rodriguez, LAG, Cea-Soriano L, Tacconelli S, Patrignani P. Coxibs: pharmacology, toxicity and efficacy in cancer clinical trials. Prospects for Chemoprevention of Colorectal Neoplasia: Springer; 2013. p. 67-93.
182. Anwar, A, Anwar IJ, Delafontaine P. Elevation of cardiovascular risk by non-steroidal anti-inflammatory drugs. Trends in cardiovascular medicine. 2015;25(8):726-35.
183. Schober, LJ, Khandoga AL, Dwivedi S, Penz SM, Maruyama T, Brandl R, Siess W. The role of PGE(2) in human atherosclerotic plaque on platelet EP(3) and EP(4) receptor activation and platelet function in whole blood. Journal of thrombosis and thrombolysis. 2011;32(2):158-66.
184. Holmes, DR, Wester W, Thompson RW, Reilly JM. Prostaglandin E2 synthesis and cyclooxygenase expression in abdominal aortic aneurysms. Journal of vascular surgery. 1997;25(5):810-5.
185. Gross, S, Tilly P, Hentsch D, Vonesch JL, Fabre JE. Vascular wall-produced prostaglandin E2 exacerbates arterial thrombosis and atherothrombosis through platelet EP3 receptors. J Exp Med. 2007;204(2):311-20.
186. Raouf, J, Mobarrez F, Larsson K, Jakobsson PJ, Korotkova M. mPGES-1 deletion affects platelet functions in mice. Clinical science (London, England : 1979). 2016.
187. Tang, SY, Monslow J, Grant GR, Todd L, Pawelzik S-C, Chen L, Lawson J, Puré E, FitzGerald GA. Cardiovascular Consequences of Prostanoid I Receptor Deletion in Microsomal Prostaglandin E Synthase-1 Deficient Hyperlipidemic Mice. Circulation. 2016:CIRCULATIONAHA. 116.022308.
188. Wang, M, Lee E, Song W, Ricciotti E, Rader DJ, Lawson JA, Pure E, FitzGerald GA. Microsomal prostaglandin E synthase-1 deletion suppresses oxidative stress and angiotensin II-induced abdominal aortic aneurysm formation. Circulation. 2008;117(10):1302-9.

189. Wang, M, Zukas AM, Hui Y, Ricciotti E, Pure E, FitzGerald GA. Deletion of microsomal prostaglandin E synthase-1 augments prostacyclin and retards atherogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2006;103(39):14507-12.
190. George, JN, Shattil SJ. The clinical importance of acquired abnormalities of platelet function. New England Journal of Medicine. 1991;324(1):27-39.
191. Semple, JW, Freedman J. Platelets and innate immunity. Cellular and Molecular Life Sciences. 2010;67(4):499-511.
192. Davì, G, Patrono C. Platelet activation and atherothrombosis. New England Journal of Medicine. 2007;357(24):2482-94.
193. Boilard, E, Larabee K, Shnayder R, Jacobs K, Farndale RW, Ware J, Lee DM. Platelets participate in synovitis via Cox-1–dependent synthesis of prostacyclin independently of microparticle generation. The Journal of Immunology. 2011;186(7):4361-6.
194. Boilard, E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, Massarotti EM, Remold-O'Donnell E, Farndale RW, Ware J. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. Science. 2010;327(5965):580-3.
195. Ertenli, I, Kiraz S, Arici M, Haznedaroglu I, Calgüneri M, Celik I, Kirazli S. P-selectin as a circulating molecular marker in rheumatoid arthritis with thrombocytosis. The Journal of rheumatology. 1998;25(6):1054-8.
196. Italiano Jr, JE, Mairuhu AT, Flaumenhaft R. Clinical relevance of microparticles from platelets and megakaryocytes. Current opinion in hematology. 2010;17(6):578.
197. Horstman, LL, Ahn YS. Platelet microparticles: a wide-angle perspective. Critical reviews in oncology/hematology. 1999;30(2):111-42.
198. Mezouar, S, Mege D, Darbousset R, Farge D, Debourdeau P, Dignat-George F, Panicot-Dubois L, Dubois C, editors. Involvement of platelet-derived microparticles in tumor progression and thrombosis. Seminars in oncology; 2014: Elsevier.
199. Distler, JH, Pisetsky DS, Huber LC, Kalden JR, Gay S, Distler O. Microparticles as regulators of inflammation: novel players of cellular crosstalk in the rheumatic diseases. Arthritis and rheumatism. 2005;52(11):3337-48.
200. Jungel, A, Distler O, Schulze-Horsel U, Huber LC, Ha HR, Simmen B, Kalden JR, Pisetsky DS, Gay S, Distler JH. Microparticles stimulate the synthesis of prostaglandin E(2) via induction of cyclooxygenase 2 and microsomal prostaglandin E synthase 1. Arthritis and rheumatism. 2007;56(11):3564-74.
201. Dovizio, M, Tacconelli S, Ricciotti E, Bruno A, Maier TJ, Anzellotti P, Di Francesco L, Sala P, Signoroni S, Bertario L. Effects of celecoxib on prostanoid biosynthesis and circulating angiogenesis proteins in familial adenomatous polyposis. Journal of Pharmacology and Experimental Therapeutics. 2012;341(1):242-50.
202. Iyu, D, Glenn JR, White AE, Johnson AJ, Fox SC, Heptinstall S. The role of prostanoid receptors in mediating the effects of PGE(2) on human platelet function. Platelets. 2010;21(5):329-42.
203. Paul, B, Ashby B, Sheth S. Distribution of prostaglandin IP and EP receptor subtypes and isoforms in platelets and human umbilical artery smooth muscle cells. British journal of haematology. 1998;102:1204-11.
204. Coleman, RA, Smith WL, Narumiya S. International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. Pharmacol Rev. 1994;46(2):205-29.

205. Mudrovcic, N, Arefin S, Craenenbroeck AH, Kublickiene K. Endothelial maintenance in health and disease: importance of sex differences. Pharmacological research. 2017.
206. Camacho, M, Lopez-Belmonte J, Vila L. Rate of vasoconstrictor prostanoids released by endothelial cells depends on cyclooxygenase-2 expression and prostaglandin I synthase activity. Circulation research. 1998;83(4):353-65.
207. Soler, M, Camacho M, Escudero JR, Iniguez MA, Vila L. Human vascular smooth muscle cells but not endothelial cells express prostaglandin E synthase. Circulation research. 2000;87(6):504-7.
208. Casos, K, Siguero L, Fernandez-Figueras MT, Leon X, Sarda MP, Vila L, Camacho M. Tumor cells induce COX-2 and mPGES-1 expression in microvascular endothelial cells mainly by means of IL-1 receptor activation. Microvascular research. 2011;81(3):261-8.
209. Charo, IF, Shak S, Karasek MA, Davison PM, Goldstein IM. Prostaglandin I2 is not a major metabolite of arachidonic acid in cultured endothelial cells from human foreskin microvessels. J Clin Invest. 1984;74(3):914-9.
210. Kirkby, NS, Lundberg MH, Wright WR, Warner TD, Paul-Clark MJ, Mitchell JA. COX-2 protects against atherosclerosis independently of local vascular prostacyclin: identification of COX-2 associated pathways implicate Rgl1 and lymphocyte networks. PLoS One. 2014;9(6):e98165.
211. Ahmetaj-Shala, B, Kirkby NS, Knowles R, Al'Yamani M, Mazi S, Wang Z, Tucker AT, Mackenzie L, Armstrong PC, Nüsing RM. Evidence That Links Loss of Cyclooxygenase-2 With Increased Asymmetric Dimethylarginine Novel Explanation of Cardiovascular Side Effects Associated With Anti-Inflammatory Drugs. Circulation. 2015;131(7):633-42.
212. Caplin, B, Leiper J. Endogenous nitric oxide synthase inhibitors in the biology of disease markers, mediators, and regulators? Arteriosclerosis, thrombosis, and vascular biology. 2012;32(6):1343-53.
213. Friesen, RW, Mancini JA. Microsomal prostaglandin E2 synthase-1 (mPGES-1): a novel anti-inflammatory therapeutic target. Journal of medicinal chemistry. 2008;51(14):4059-67.
214. Bondesen, BA, Jones KA, Glasgow WC, Pavlath GK. Inhibition of myoblast migration by prostacyclin is associated with enhanced cell fusion. The FASEB Journal. 2007;21(12):3338-45.
215. Zalin, RJ. The role of hormones and prostanoids in the in vitro proliferation and differentiation of human myoblasts. Experimental cell research. 1987;172(2):265-81.
216. Otis, JS, Burkholder TJ, Pavlath GK. Stretch-induced myoblast proliferation is dependent on the COX2 pathway. Experimental cell research. 2005;310(2):417-25.
217. Korotkova, M, Lundberg IE. The skeletal muscle arachidonic acid cascade in health and inflammatory disease. Nature reviews Rheumatology. 2014;10(5):295-303.
218. Jansen, KM, Pavlath GK. Prostaglandin F2alpha promotes muscle cell survival and growth through upregulation of the inhibitor of apoptosis protein BRUCE. Cell death and differentiation. 2008;15(10):1619-28.
219. Velica, P, Khanim FL, Bunce CM. Prostaglandin D2 inhibits C2C12 myogenesis. Molecular and cellular endocrinology. 2010;319(1-2):71-8.
220. Kozlovsky, N, Shohami E, Bashan N. Increased PLA 2 activity is not related to increased GLUT1 expression in L6 myotubes under hypoxic conditions. Prostaglandins, leukotrienes and essential fatty acids. 1997;56(1):17-22.

221. Lee, AL, Park Y. The association between n-3 polyunsaturated fatty acid levels in erythrocytes and the risk of rheumatoid arthritis in Korean women. Annals of Nutrition and Metabolism. 2013;63(1-2):88-95.
222. Gorska, A, Nawrocki A, Urban M, Florys B. Composition of phospholipid fatty acids in erythrocyte membranes of children with chronic juvenile arthritis: clinical and biochemical correlations. Medical science monitor : international medical journal of experimental and clinical research. 2000;6(1):30-9.
223. Aghdassi, E, Ma DW, Morrison S, Hillyer LM, Clarke S, Gladman DD, Urowitz MB, Fortin PR. Alterations in circulating fatty acid composition in patients with systemic lupus erythematosus: a pilot study. JPEN Journal of parenteral and enteral nutrition. 2011;35(2):198-208.
224. Andersson, A, Sjödin A, Olsson R, Vessby B. Effects of physical exercise on phospholipid fatty acid composition in skeletal muscle. American Journal of Physiology-Endocrinology And Metabolism. 1998;274(3):E432-E8.
225. Helge, JW, Wu BJ, Willer M, Daugaard JR, Storlien LH, Kiens B. Training affects muscle phospholipid fatty acid composition in humans. Journal of Applied Physiology. 2001;90(2):670-7.
226. Loell, I, Raouf J, Chen YW, Shi R, Nennesmo I, Alexanderson H, Dastmalchi M, Nagaraju K, Korotkova M, Lundberg IE. Effects on muscle tissue remodeling and lipid metabolism in muscle tissue from adult patients with polymyositis or dermatomyositis treated with immunosuppressive agents. Arthritis research & therapy. 2016;18(1):136.
227. Lee, JH, Tachibana H, Morinaga Y, Fujimura Y, Yamada K. Modulation of proliferation and differentiation of C2C12 skeletal muscle cells by fatty acids. Life Sci. 2009;84(13-14):415-20.
228. Ayre, KJ, Hulbert A. Dietary fatty acid profile affects endurance in rats. Lipids. 1997;32(12):1265-70.
229. Peoples, GE, McLennan PL. Dietary fish oil reduces skeletal muscle oxygen consumption, provides fatigue resistance and improves contractile recovery in the rat in vivo hindlimb. British journal of nutrition. 2010;104(12):1771-9.
230. Rodacki, CL, Rodacki AL, Pereira G, Naliwaiko K, Coelho I, Pequito D, Fernandes LC. Fish-oil supplementation enhances the effects of strength training in elderly women. Am J Clin Nutr. 2012;95(2):428-36.
231. Lee, JY, Sohn KH, Rhee SH, Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. Journal of Biological Chemistry. 2001;276(20):16683-9.
232. Lee, JY, Zhao L, Youn HS, Weatherill AR, Tapping R, Feng L, Lee WH, Fitzgerald KA, Hwang DH. Saturated fatty acid activates but polyunsaturated fatty acid inhibits Toll-like receptor 2 dimerized with Toll-like receptor 6 or 1. Journal of Biological Chemistry. 2004;279(17):16971-9.
233. Briolay, A, Jaafar R, Nemoz G, Bessueille L. Myogenic differentiation and lipid-raft composition of L6 skeletal muscle cells are modulated by PUFAs. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2013;1828(2):602-13.
234. Smith, GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, Mittendorfer B. Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia–hyperaminoacidaemia in healthy young and middle-aged men and women. Clinical science. 2011;121(6):267-78.
235. Smith, GI, Julliand S, Reeds DN, Sinacore DR, Klein S, Mittendorfer B. Fish oil–derived n–3 PUFA therapy increases muscle mass and function in healthy older adults. The American journal of clinical nutrition. 2015;102(1):115-22.
236. Jiang, J, Li K, Wang F, Yang B, Fu Y, Zheng J, Li D. Effect of Marine-Derived n-3 Polyunsaturated Fatty Acids on Major Eicosanoids: A Systematic Review and

Meta-Analysis from 18 Randomized Controlled Trials. PLoS One. 2016;11(1):e0147351.
237. Morgenstern, R, Lundqvist G, Andersson G, Balk L, Depierre JW. The distribution of microsomal glutathione transferase among different organelles, different organs, and different organisms. Biochemical pharmacology. 1984;33(22):3609-14.
238. Estonius, M, Forsberg L, Danielsson O, Weinander R, Kelner MJ, Morgenstern R. Distribution of microsomal glutathione transferase 1 in mammalian tissues. European Journal of Biochemistry. 1999;260(2):409-13.
239. Cohen, SN, Chang AC, Boyer HW, Helling RB. Construction of biologically functional bacterial plasmids in vitro. Proceedings of the National Academy of Sciences. 1973;70(11):3240-4.
240. Makrides, SC. Strategies for achieving high-level expression of genes in Escherichia coli. Microbiological reviews. 1996;60(3):512-38.
241. Baneyx, F, Mujacic M. Recombinant protein folding and misfolding in Escherichia coli. Nature biotechnology. 2004;22(11):1399-408.
242. Baneyx, F. Recombinant protein expression in Escherichia coli. Current opinion in biotechnology. 1999;10(5):411-21.
243. Sørensen, HP, Mortensen KK. Advanced genetic strategies for recombinant protein expression in Escherichia coli. Journal of biotechnology. 2005;115(2):113-28.
244. Thorén, S, Weinander R, Saha S, Jegerschöld C, Pettersson PL, Samuelsson B, Hebert H, Hamberg M, Morgenstern R, Jakobsson P-J. Human Microsomal Prostaglandin E Synthase-1 Purification, Functional Characterization, and Projection Structure Determination. Journal of Biological Chemistry. 2003;278(25):22199-209.
245. Hamberg, M, Samuelsson B. Detection and isolation of an endoperoxide intermediate in prostaglandin biosynthesis. Proceedings of the National Academy of Sciences. 1973;70(3):899-903.
246. Burnette, WN. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Analytical biochemistry. 1981;112(2):195-203.
247. Taylor, SC, Posch A. The design of a quantitative western blot experiment. BioMed research international. 2014;2014.
248. Ornatsky, O, Bandura D, Baranov V, Nitz M, Winnik MA, Tanner S. Highly multiparametric analysis by mass cytometry. Journal of immunological methods. 2010;361(1):1-20.
249. De Cuyper, IM, Meinders M, van de Vijver E, de Korte D, Porcelijn L, de Haas M, Eble JA, Seeger K, Rutella S, Pagliara D. A novel flow cytometry–based platelet aggregation assay. Blood. 2013;121(10):e70-e80.
250. Walker, R. Quantification of immunohistochemistry—issues concerning methods, utility and semiquantitative assessment I. Histopathology. 2006;49(4):406-10.
251. Taylor, C, Levenson RM. Quantification of immunohistochemistry—issues concerning methods, utility and semiquantitative assessment II. Histopathology. 2006;49(4):411-24.
252. Hennion, M-C. Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography. Journal of chromatography A. 1999;856(1):3-54.
253. Augusto, F, Hantao LW, Mogollon NG, Braga SC. New materials and trends in sorbents for solid-phase extraction. TrAC Trends in Analytical Chemistry. 2013;43:14-23.

254. Rodriguez-Mozaz, S, de Alda MJL, Barceló D. Advantages and limitations of on-line solid phase extraction coupled to liquid chromatography–mass spectrometry technologies versus biosensors for monitoring of emerging contaminants in water. Journal of Chromatography A. 2007;1152(1):97-115.
255. Rezaee, M, Assadi Y, Hosseini M-RM, Aghaee E, Ahmadi F, Berijani S. Determination of organic compounds in water using dispersive liquid–liquid microextraction. Journal of Chromatography A. 2006;1116(1):1-9.
256. Viñas, P, Campillo N, López-García I, Hernández-Córdoba M. Dispersive liquid–liquid microextraction in food analysis. A critical review. Analytical and bioanalytical chemistry. 2014;406(8):2067-99.
257. Pawelzik, S-C, Uda NR, Spahiu L, Jegerschöld C, Stenberg P, Hebert H, Morgenstern R, Jakobsson P-J. Identification of key residues determining species differences in inhibitor binding of microsomal prostaglandin E synthase-1. Journal of Biological Chemistry. 2010;285(38):29254-61.
258. Gawaz, M, Langer H, May AE. Platelets in inflammation and atherogenesis. The Journal of clinical investigation. 2005;115(12):3378-84.
259. Yuri Gasparyan, A, Ayvazyan L, Pretorius E, D Kitas G. Platelets in rheumatic diseases: friend or foe? Current pharmaceutical design. 2014;20(4):552-66.
260. Vincent, J-L, Yagushi A, Pradier O. Platelet function in sepsis. Critical care medicine. 2002;30(5):S313-S7.
261. Shibazaki, M, Kawabata Y, Yokochi T, Nishida A, Takada H, Endo Y. Complement-dependent accumulation and degradation of platelets in the lung and liver induced by injection of lipopolysaccharides. Infection and immunity. 1999;67(10):5186-91.
262. Lievens, D, Zernecke A, Seijkens T, Soehnlein O, Beckers L, Munnix IC, Wijnands E, Goossens P, van Kruchten R, Thevissen L. Platelet CD40L mediates thrombotic and inflammatory processes in atherosclerosis. Blood. 2010;116(20):4317-27.
263. Salazar, F, Vazquez ML, Masferrer JL, Mbalaviele G, Llinas MT, Saez F, Arhancet G, Salazar FJ. Renal effects induced by prolonged mPGES1 inhibition. American Journal of Physiology-Renal Physiology. 2014;306(1):F68-F74.
264. Walsh, RJ, Kong SW, Yao Y, Jallal B, Kiener PA, Pinkus JL, Beggs AH, Amato AA, Greenberg SA. Type I interferon–inducible gene expression in blood is present and reflects disease activity in dermatomyositis and polymyositis. Arthritis & Rheumatism. 2007;56(11):3784-92.
265. Greenberg, S, Higgs B, Morehouse C, Walsh R, Kong SW, Brohawn P, Zhu W, Amato A, Salajegheh M, White B. Relationship between disease activity and type 1 interferon-and other cytokine-inducible gene expression in blood in dermatomyositis and polymyositis. Genes and immunity. 2012;13(3):207-13.
266. Nagaraju, K, Ghimbovschi S, Rayavarapu S, Phadke A, Rider LG, Hoffman EP, Miller FW. Muscle myeloid type I interferon gene expression may predict therapeutic responses to rituximab in myositis patients. Rheumatology (Oxford, England). 2016;55(9):1673-80.
267. Dastmalchi, M, Alexanderson H, Loell I, Ståhlberg M, Borg K, Lundberg IE, EsbJörnsson M. Effect of physical training on the proportion of slow-twitch type I muscle fibers, a novel nonimmune-mediated mechanism for muscle impairment in polymyositis or dermatomyositis. Arthritis Care & Research. 2007;57(7):1303-10.
268. Loell, I, Helmers S, Dastmalchi M, Alexanderson H, Munters L, Nennesmo I, Lindroos E, Borg K, Lundberg I, Esbjörnsson M. Higher proportion of fast-twitch (type II) muscle fibres in idiopathic inflammatory myopathies–evident in chronic but not in untreated newly diagnosed patients. Clinical physiology and functional imaging. 2011;31(1):18-25.

269. Schmitz-Peiffer, C. Targeting ceramide synthesis to reverse insulin resistance. Diabetes. 2010;59(10):2351-3.
270. Nikolova-Karakashian, MN, Reid MB. Sphingolipid metabolism, oxidant signaling, and contractile function of skeletal muscle. Antioxidants & redox signaling. 2011;15(9):2501-17.
271. Bryner, RW, Woodworth-Hobbs ME, Williamson DL, Alway SE. Docosahexaenoic acid protects muscle cells from palmitate-induced atrophy. ISRN obesity. 2012;2012.
272. Morgan, SA, Gathercole LL, Simonet C, Hassan-Smith ZK, Bujalska I, Guest P, Abrahams L, Smith DM, Stewart PM, Lavery GG. Regulation of lipid metabolism by glucocorticoids and 11β-HSD1 in skeletal muscle. Endocrinology. 2013;154(7):2374-84.
273. Huang, Y-S, Huang W-C, Li C-W, Chuang L-T. Eicosadienoic acid differentially modulates production of pro-inflammatory modulators in murine macrophages. Molecular and cellular biochemistry. 2011;358(1-2):85-94.