Exercise-induced lipid mediator inflammatory signalling in male recreational runners versus untrained males considering potential modulating factors

E Bezuidenhout

orcid.org/ 0000-0003-1176-2185

Dissertation submitted in fulfilment of the requirements for the degree Masters of Science in Nutrition at the North West University

Supervisor:	Dr L Malan
Co-supervisor:	Dr L Zandberg
Co-supervisor:	Ms A Nienaber

Examination November 2019

Student number: 24185264

ACKNOWLEDGEMENTS

There are numerous people I would like to thank for all their help, guidance and patience throughout these two years.

Firstly, I would like to thank Dr. Linda Malan for the interesting topic for my Masters. I would also like to give a very special thanks to Dr. Malan, as my supervisor. Thank you for all your help, support and kindness throughout these two years, most of all your patience, as I was not able to be in Potchefstroom a lot. With my having a baby, the time spent on my Masters was not always my first priority. Without you this dissertation would not be what it is today. It was such a pleasure to work with you and I have learnt so much from you.

Secondly, to my parents, without you I would not be sitting here today. Thank you for everything, the financial support, all of your love and definitely all off your support.

To my Husband, Albert, thank you for every hug, every cup of milo, every word of encouragement and every second you had to deal with a crying baby while I worked throughout this year. Thank you for keeping me going when I thought I could not anymore. You truly are a blessing to me and I cannot thank you enough.

To my precious little boy, JB. Thank you for being my biggest encouragement to finish my Masters, even though I could not finish before you arrived.

Most importantly, thank you to my Heavenly Father, YHWE Yeshua our Messiah, for everything. You have guided, encouraged and supported me most throughout these years. Without You nothing would have been possible. You have chosen this path for me and I truly hope I have made You proud.

ABSTRACT

Background: Resistance exercise can cause inflammation and it is known that inflammation is closely related to oxidative stress. Oxidative stress can be defined as an imbalance between free radicals and anti-oxidative mechanisms, or the excess of reactive oxygen species (ROS). The body reacts to injury or infection, as well as to excessive ROS production during exercise, with an inflammatory response. A complex network of lipid mediators regulates the inflammatory response. Lipid mediators are also known as eicosanoids and can regulate inflammation by eliciting either a pro-inflammatory or anti-inflammatory response. There is still little evidence to the question as to whether there is a difference in pro-inflammatory lipid mediator status between recreational runners and untrained individuals, and which factors can modulate this response.

Objectives: The aim of the study is to compare exercise-induced lipid mediator inflammatory signalling in male recreational runners with untrained males considering potential modulating factors such as fatty acid and antioxidant status, as well as antioxidant nutrient intake.

Study design: This was a cross-sectional study on healthy, male, untrained individuals (n=20) and recreational runners (n=20). A medical screening was done (blood pressure, heart rate, temperature and fingerprick blood glucose). The participants' height and weight were measured, and a three-day dietary record was administer red. Blood samples were collected before both groups participated in resistance exercises at 80% of their 1 repetition max and immediately on completion of that exercise challenge, as well as 1, 2, 24 and 72 hours after cessation of the exercise.

Results: The runners were taller and heavier than the non-runners ($P = 0.018$ and $P = 0.04$, respectively). Runners did significantly more running exercise than non-runners ($P < 0.001$). Eicosapentaenoic acid (EPA), monounsaturated fatty acids (MUFA), n-3 polyunsaturated fatty acids (PUFA) and n-3 long chain (LC) PUFA were higher in the runner group compared to the non-runner group ($P = 0.001$, $P = 0.045$, $P = 0.040$ and $P = 0.041$). On the other hand, the total n-6:n-3 PUFA and the n-6:n-3 LCPUFA ratios were higher in the non-runner group compared to the runner group ($P = 0.032$ and $P = 0.031$) . The runners' linoleic acid, vitamin C and Vitamin E intake were higher than the intake of the non-runners ($P = 0.035$; $P = 0.028$ and $P = 0.013$), respectively. There was a significant difference in response over time in the runners compared to the non-runners for 5-HETE and 12-HEPE ($P = 0.005$ and 0.008). When adjusted for BMI and diet, 5-HETE, 15-HETE and 8-HETE showed a significantly different response over time between the two groups ($P = 0.012$, $P = 0.033$, $P = 0.016$), respectively. There was also a significant

different response for 8-HETE, 12-HEPE and 18-HEPE adjusted for BMI and oxidative stress (P = 0.041, P = 0.059 and P = 0.085), respectively. When adjusting for BMI, diet and oxidative stress this resulted in a significant difference between the groups for 5-HETE (P = 0.016) and 8-HETE (P = 0.043).

Conclusion: This study showed that pro- and anti-inflammatory lipid mediator responses are different among runners and non-runners and are influenced by BMI, vitamin C and E intake, the n6:n3 LCPUFA status ratio and endogenous oxidative stress adaptation.

Key words: Exercise; fatty acids; inflammation; lipid mediators; oxidative stress; resolution.

ABBREVIATIONS

˚C:	Degree Celsius
µg/day:	Microgram per day
1 RM:	1 repetition maximum
17HDHA:	Neuroprostanes,17-hydroxy DHA
A:	Athlete
A:	Adrenaline
AA:	Arachidonic acid
AE:	Adverse event
ALA:	Alpha-linolenic acid
BMI:	Body mass index
bpm:	Beats per minute
CAT:	Catalase
CHO:	Carbohydrates
cm:	Centimetre
COX:	Cyclooxygenase
Cu-SOD:	Copper- superoxide dismutase
DA:	Dopamine
DEPPD:	Diethyl-para-phenylenediamine
DHA:	Docosahexaenoic acid
DNA:	Deoxyribonucleic acid
EPA:	Eicosapentaenoic acid
ERK:	Extracellular signal-regulated kinases
ETA:	Exercise Teachers Academy
ETC:	Electron transport chain
FAMEs:	Fatty acid methyl esters
FRAP:	Ferric reducing ability of plasma
g/dL:	Gram per decilitre
GHS:	Glutathione
GPX:	Glutathione peroxidase
GSH:	Glutathione
H:	Hour
Hb:	Haemoglobin
HDHA:	Hydroxydocosahexaenoic aid
HEPE:	Hydroxyeicosapentaenoic acids
HETE:	Hydroxyeicosatetraenoic acids
HOC1:	Hypochlorous acid

HpDHA:	Hydroperoxydocosahexaenoic acid
HpETE:	Hydroperoxyeicosatetraenoic acid
HREC:	Health Research Ethics Committee
ICF:	Information and written informed consent
IFN:	Interferon
IFN-y:	Interferon-y
IL-1:	Interleukin-1
IL-6:	Interleukin 6
ISAK:	International Society for the Advancement of Kinanthropometry
ISTD:	Internal standard
kg/m^2:	Kilogram per square meter
kg:	Kilogram
LA:	Linoleic acid
LCFA:	Long chain fatty acids
LCMSMS:	Liquid chromatography tandem mass spectrometry
LCPUFA:	Long-chain polyunsaturated fatty acids
LOX:	Lipoxygenase
LT:	Leukotriene
LTB_4:	Leukotriene B_4
LX:	Lipoxin
m:	Meter
MAPK:	Mitogen-activated protein kinase
mcg:	Microgram
min/session:	Minutes per session
mm/dL:	Millimetre per decilitre
mm:	Millimetre
mmHg:	Millimetre mercury
Mn-SOD:	Manganese superoxide dismutase
MRC:	Medical Research Council
MRM:	Multiple reaction monitoring
MUFAs:	Monounsaturated fatty acids
n:	Number of participants
n-3:	Omega-3
n-6:	Omega-6
n-9:	Omega-9
NA:	Non-athlete
NA:	Noradrenalin
NADPH:	Nicotinamide adenine dinucleotide phosphate-oxidase

NCDs:	Non-communicable diseases
NF:	Nuclear factor
Nfκβ:	Nuclear factor kappa β
NO:	Nitroxide
NOX:	Nicotinamide adenine dinucleotide phosphate oxidase
NSAIDS:	Non-steroidal anti-Inflammatory drugs
NWU:	North-West University
P:	Probability
PARQ:	Physical activity readiness questionnaire
PBMC:	Peripheral mononuclear cells
PD:	Protectins
pg/µl:	Picogram per microliter
PG:	Prostaglandins
PGD_2:	Prostaglandins D2
PGE_2:	Prostaglandins E2
PLA_2:	Phospholipase A_2
PM:	Post meridiem
PMN:	Polymorphonuclear
PUFA:	Polyunsaturated fatty acids
QFFQ:	Quantified food frequency questionnaire
RD:	Registered dietician
RDA:	Recommended daily allowance
ROS:	Reactive oxygen species
RT:	Retention Time
Rv:	Resolving
RvD:	Resolvins
SAE:	Serious adverse event
SASA:	South African Sugar Association
SOD:	Superoxide dismutase
SR:	Sarcoplasmic reticulum
TNF:	Tumor necrosis factor
TNF-α:	Tumour necrosis factor alpha
TX:	Thromboxane
TxnRd2:	Thioredoxin reductase 2
VitC:	Vitamin C
VitE:	Vitamin E
Vol:	Volume
XO:	Xanthine oxidase

yr:	Year
Zn-SOD:	Zinc-Superoxide dismutase
µm:	Micrometer

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 INTRODUCTION

1 INTRODUCTION

1.2 Rationale and background

The evidence of the health benefits of an optimal inflammation-resolving capacity is mounting. There is increasing evidence that the capacity to resolve inflammation improves immune competence and the response to infection (Calder, 2007; Fullerton *et al.*, 2014; Pradelli *et al.*, 2012). Moreover, resolution therapies (such as optimising omega-3 (n-3) polyunsaturated fatty acid (PUFA) status), which involve manipulation of, and impacting on the lipid mediator profiles, show promising results in reducing susceptibility to hospital-acquired infections (Eisen *et al.*, 2012; O'Neal Jr *et al.*, 2011; Otto *et al.*, 2013; Sossdorf *et al.*, 2013; Winning *et al.*, 2009). In addition, the pathogenesis of many non-communicable diseases (NCDs), such as Type 2 diabetes, hypertension and insulin resistance, atherosclerosis, neurodegeneration and tumour growth, is associated with oxidative stress and chronic inflammation (Camps & García-Heredia, 2014; Prescott, 2014). Physical inactivity appears to be an independent and strong risk factor for accumulation of visceral fat, which is more inflamed than other fat, and is a source of systemic inflammation (Pedersen, 2009). Exercise offers anti-inflammatory effects and can therefore protect against NCD; similarly, n-3 PUFA status may also, to some extent, be ascribed to improving the capacity to resolve chronic inflammation (Li, 2015; Minihane *et al.*, 2015; Pedersen, 2009).

Inflammation is a defence mechanism that protects the host / body / organism against infections, pathogens and other physical insults (Calder, 2010). High intensity exercise can activate an inflammatory response via muscle tissue damage and / or oxidative stress, as a result of increased production of reactive oxygen species (ROS) (Cabral-Santos *et al.*, 2015; Jackson *et al.*, 2016; Markworth *et al.*, 2013; Powers *et al.*, 2011).

Trained individuals have a lower level of oxidative damage (lipid peroxidation) than their sedentary counterparts when subjected to the same relative exercise intensity (Powers & Jackson, 2008; Powers *et al.*, 2011). This is a known result of exercise adaptation. Exercise adaptation is characterised by an up-regulation of anti-oxidative mechanisms (Powers & Jackson, 2008; Powers *et al.*, 2011). Irrespective of the cause of inflammation, the inflammatory response involves the release of various mediators, including lipid-derived mediators from muscle and immune cells at the site of inflammation (Calder, 2010; Pedersen,

2009; Pedersen & Febbraio, 2008). Changes in lipid mediator concentrations are detectable in the peripheral blood and urine (Malan *et al.*, 2016; Tsikas, 2016). Furthermore, associated inflammatory signalling (e.g. nuclear factor kappa β [Nfκβ] and tumor necrosis factor α [TNFα]), lipid mediator signalling and biosynthesis (e.g. cyclooxygenase and lipoxygenase), as well as oxidative stress–related enzymes gene expression (e.g. superoxide dismutase and glutathione peroxidase), can be detected in peripheral blood mononuclear cells (PBMC) (Malan, 2016; Pingitore *et al.*, 2015).

Lipid mediators are locally produced molecules, derived mostly from the long chain polyunsaturated fatty acids (LCPUFA) (Buckley *et al.*, 2014), mostly from arachidonic acid (AA; 20:4n-6), eicosapentaenoic acid (EPA; 22:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (Buckley *et al.*, 2014), in the phospholipid bilayer of cells upon inflammatory stimuli (Calder, 2010). The membrane LCPUFA composition of the cells, in turn, is largely dependent on the dietary intake and to a lesser extend the endogenous synthesis (desaturation and elongation) of LCPUFAs. Linoleic acid (LA, 18:2n-6, an essential omega 6 (n-6) LCPUFA) and alpha-linolenic acid (ALA, 18:3n-3, an essential omega 3 (n-3) LCPUFA), are largely dependent on intake, whereas AA, EPA and DHA can be endogenously synthesised from LA and ALA, as well as taken in as part of the diet (Calder, 2015; Innes & Calder, 2018). These fatty acids (LCPUFAs, AA, EPA and DHA) in particular, influence the biosynthesis of lipid mediators (Calder, 2010). Thus, the fatty acid composition of the cell membrane phospholipid bilayer serves as a reservoir for lipid mediators and directly affects cell signalling.

Lipid mediators generally play a role in host defence, but if produced inappropriately, they can cause damage to host tissues (Buckley *et al.*, 2014; Calder, 2007; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). Lipid mediators are also known as eicosanoids and can regulate inflammation by eliciting either a pro-inflammatory or anti-inflammatory response

(Buckley *et al.*, 2014; Calder, 2007). Lipid-mediators derived from n-6 LCPUFA (mainly AA, e.g. prostaglandins and leukotrienes) are mostly pro-inflammatory, whilst the lipid mediators derived from n-3 LCPUFA (EPA and DHA, e.g. neuroprotectin D1) are more anti-inflammatory by means of their resolving ability (Serhan, 2017b). Among the first signalling events after infection or tissue injury, is the release of pro-inflammatory lipid-mediators that stimulate a series of signalling cascades with the main aim to destroy the invading pathogens and repair the damaged tissue (Calder, 2010; Serhan *et al.*, 2017a). Consequently, in a time-dependant manner, lipid mediators that signal for the termination of inflammation are released (Serhan *et*

al., 2017a). Changes in lipid mediator concentrations are detectable in the peripheral blood and urine (Malan, 2016; Tsikas, 2016).

Resistance exercise activates an inflammatory response via the increased production of ROS and / or muscle tissue damage (Powers *et al.*, 2011). The magnitude of this response is dependent on the intensity and duration of the Resistance exercise stimulus, as well as the physical training status of the individual (Jackson *et al.*, 2016; Neto *et al.*, 2011; Pedersen, 2009; Seifi-skishahr *et al.*, 2016). Regular moderate exercise training has been shown to produce an optimal amount of ROS to stimulate an adaptive response and has consistently been shown to up-regulate anti-oxidative capacity by increasing antioxidant enzymes in skeletal muscle via up-regulated gene expression (Falone *et al.*, 2010; Powers *et al.*, 2011; Seifi-skishahr *et al.*, 2016). Furthermore, trained individuals have a lower level of oxidative damage (lipid peroxidation) than their sedentary counterparts when subjected to the same relative work-load (Powers *et al.*, 2011). A study by Falone *et al.* (2010) has demonstrated that even amateur runners, representing the average man in the street, interested in achieving and maintaining good fitness levels for health purposes, have an enhanced antioxidant defence system in response to an acute bout of exercise compared to untrained controls.

Oxidative stress has been defined as “an imbalance between pro-oxidants and antioxidants in favour of the oxidants, leading to a disruption of redox signalling and control, and / or molecular damage” (Powers *et al.*, 2011; Sies & Jones, 2007). Furthermore, an optimal antioxidant status is also associated with the formation of less inflammation. An antioxidant is defined as a substance that can lower oxidative damage caused by (amongst other) ROS (Knez *et al.*, 2006; Niess, 2007; Powers & Jackson, 2008; Steinbacher & Eckl, 2015). Endogenous enzymatic antioxidants include: catalase (Zhao *et al.*), superoxide dismutase (Shelton & Kumar, 2010) and glutathione peroxidase (GPX), whereas non-enzymatic antioxidants include: glutathione (GHS), bilirubin, uric acid. (Powers & Jackson, 2008; Steinbacher & Eckl, 2015; Urso & Clarkson, 2003). Dietary micronutrients and / or micronutrient supplements with pro- or anti-oxidative and / or pro- or anti-inflammatory properties mainly include iron, vitamin C, vitamin E, zinc, vitamin D, selenium and carotenoids (e.g. the precursors of vitamin A) (Knez *et al.*, 2006; Powers & Jackson, 2008; Steinbacher & Eckl, 2015; Urso & Clarkson, 2003).

1.3 Problem statement

It has been well established that resistance exercise can induce the production of ROS, as well as inflammation and, consequently, cause muscle damage (Davis *et al*., 2007; Malm, 2001; Markworth *et al*., 2013). Limited data is available on the lipid mediator profiles in response to unaccustomed resistance exercise. Furthermore, it is not clear if aerobic exercise training induces adaptation in terms of inflammation-resolving capacity independent of anti-oxidative adaptation, and whether the proposed adaptation is modulated by fatty acid and micronutrient intake and status. These questions are important in the global health context for addressing communicable and NCD's associated with perpetuating inflammation, with sustainable intervention strategies with impact. The *in vivo* model of acute inflammation through exercise stimuli in the muscle is relevant for the fact that skeletal muscle is a major source of a number of inflammatory signalling molecules, including cytokines released in response to inflammatory stimuli (Neto *et al.*, 2011; Pedersen, 2009).

1.4 Research aim and objectives

The aim of the study was to compare exercise-induced lipid mediator inflammatory signalling in male recreational runners with untrained males, considering potential modulating factors such as blood fatty acid and antioxidant status.

The primary objectives were, in male recreational runners (n=20) and untrained male controls (n=20), to:

- Measure the plasma lipid mediator concentrations (17-hydroxydocosahexaenoic aid (HDHA), 5- and 12-hydroxyeicosapentaenoic acid (HEPE), 5-, 8-, 11-, and 12- hydroxyeicosatetraenoic acid (HETE) and prostaglandin (PG) PGE_2) pre and periodically post an unaccustomed resistance exercise session.
- Measure markers of oxidative stress status, namely ROS, ferric reducing antioxidant power (FRAP) and glutathione (GSH) levels before an unaccustomed resistance exercise session.
- Assess the red blood cell fatty acid status and habitual antioxidant micronutrient intake, specifically of vitamin C, E, total carotenoids and zinc, before an unaccustomed resistance exercise session.

- Compare the plasma lipid mediator response to acute unaccustomed resistance exercise between the two groups when controlling for potential confounding factors, including fatty acid and oxidative stress status, as well as antioxidant micronutrient intake.

1.5 Ethics approval

Ethical approval was obtained for the study from the Health Research Ethics Committee (HREC) of the Faculty of Health Sciences of the North West University, Potchefstroom (NWU-00061-16-A1). Participants signed indemnity forms and the study was insured by the NWU.

1.6 Study team

The members of the research team and their roles and expertise are listed in Table 1-6. None of the team members had a conflict of interest.

Table 1-1: Study team members and their roles and expertise

Affiliation	Name	Role	Relevant Expertise
North-West University	Erika Bezuidenhout (Botes)	MSc student, data collection of 25 participants, lipid mediator analysis, quantitation of lipid mediator analysis, quantitation of fatty acid analysis, coding and capturing of 3-day dietary data, data quality control, statistics, writing of dissertation.	Honours in Nutrition and completed honours project in the Immunex study where she assisted with data collection (13 participants).

Affiliation	Name	Role	Relevant Expertise
North-West University	Dr. Linda Malan	Master student supervisor and Principal Investigator of the study	Essential fatty acid and micronutrient nutrition, role of essential fatty acids and iron in immune development and functioning, community-based clinical trials, laboratory specialist, lipid mediator analysis
North-West University	Dr. Lizelle Zandberg	Master student co-supervisor, Investigator and post-doctoral fellow and investigator in the study	Biochemist and antioxidant status analysis.
North-West University	Arista Nienaber	Master student co-supervisor, Investigator in the study	Sport nutrition expert and Registered Dietician
North-West University	Dr. Cindy Pienaar	Team member, exercise manipulation expert.	Sport Scientist, Exercise Teachers Academy (ETA) personal trainer and International Society for the Advancement in Kinanthropometry (ISAK) level 2 Anthropometrist
North-West University	Dr. Chrisna Botha-Ravyse	Team member, exercise manipulation expert.	Registered Biokineticist and qualified ISAK level 3 Anthropometrist
North-West University	Marike Cockeran	Biostatistician	Statistics and data analysis

CHAPTER 2 LITERATURE REVIEW

2 LITERATURE REVIEW

2.1 Inflammation and inflammatory signalling responses

2.1.1 Inflammation

Inflammation can be defined as the body's normal defence mechanism that reacts to injury or infection, as well as to excessive reactive oxygen species (ROS), by restoring homeostasis at the infected site (Calder, 2006a; Calder, 2006b; Calder, 2007; Calder, 2010; Minihane *et al.*, 2015; Rangel-Huerta *et al.*, 2012; Sorci & Faivre, 2009; Walsh *et al.*, 2011). There are two types of inflammation: acute inflammation that occurs within a short period and chronic inflammation that persist over a longer period of time (Rangel-Huerta *et al.*, 2012). Acute inflammation is characterised by swelling, pain, heat and redness (Calder, 2006a; Calder, 2010). These symptoms occur because of increased permeability across blood capillaries and increase blood flow (Calder, 2006a; Calder, 2006b; Minihane *et al.*, 2015). Inflammation is known to be closely related to oxidative stress (Hensley *et al.*, 2000; Pingitore *et al.*, 2015).

A person's immune system protects him from environmental infections agents such as bacteria, viruses, parasites. (Calder, 2007). This system consists of the innate immune system (inflammation) and the adaptive immune system (Calder, 2007). The origin of immune cells is in the bone marrow and they can be found circulating in the blood, assembled in lymphoid organs or spread out in other areas (Calder, 2007). Monocytes, granulocytes, and macrophages are the initial cells that move to the inflamed sites; these cells play a role in the killing of pathogens; cleaning cellular waste and tissue repair (Calder, 2006a; Calder, 2006b; Calder, 2007; Minihane *et al.*, 2015; Rangel-Huerta *et al.*, 2012).

2.1.2 The inflammatory response and the resolution of inflammation

Wherever inflammation does occur, it is well regulated (Calder, 2006b; Calder, 2010). Regardless of the cause of inflammation, the response usually includes four big events, namely:

- a rise of blood supply to the inflamed site (Calder, 2006a; Calder, 2006b; Calder, 2007; Minihane *et al.*, 2015; Rangel-Huerta *et al.*, 2012; Serhan & Petasis, 2011);

- an increase of capillary permeability, that is triggered by the withdrawal of endothelial cells (Calder, 2006a; Calder, 2006b; Calder, 2007; Calder, 2010; Minihane *et al.*, 2015; Rangel-Huerta *et al.*, 2012; Serhan & Petasis, 2011) that allows larger molecules to transfer through the endothelium to deliver soluble mediators to the inflamed site (Calder, 2010);
- the movement of leukocytes from the capillaries into the adjacent tissue (Calder, 2006b; Calder, 2007; Calder, 2010; Minihane *et al.*, 2015; Rangel-Huerta *et al.*, 2012; Serhan & Petasis, 2011), which is encouraged by the release of chemo-attractants from the inflammation site and the up-regulation of the endothelium's connection molecules (Calder, 2010); thus, when the leukocytes are in the tissue, they move to the inflamed site (Calder, 2010);
- the release of mediators from the leukocytes at the inflamed site (Calder, 2006b; Calder, 2007; Calder, 2010; Minihane *et al.*, 2015; Rangel-Huerta *et al.*, 2012; Serhan & Petasis, 2011), including peptide mediators (e.g. cytokines); amino acid derivatives (e.g. histamine); enzymes (e.g. matrix proteases); ROS (e.g. superoxide) and lipid-mediators (e.g. leukotrienes (LTs) and prostaglandins (PGs), depending on the nature of the inflammatory stimulus, cell type involved, the stage during the response to inflammation and the anatomical site involved (Calder, 2006b; Calder, 2007; Calder, 2010; Minihane *et al.*, 2015; Rangel-Huerta *et al.*, 2012; Serhan & Petasis, 2011).

The resolution of inflammation can be explained as follows: Resolution is known as the rate of polymorphonuclear (PMN) leukocyte clearance until they are absent from the site of injury (Buckley *et al.*, 2014). One of the first events that occur after tissue damage, is the release of pro-inflammatory lipid mediators, such as prostaglandins (PGs) and leukotrienes (LTs), which start a sequence to eventually destroy the invading pathogens and restore damaged tissue (Buckley *et al.*, 2014; Serhan & Petasis, 2011). Therefore, the production and release of leukotriene B_4 (LTB_4) increase the attraction of neutrophils to the inflamed sites, while the synthesis of prostaglandins E_2 and D_2 (PGE_2 and PGD_2) further speeds up the inflammatory process, finally resulting in acute inflammation (Buckley *et al.*, 2014; Serhan & Petasis, 2011).

Irrespective of its protective function, if acute inflammation is not resolved adequately, it can give rise to chronic inflammation that may eventually contribute to a wide range of diseases (Buckley *et al.*, 2014; Serhan & Petasis, 2011). Normally, the pharmacological treatment of chronic inflammation involves the inhibition of pro-inflammatory mediators via COX-2 pathway,

but this is not always very effective (Serhan & Petasis, 2011). Therefore, a novel treatment method, using anti-inflammatory and / pro-resolving lipid-mediators may be useful (Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). *In vivo*, switching lipid mediator class from the original pro-inflammatory lipid mediators to the anti-inflammatory and pro-resolving actions of protectins, resolvins, lipoxins, and maresins, takes place as a natural process (Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). Finally, through the combination of the actions of these mediators, inflammation can be resolved and homeostasis reached (Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). This process is illustrated in **Figure 2-1**.

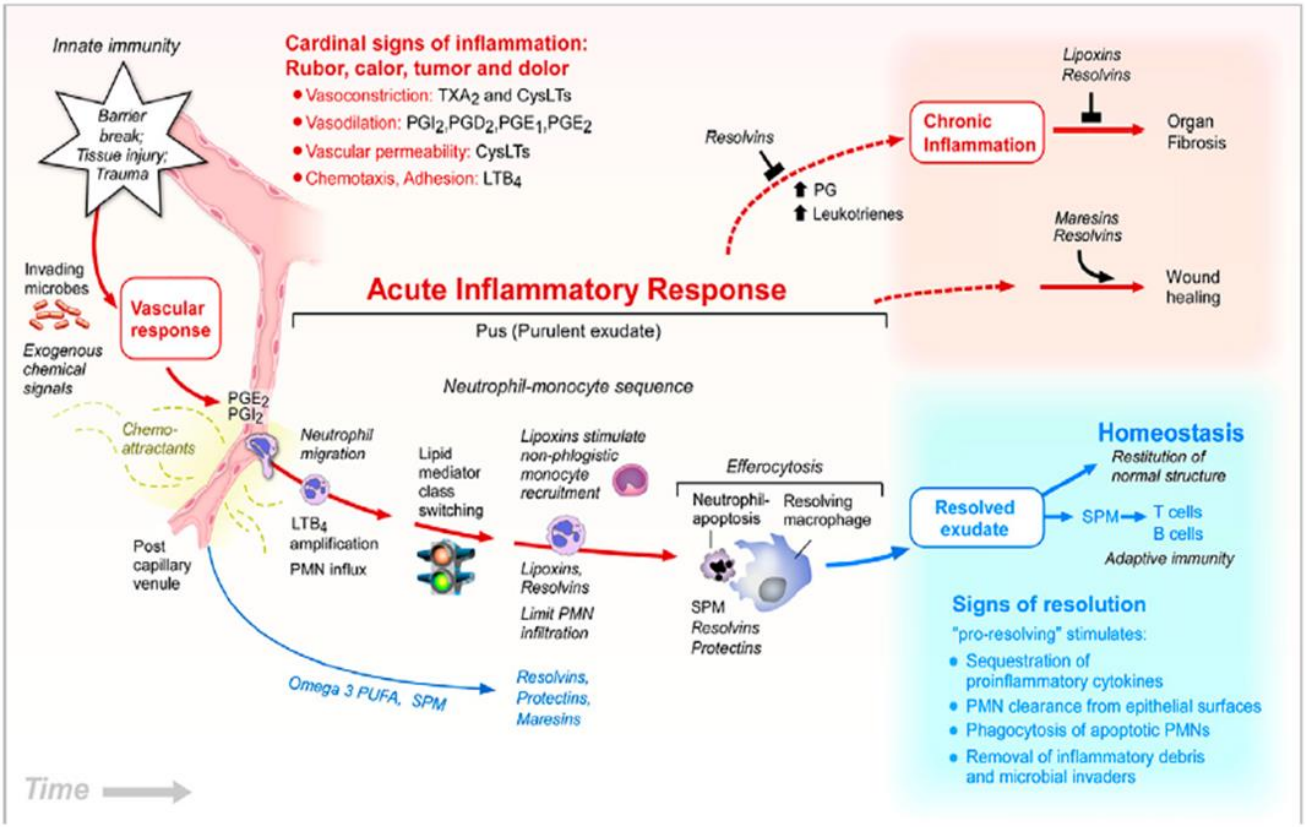

Figure 2-1: The resolution of acute inflammation (Serhan, 2017b)

Pro-resolving mediators and eicosanoids in initiation and resolution of inflammation. Prostaglandin E2 (PGE_2) and PGI_2 allow neutrophils to travel along a gradient of leukotriene B4 (LTB4). A lipid mediator class switching then occurs and lipoxins stimulate monocyte recruitment. Lipoxins, resolvins and other specialised pro-resolving mediators are generated to limit or stop further neutrophil tissue infiltration. Resolving macrophages and neutrophils also produce specialised pro-resolving mediators. Specialised pro-resolving mediators stimulate the signs of resolution. Resolvins and specialised pro-resolving mediators block chronic inflammation (Serhan, 2017b).

2.1.3 Inflammatory signalling and the health impact of inflammation

Even though the inflammatory response is protective, failing to resolve inflammation can result in systematic and chronic inflammatory disorders / conditions (Serhan & Petasis, 2011). These disorders / conditions include, but are not limited to, diabetes, cardiovascular diseases, cancer, obesity, asthma, rheumatoid arthritis and neurodegenerative diseases (Calder, 2006a; Calder, 2006b; Rangel-Huerta *et al.*, 2012). Research have shown that people who have diseases such as rheumatoid arthritis, asthma and inflammatory bowel diseases, have very high infiltration of inflammatory cells where the disease occurs (Calder, 2015). They also have an increased concentration of inflammatory mediators at the location of the disease, as well as in their systemic circulation (Calder, 2015). This is then treated with anti-inflammatory drugs (Calder, 2015). Other research showed that people with atherosclerosis and obesity, have infiltration of inflammatory cells at the blood vessel walls of the adipose tissue, whilst the inflammatory mediators concentration is moderately increased in their systemic circulation (Calder, 2015). Nonetheless, these people were not treated with anti-inflammatory drugs per se (Calder, 2015).

2.2 The role of phospholipid fatty acids in the inflammatory process

2.2.1 Phospholipid fatty acids

Hydrocarbons that have a terminal carboxyl group, are called fatty acids (Burdge & Calder, 2015; Edwards & O'Flaherty, 2008). Based on the level of saturation of these hydrocarbon chains, saturated fatty acids, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) are distinguished (Edwards & O'Flaherty, 2008). The most common fatty acids have straight chains with even numbers of carbon atoms (Burdge & Calder, 2015). The chain lengths can differ from four to 30 carbons and some contain double bonds (Burdge & Calder, 2015). Single bonds connect the carbons of saturated fatty acids, while, in addition to some single bonds, one or more double bonds also connect the carbons of MUFAs and PUFAs (Burdge & Calder, 2015; Edwards & O'Flaherty, 2008).The physical properties of a fat are determined by the length of the chain and degree of unsaturation (Burdge & Calder, 2015). An unsaturated fatty acid is named according to the location of the first double bond closest to the omega carbon, for example, n-3 fatty acids have the first double on the third carbon relative to methyl end of the molecule, and n-6 fatty acids have the first double bond on the sixth carbon from the methyl end of the carbon chain (**Figure 2-2**) (Burdge & Calder, 2015;

Edwards & O'Flaherty, 2008). Similarly oleic acid (18:1) has its double bond between carbons nine and 10, and is therefore named n-9 or omega-9 MUFA (Edwards & O'Flaherty, 2008). The human diet contains a large diversity of fatty acids (Burdge & Calder, 2015). Omega-3 and -6 PUFAs are considered essential as they cannot be produced by the human body and therefore need to be consumed through the diet (Burdge & Calder, 2015). Oils that contain PUFAs, especially n-3 and n-6, are fatty fish, fish oils, vegetable oils, soybean oil (Burdge & Calder, 2015).

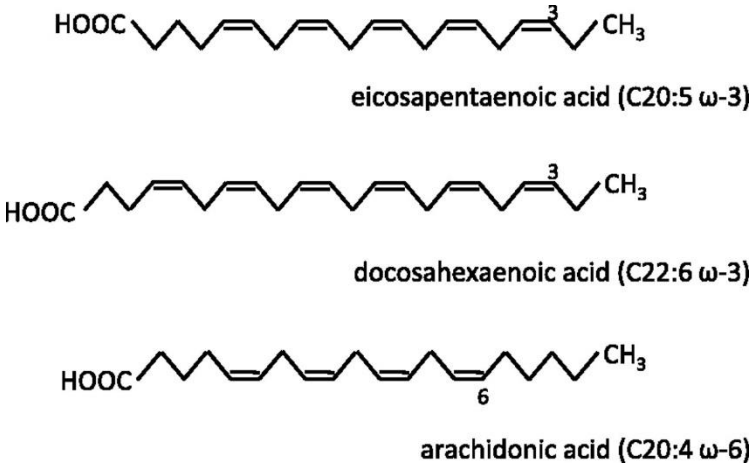

Figure 2-2: Structures of some fatty acids (Cockbain et al., 2012)

Structure and classification of polyunsaturated fatty acids (PUFAs). Cx:y u-z signify the chemical structure where x is the number of carbon atoms, y is the number of carbone carbon double bonds and z is the location of the first carbone carbon double bond away from the methyl (u) end of the hydrocarbon chain. Note that all double bonds in these fatty acids are in the cis formation (Cockbain *et al.*, 2012).

2.2.2 Lipid mediators

Biomarkers of inflammation can be described as chemical mediators of inflammation and these consist of eight groups, of which lipid mediators is one (Rangel-Huerta *et al.*, 2012). Lipid mediators are hormone-like molecules, derived mostly from long-chain PUFA (LCPUFA), that regulate the inflammatory response (Calder, 2006a; Calder, 2006b; Fullerton *et al.*, 2014; Rangel-Huerta *et al.*, 2012). Lipid mediators are key mediators of immunity and inflammation (Calder, 2010) and can essentially turn the inflammatory response on or off. They are also described as a variety of bioactive autocrine- or paracrine-signalling molecules that are metabolised from n-3 and n-6 fatty acids (Fullerton *et al.*, 2014; Markworth *et al.*, 2013). The phospholipid fatty acids that produce the main bioactive lipid mediators, are eicosapentaenoic

acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) (Smith, 1989). Pro-inflammatory eicosanoids are generally produced from AA, whilst anti-inflammatory and / pro-resolving eicosanoids are generally produced from EPA and DHA (Calder, 2006a; Calder, 2006b; Calder, 2007; Calder, 2010). Eicosapentaenoic acid and DHA are both n-3 polyunsaturated fatty acids, synthesised from the essential fatty acid. alpha-linolenic acid (ALA) (Masoodi *et al.*, 2008). The series-3-prostanoids, hydroxyeicosapentaenoic acids (HEPE) and 5-series leukotrienes are all EPA derived mediators, whereas neuroprostanes,17-hydroxy DHA (17-HDHA), protectins, maresins and resolvins are DHA derived mediators (Masoodi *et al.*, 2008; Serhan & Petasis, 2011). Arachidonic acid is synthesised from the essential n-6 fatty acid, linoleic acid (LA) (Masoodi *et al.*, 2008). Arachidonic acid is the precursor of lipid mediators such as hydroxyeicosatetraenoic acids (HETEs), thromboxanes, prostaglandins, 4-series leukotrienes and f_2- isoprostanes (Malan, 2016; Masoodi *et al.*, 2008; Nelson *et al.*, 1997).

2.2.3 The pathways of lipid mediators biosynthesis from arachidonic acid

The production of lipid mediators from AA is demonstrated in **Figure 2-3**. Arachidonic acid is cleaved off the cell membrane by the enzyme phospholipase A_2 (PLA_2) and is then metabolised by the cyclooxygenase (Sellayah *et al.*) pathway and lipoxygenase (LOX) pathway (**Figure 2-3**).

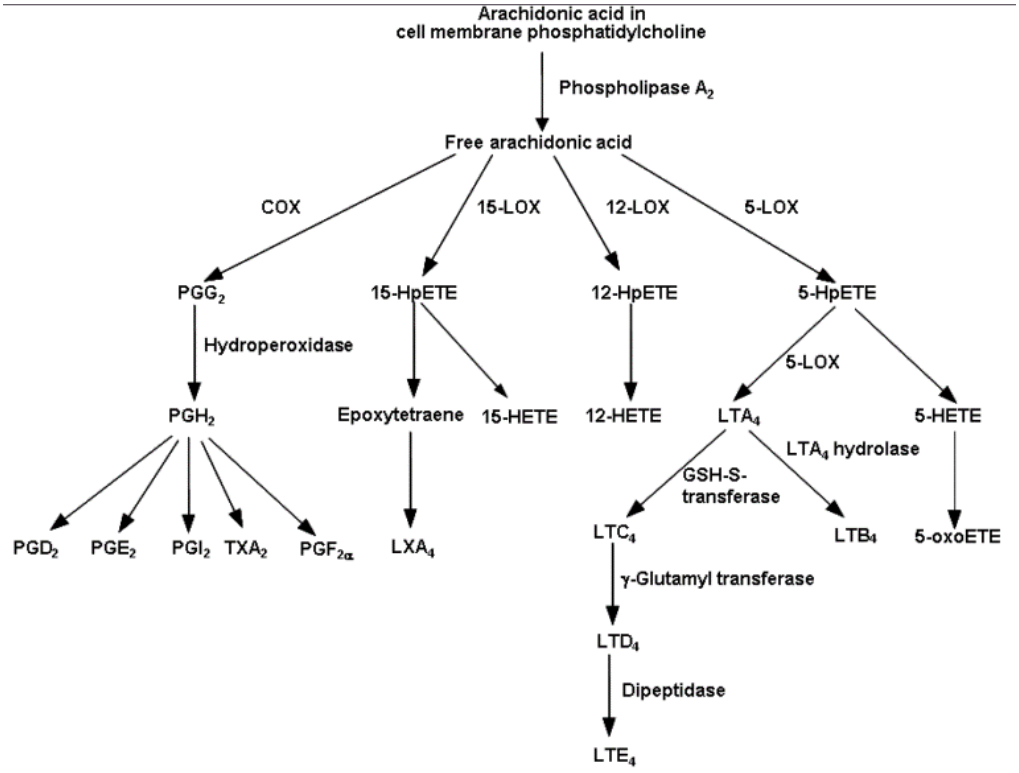

Figure 2-3: The pathways of eicosanoids biosynthesis of arachidonic acid (Calder, 2010)

The outline of the pathway for eicosanoid production from arachidonic acid. Cyclooxygenase (Sellayah *et al.*) , lipoxygenase (LOX), hydroxyeicosatetraenoic acid (HETE), hydroperoxyeicosatetraenoic acid (HpETE), leukotriene (LT), lipoxin (LX), prostaglandin (PG), thromboxane (TX) (Calder, 2010).

The LOX pathway generates hydroperoxyeicosatetraenoic acids (HPETEs) which are metabolised to hydroxyeicosatetraenoic acids (HETEs) [5-HETE, 8-HETE, 12-HETE and 15-HETE] as well as leukotriene A_4, which, in turn, is metabolised to the peptide leukotrienes [LTC_4, LTD_4, LTE_4], leukotriene B_4 and lipoxins (**Figure 2-3**). The COX pathway gives rise to the prostanoids, which are prostaglandins and thromboxanes [PGD_2, PGE_2, PGF_2, PGI_2, TxA_2] (**Figure 2-3**) (Calder, 2006a; Calder, 2006b; Calder, 2010; Masoodi *et al.*, 2008; Smith, 1989).

2.2.4 The pathways of lipid mediator biosynthesis from Eicosapentaenoic acid and Docosahexaenoic acid.

The production of lipid mediators from EPA and DHA is demonstrated in **Figure 2-4**. Eicosapentaenoic acid is cleaved off the cell membrane by the enzyme PLA_2 and is then metabolised by the COX-2, LOX or free radical catalysed pathways (Calder, 2010; Fredman

& Serhan, 2011; Masoodi *et al.*, 2008; Serhan & Petasis, 2011). The COX-2 pathway generates mediators including 18(R)-HEPE which is further broken down by the LOX-5 pathway to the E-series resolvins (RvE) (Calder, 2010; Fredman & Serhan, 2011; Masoodi *et al.*, 2008; Serhan & Petasis, 2011). A series of HEPEs is produced from free radical oxidation pathway which includes 5-, 8-, 9-, 12-, 15-, and 18-HEPE. From the LOX pathway LTB_5 and 5-, 8-, 12- and 15-HEPE is formed (Calder, 2010; Fredman & Serhan, 2011; Masoodi *et al.*, 2008; Serhan & Petasis, 2011).

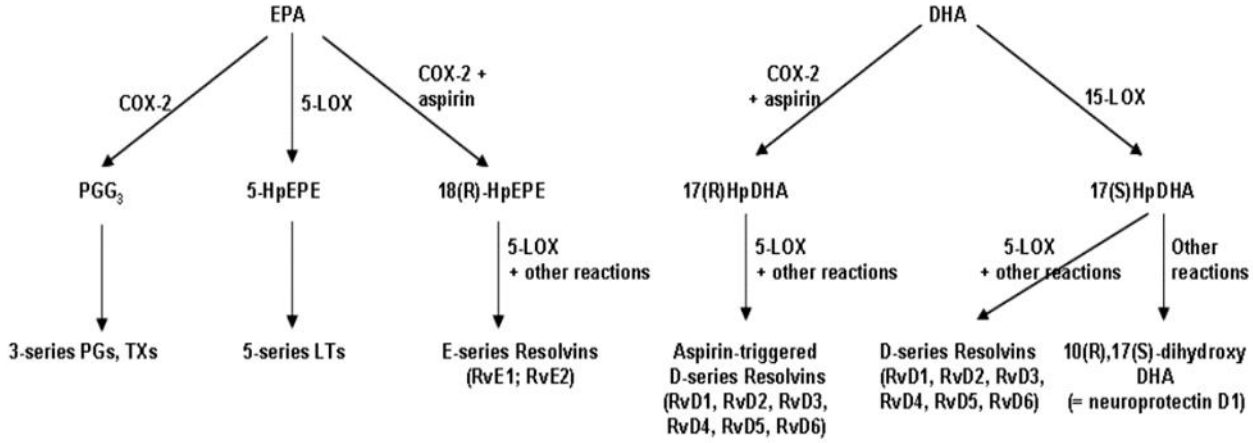

Figure 2-4: The pathways of lipid mediator biosynthesis from eicosapentaenoic acid and docosahexaenoic acid (Calder, 2010)

Outline of the pathway for production of resolvins and related mediators from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Cyclooxygenase (COX), lipoxygenase (LOX), hydroperoxyeicosatetraenoic acid (HpETE), leukotriene (LT), lipoxin (LX), prostaglandin (PG), thromboxane (TX) hydroperoxydocosahexaenoic acid (HpDHA) and resolving (Rv) (Calder, 2010).

Docosahexaenoic acid (DHA) is cleaved off the phospholipid bilayer of the cell membrane by the enzyme PLA_2 and is then metabolised by the LOX pathway (Calder, 2010; Fredman & Serhan, 2011; Masoodi *et al.*, 2008; Serhan & Petasis, 2011). This pathway produces 17(S)-HDHA or 17-HDHA that is further metabolised to form the D-series resolvins (RvD) and protectins (PD) (Calder, 2010; Fredman & Serhan, 2011; Masoodi *et al.*, 2008; Serhan & Petasis, 2011). This pathway also generates 17S-HDHA, which produces RvD1 (Calder, 2010; Fredman & Serhan, 2011; Masoodi *et al.*, 2008; Serhan & Petasis, 2011).

2.2.5 The balance between omega-3 and -6 PUFA and its role in inflammation

The balance between n-3 and n-6 fatty acids is significant since mediators derived from AA (n-6) are mainly pro-inflammatory, whilst mediators derived from EPA and DHA (n-3) are mainly anti-inflammatory and pro-resolving (Pradelli *et al.*, 2012). Some of these anti-inflammatory effects of n-3 include: decrease of AA-derived eicosanoids; decrease in ROS production and increase in EPA and DHA mediators (resolvins and protectins) (Calder, 2010). AA-derived eicosanoids normally exert a pro-inflammatory action (Calder, 2010), for instance, PGE_2 can induce fever, enhance pain, increase vasodilation and vascular permeability, and enhance oedema (Calder, 2006a; Calder, 2006b). However, PGE_2 is also recognised to have anti-inflammatory effects by inhibiting the LOX pathway and, therefore; decreasing the 4-series leukotriene production (Calder, 2006a; Calder, 2006b; Calder, 2010). Lipoxin A_2, derived from AA, is also recognised to be anti-inflammatory (Calder, 2006a; Calder, 2006b; Calder, 2010). Arachidonic acid can be partially replaced by EPA and DHA in the phospholipid bilayer of membranes, consequently leading to less inflammation (Toft *et al.*, 2000). In addition, an increased consumption of n-3 PUFA such as EPA and DHA, in itself also has anti-inflammatory and pro-resolving effects (Calder, 2006a; Calder, 2006b; Calder, 2010; Masoodi *et al.*, 2008; Serhan, 2017b; Serhan *et al.*, 2017a). Therefore, EPA and DHA also give rise to resolving compounds, which are important because it can resolve the on-going inflammation and; therefore, limit tissue damage that is caused by inflammation (Calder, 2010; Serhan, 2017b; Serhan *et al.*, 2017a). EPA and DHA mediators or pro-resolving mediators act to resolve inflammation by terminating the infiltration of neutrophils and the stimulation of the macrophage function (Serhan *et al.*, 2017a). Fatty acids are usually part of the diet and can be saturated or unsaturated (Calder, 2012).

Apart from PUFA directly influencing signalling through lipid mediators, they can also influence the inflammatory process by means of the following mechanisms (Calder, 2010):

- The intake of PUFAs has an influence on lipoproteins, complex lipids, hormone concentrations and metabolite concentrations which then influence inflammation;
- non-esterified PUFAs are able to act directly on inflammatory cells via intracellular fatty acid receptors;
- PUFAs can be oxidised and then act directly on inflammatory cells; and
- PUFAs can be incorporated into the inflammatory cell membranes (Calder, 2010; Calder, 2012).

2.3 The role of oxidative stress markers in the inflammatory process

2.3.1 The link between oxidative stress markers and inflammation

Numerous studies show that inflammation and oxidative stress have an interdependent relationship (Biswas, 2016; Castellani *et al.*, 2014). It is known that oxidative stress and inflammation are connected to several chronic diseases, including cardiovascular diseases, hypertension, diabetes, neurodegenerative diseases, chronic kidney disease, alcoholic liver disease, ageing and cancer (Biswas, 2016). Inflammatory cells (e.g. macrophages, PMNs, and mast cells) produce and release several ROS at the location of inflammation that can lead to excessive oxidative stress (Biswas, 2016). Throughout the inflammatory process, the activated phagocytic cells, such as macrophages and neutrophils, produce large amounts of ROS and reactive nitrogen, including hydroxyl free radical, hydrogen peroxide, superoxide, hypochlorous acid, peroxynitrite and nitric oxide, to kill the invading agents (Biswas, 2016). Under uncontrolled inflammatory conditions, there may be excessive generation of ROS that cause localised oxidative stress and tissue injury (Biswas, 2016). In return, oxidative stress itself can also cause inflammation through activation of several pathways (Biswas, 2016). **Figure 2-5** represents this close and interdependent relationship between inflammation and oxidative stress, even though the order of events is not quite so simple (Biswas, 2016). If the main abnormality in an organ is oxidative stress, ultimately, inflammation will develop, which in turn, will further increase oxidative stress (Biswas, 2016). Thus, the identification of the main abnormality can clinically be very important, because the treatment of the main disorder is likely to ensure a constant relief from the problem (Biswas, 2016).

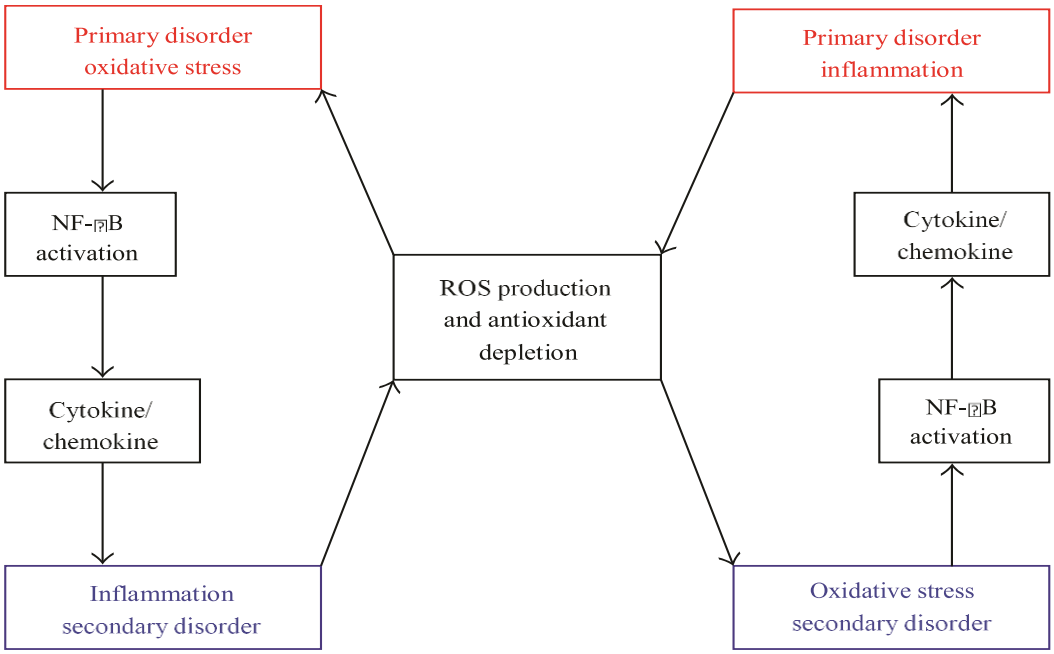

Figure 2-5: Overview of interdependence between oxidative stress and inflammation (Biswas, 2016)

Summary of the interdependence between inflammation and oxidative stress. When oxidative stress appears as a main disorder, inflammation progresses as a secondary disorder and further increases oxidative stress. However, when inflammation occurs as the main disorder, it can generate oxidative stress as a secondary disorder which, in turn, can further increase inflammation (Biswas, 2016).

2.3.2 The chemistry of oxidative stress markers and reactive oxygen species (ROS)

Oxidative stress can be defined as an imbalance between free radicals and anti-oxidative mechanisms, or as the excessive production of ROS (Betteridge, 2000; Bloomer *et al.*, 2009; Durackova, 2010; Hensley *et al.*, 2000; Pingitore *et al.*, 2015; Storz & Imlayt, 1999; Turrens, 2003). Research has shown that excessive oxidative stress is associated with many chronic diseases such as cancer, diabetes mellitus, Alzheimer's, atherosclerosis, Parkinson's disease and inflammatory disease (Durackova, 2010; Pingitore *et al.*, 2015; Storz & Imlayt, 1999).

Free radicals are chemical species that has one or more unpaired electrons in their outer orbital, making them unstable and; therefore, very reactive (Betteridge, 2000; Turrens, 2003). For this reason, free radicals react with nearby molecules, accepting or donating electrons, to become more stable (Betteridge, 2000). Most molecules exist as non-radicands (not free radicals) (Betteridge, 2000). When a free radical reacts with a non-radicand, it produces a free radical chain reaction, forming new free radicals (ROS), which can then further react with other macromolecules (Betteridge, 2000). Such free radical chain reactions can be caused by an uncontrolled elevation in oxidant concentrations (Turrens, 2003).

Free radicals are formed in large amounts as an inescapable product of normal biochemical processes (Betteridge, 2000). They are mostly obtained from chain reactions (described

above) which are triggered by processes that produce basic radical molecules such as superoxide O_2 or nitroxide (NO) (Durackova, 2010). A number of such normal; biochemical processes in the body that produce free radicals, include:

- the reduction of molecular oxygen during aerobic respiration, producing hydroxyl radicals and superoxide;
- the reduction of molecular oxygen to superoxide through the oxidation of catecholamines and the beginning of the AA cascade product electrons;
- the production of hypochlorous acid (HOCl) - a powerful oxidant- and superoxide through the activation of phagocytes; and finally
- vascular endothelium and other cells that produce NO (Betteridge, 2000).

In other words, these reactive oxygen intermediates, superoxide O_2 or NO, can cause oxidative stress (Storz & Imlayt, 1999).

Reactive oxygen species are, thus, a variety of free radicals and molecules which are obtained from molecular oxygen (Turrens, 2003); as part of metabolic processes, ROS and free radicals are constantly produced by cells (Urso & Clarkson, 2003). Under normal circumstances, ROS are used as second messengers to spread growth stimulatory molecular species or pro-inflammatory signals (Hensley *et al.*, 2000). Excessive ROS producing sources include the mitochondrial electron transport chain (ETC) or the production of ROS by xanthine oxidase (Dröge, 2002). Excess production of ROS can lead to the damaging of lipids, nucleic acids, deoxyribonucleic acid and proteins (Pingitore *et al.*, 2015; Thannickal & Fanburg, 2000).

2.3.3 Antioxidant defence mechanisms

An antioxidant is defined as a substance that can lower the oxidative damage that oxidative stress produces, and include both exogenous (dietary) and endogenous (enzymatic and non-enzymatic) antioxidants (Knez *et al.*, 2006; Niess, 2007; Powers & Jackson, 2008; Steinbacher & Eckl, 2015). Antioxidants can reduce oxidative stress through either quenching the damaging free radical chain reaction, or by generating a less active radical (Knez *et al.*, 2006). These antioxidants contribute to protect against ROS that are formed during oxidative stress producing circumstances such as exercise (Powers & Jackson, 2008; Steinbacher & Eckl, 2015). Enzymatic endogenous antioxidants include: catalase (Zhao *et al.*), superoxide dismutase (SOD), and glutathione peroxide (GPX), whereas non-enzymatic endogenous

antioxidants include: glutathione (GSH) (Knez *et al.*, 2006; Niess, 2007; Powers & Jackson, 2008; Steinbacher & Eckl, 2015; Urso & Clarkson, 2003). Dietary antioxidants include but are not limited to: vitamin C, vitamin E, vitamin D, zinc, selenium and carotenoids (Knez *et al.*, 2006; Powers & Jackson, 2008; Steinbacher & Eckl, 2015; Urso & Clarkson, 2003). It has been shown that a low dietary intake of antioxidants can decrease an athlete's capability to detoxify exercise-induced ROS, leading to increased levels of oxidative stress (Slattery *et al.*, 2015). Conversely, research has shown that the intake of antioxidant supplements can reduce oxidative damage and markers of inflammation (Slattery *et al.*, 2015).

2.3.3.1 Dietary antioxidants

2.3.3.1.1 Vitamin C

Vitamin C, the reduced from of ascorbic acid, is a powerful antioxidant, which can reduce ROS (Cárcamo et al., 2004; Frei et al., 1989). This hydrophilic vitamin can directly scavenge hydroxyl, lipid hydroxide and superoxide radicals (Powers & Jackson, 2008). A vitamin C radical (semi-ascorbyl) can also be formed through a process where vitamin E is recycled, but can be transformed back to vitamin C by NADH semi-ascorbyl reductase (Powers & Jackson, 2008). Food sources high in vitamin C include: citrus foods, strawberries, lettuce, tomatoes, potatoes and mangoes (Whitney & Rolfes, 2015). The recommended daily allowance (RDA) of vitamin C is 90 mg/day for men and 75 mg/day for women, and an extra 30g for smokers due to the added ROS smoking causes (Whitney & Rolfes, 2015).

2.3.3.1.2 Vitamin E

Vitamin E acts as a peroxyl radical scavenger which limits the production of damaging free radicals in tissues, by reacting with them to produce a tocopheryl radical (Traber & Stevens, 2011). This radical will then be reduced (redox–oxidation reaction) by a hydrogen donor (such as vitamin C) and return to its reduced state (Traber & Stevens, 2011). Some studies have shown that there is a decrease in muscle vitamin E concentrations when resistance exercise is performed, whilst others reported no effect (Powers & Jackson, 2008). Food sources of vitamin E include: green leafy vegetables, wheat germ, whole grains with the endosperm included, liver, egg yolks, nuts and seeds. The RDA is 15 mg/day for adults (Whitney & Rolfes, 2015).

2.3.3.1.3 Carotenoids

Beta-carotene is another lipid-soluble antioxidant that is mainly found in the membranes of tissues (Powers & Jackson, 2008). Due to its scavenging capacity and location, it serves an important function to oppose lipid peroxidation (Powers & Jackson, 2008). Food sources rich in beta-carotene include: spinach, broccoli and orange fruits. The RDA is 900 µg/day for men and 700 µg/day for women (Whitney & Rolfes, 2015).

2.3.3.2 Enzymatic antioxidants

2.3.3.2.1 Catalase (CAT)

Catalase (CAT) can mainly be found in peroxisomes of most cells, but other intracellular organelles like the endoplasmic reticulum and mitochondria, can also contain the enzyme (Niess, 2007). The liver has the highest amount of CAT, whereas lower levels are found in the skeletal muscle (Niess, 2007). Catalase acts as an antioxidant by catalysing the conversion of hydrogen peroxide to water and oxygen (Niess, 2007).

2.3.3.2.2 Superoxide dismutase (SOD)

There are three forms of SOD's: Zn- and Cu-SOD [SOD_1] (found in the cytosol in the intracellular spaces); Mn-SOD [SOD_2] (found in the mitochondria); as well as Zn- and Cu-SOD [SOD_3] (found in the extracellular spaces) (Knez *et al.*, 2006; Niess, 2007; Powers & Jackson, 2008; Steinbacher & Eckl, 2015). The highest amounts of SOD are found in the skeletal muscle (Powers & Jackson, 2008). Superoxide is catalysed to hydroperoxide by SOD (Knez *et al.*, 2006; Niess, 2007; Powers & Jackson, 2008; Steinbacher & Eckl, 2015).

2.3.3.2.3 Glutathione peroxide (GPx)

Glutathione peroxide is found in both the mitochondria and the cytosol close to where hydrogen peroxide is formed (Niess, 2007; Powers & Jackson, 2008). Hydrogen peroxide and fatty acid peroxide are catalysed by GPx to oxidised GSH and water, and a reduced form of GSH is used as an electron donor (Knez *et al.*, 2006; Niess, 2007; Powers & Jackson, 2008). Skeletal muscle fibres contain the highest activity of GPx (Niess, 2007; Powers & Jackson, 2008).

2.3.3.3 Non-enzymatic antioxidants

2.3.3.3.1 Glutathione (GSH)

Glutathione has multiple purposes in cells (Powers & Jackson, 2008). Firstly, as mentioned above, GSH serves as a substrate for GPx to eliminate organic hydroperoxides and hydrogen peroxide (Niess, 2007; Powers & Jackson, 2008). Secondly, by donating a hydrogen atom, GSH can react directly with various radicals (Powers & Jackson, 2008). Glutathione can also reduce other antioxidants such as vitamins E and C in the cells (Powers & Jackson, 2008). Recent research showed that skeletal muscles adapt to exercise by increased GSH levels (Powers & Jackson, 2008). This can be due to the fact that a key enzyme involved in GSH synthesis, increases during exercise (Powers & Jackson, 2008).

2.4 The role of resistance exercise in oxidative stress marker and inflammation

2.4.1 Resistance exercise-induced oxidative stress (sources of ROS in muscle)

It has been well established that resistance exercise can induce the production of ROS, as well as inflammation and, consequently, cause muscle damage (Davis *et al.*, 2007; Malm, 2001; Markworth *et al.*, 2013). Contracting skeletal muscle produces free radicals (ROS) (Durackova, 2010; Niess, 2007; Powers & Jackson, 2008; Steinbacher & Eckl, 2015), leading to increased oxidative stress (Niess, 2007; Powers & Jackson, 2008; Urso & Clarkson, 2003). The intensity, duration, and mode of the resistance exercise play a key role in the amount of muscle damage; ROS and inflammation that the exercise produces (Cabral-Santos *et al.*, 2015; Davis *et al.*, 2007; Malm, 2001). According to Cabral-Santos *et al.* (2015), workload is the factor that has the greatest impact on inflammation, because workload depends on the intensity and duration of the exercise session. Exercise that involves a large component of eccentric contraction will result in more damage and inflammation compared to exercise that involves a larger concentric component at the same duration and intensity (Cabral-Santos *et al.*, 2015; Davis *et al.*, 2007; Malm, 2001; Markworth *et al.*, 2013). The production of free radicals while exercising can arise in several ways, for instance, through the use of oxygen in the mitochondria to produce energy, or through the catecholamines that are released during exercise (Urso & Clarkson, 2003). Also, exercise-induced ROS production can be caused by a leakage of electrons at the mitochondria, as well as the increased activity of metabolic processes and the immune response (Knez *et al.*, 2006). The human body seems to be able to tolerate a limited amount of increased free radicals and, for muscle adaptation to occur, an increase of ROS is indeed a necessity (Bloomer *et al.*, 2009; Urso & Clarkson, 2003).

2.4.1.1 Sources of ROS in muscle

Several producers of ROS, which are activated by different stimuli, have been identified in muscle cells. Some of them are discussed in more detail below and can be seen in **Figure 2-6:**

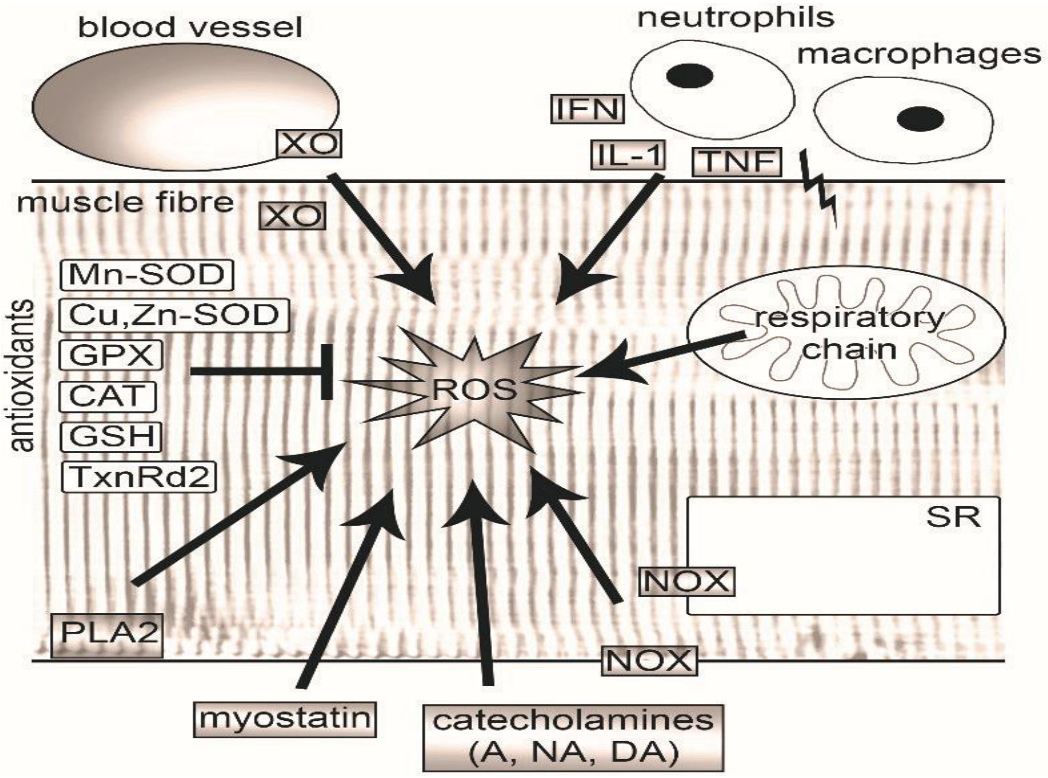

Figure 2-6: Sources of ROS in muscle (Steinbacher & Eckl, 2015)

Reactive oxygen species (ROS) sources and endogenous antioxidants in skeletal muscle fibers. After exercise, ROS are produced by the mitochondria, PLA2, XO and NOXs. Furthermore, exercise increases ROS production by macrophages and neutrophils, as well as by catecholamines and endothelia of blood vessels. Endogenous antioxidants are increased by regular exercise, which can neutralise free radicals. Adrenaline (A), catalase (Zhao *et al.*) , copper-zinc superoxide dismutase (Cu, Zn-SOD), dopamine (DA), glutathione peroxidase (GPX), glutathione (GSH), interferon (IFN), interleukin-1 (IL-1), manganese superoxide dismutase (Mn-SOD), noradrenaline (NA), nicotinamide adenine dinucleotide phosphate oxidase (NOX), phospholipase A2 (PLA2), sarcoplasmic reticulum (SR), tumour necrosis factor (TNF), thioredoxin reductase 2 (TxnRd2), xanthine oxidase (XO) (Steinbacher & Eckl, 2015).

2.4.1.1.1 Mitochondria

The mitochondria are thought to be the main producer of ROS through the leakage of electrons which subsequently combine with oxygen and causes the formation of superoxide (Niess, 2007; Steinbacher & Eckl, 2015). Complexes I (NADH dehydrogenase) and III (co-enzyme Q

and cytochrome C oxidoreductase) of the electron transport chain are the main producers of superoxide, but recently, complex II (succinate dehydrogenase) was also identified as a major source (Barja, 1999; Muller et al., 2004; Niess, 2007; Steinbacher & Eckl, 2015). As exercise increases muscle metabolic rate, the continuously increased oxygen utilisation in the mitochondria is, thus, considered to be the main mechanism of exercise-induced formation of superoxide (Niess, 2007; Sjödin et al., 1990; Steinbacher & Eckl, 2015).

2.4.1.1.2 Xanthine oxidase (XO)

A key enzyme in the purine catabolism, is a cytosolic molybdoflavoenzyme xanthine oxidase (XO), which also catalyses hydroxylation (of hypoxanthine) to xanthine and xanthine to uric acid (Harrison, 2002; Steinbacher & Eckl, 2015). Xanthine oxidase can be found in the cytosol and the endothelial cells of the muscle (Powers & Jackson, 2008; Steinbacher & Eckl, 2015). When the muscle contracts, XO increases significantly and this causes an increase in protein oxidation, oedema, lipid peroxidation and muscle damage (Judge & Dodd, 2004; Steinbacher & Eckl, 2015). Xanthine oxidase generates ROS when xanthine and hypoxanthine levels rise and serves as substrates, usually when intense exercise is performed and; therefore, large amounts of ATP is used (Radak *et al.*, 2013; Steinbacher & Eckl, 2015). In addition, ROS that is generated from XO, plays a role in the management of exercise-induced mitochondrial biogenesis through the peroxisome proliferator-activated receptor-*y*-coactivator-1a (PGC-1a) (Kang *et al.*, 2013; Steinbacher & Eckl, 2015).

2.4.1.1.3 Phospholipase A_2

During muscle contraction, phospholipase A_2 (PLA_2) enzymes also produce intra- and extracellular ROS (Steinbacher & Eckl, 2015). These enzymes excise AA from the phospholipids in the sarcoplasmic reticulum, plasma membrane or mitochondrial membranes (Steinbacher & Eckl, 2015). Arachidonic acid is an essential lipid signalling molecule and a substrate for lipoxygenases for the formation of ROS (Steinbacher & Eckl, 2015; Zhao *et al.*, 2002). In addition, the cytosolic PLA_2 enzyme also increases the production of ROS by stimulating nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidases (NOXs) (Steinbacher & Eckl, 2015; Zhao *et al.*, 2002).

2.4.1.1.4 Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidases (NOXs)

Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidases (NOXs, flavoprotein enzymes) are activated by free fatty acids, calcium, posttranslational modifications and protein-protein interactions (Bedard & Krause, 2007; Brandes et al., 2014; Steinbacher & Eckl, 2015). They are found in the transverse tubules and the sarcoplasmic reticulum, because they are transmembrane proteins and transport electrons to reduce H_2O_2 or superoxide across biological membranes (Bedard & Krause, 2007; Brandes et al., 2014; Steinbacher & Eckl, 2015). Studies have shown that the NOX family adds to cytosolic superoxide production at rest and during exercise in skeletal muscle (Sakellariou et al., 2013; Steinbacher & Eckl, 2015; Xia et al., 2003).

2.4.1.1.5 Myostatin

Myostatin causes oxidative stress by producing ROS in muscle cells (Sriram *et al.*, 2014; Steinbacher & Eckl, 2015). Myostatin is a blocker of differentiation and signals the production of ROS during exercise through nuclear factor-kB, tumour necrosis factor alpha (TNF-α) and Smad3, the protein coding gene, in muscle cells (Sriram *et al.*, 2014; Steinbacher & Eckl, 2015). When there is no Smad3, myostatin generates ROS through the activation of extracellular signal-regulated kinases (Kerksick & Willoughby, 2005) mitogen-activated protein kinase (MAPK) and ERK pathways through interleukin 6 (IL-6), NOX, TNF-α and XO (Sriram *et al.*, 2014; Steinbacher & Eckl, 2015).

2.4.1.1.6 Non-muscle sources

Vigorous exercise can cause muscle injuries, which then activates neutrophils and macrophages through interferon-*y* (IFN-*y),* TNF-α and IL-1 (Moylan & Reid, 2007; Peake & Suzuki, 2004; Steinbacher & Eckl, 2015). These immune cells cause an oxidative burst when immune cells produce excessive amounts of ROS (Moylan & Reid, 2007; Peake & Suzuki, 2004; Steinbacher & Eckl, 2015). Exercise also increases catecholamines, which plays a role in the production of ROS (Gomes *et al.*, 2012) and the ROS that is prduced from the endothelium (Duarte *et al.*, 1993; Steinbacher & Eckl, 2015).

2.5 Oxidative stress adaptation

One of the principles of the homeostasis response, is the adaptation of a biological system to a specific stress, which then produces an increased resistance to the given stress (Radak et al., 2001). Stress that is caused by exercise, seems to be controlling the adaptation of skeletal muscle (Niess, 2007). Exercise adaptation refers to the body's changing to keep a stable environment after exercising (Radak et al., 2001). As previously mentioned, exercise produces free radicals and ROS (Durackova, 2010; Niess, 2007; Powers & Jackson, 2008; Steinbacher & Eckl, 2015). In addition, recent research has shown that ROS may have a beneficial role in advancing the adaptive responses of skeletal muscle to training (Radak et al., 2001; Steinbacher & Eckl, 2015). According to Steinbacher et al. (2015), a single session of exercise causes oxidative stress in an untrained individual, but in trained individuals, this is not the case. This can be due to the fact that trained individuals have a higher resistance to the oxidative stress mediated by an adaptation response (Radak et al., 2001; Steinbacher & Eckl, 2015).

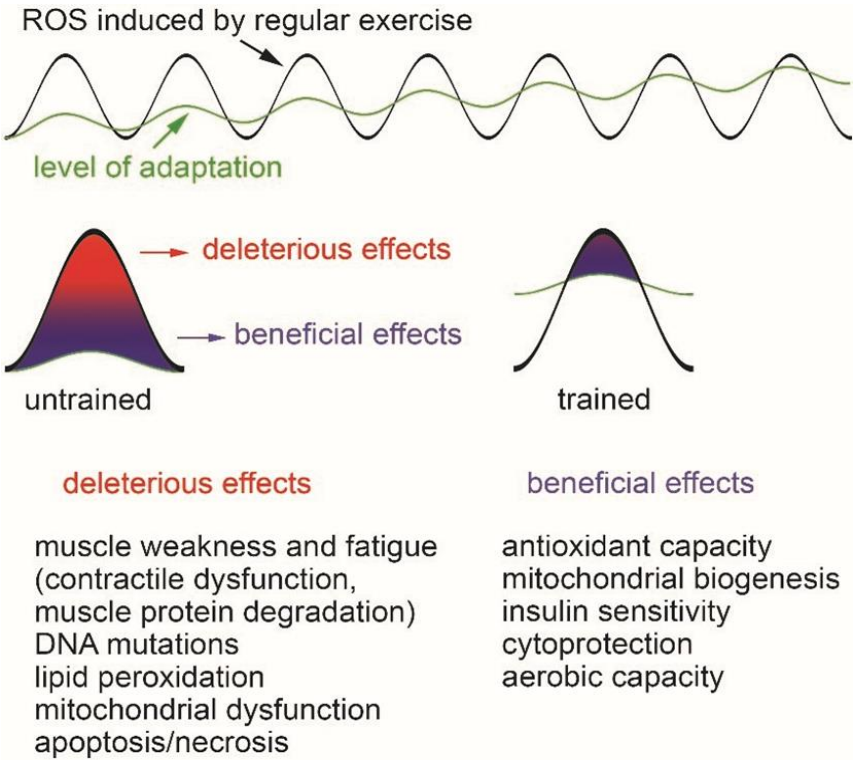

Figure 2-7: Exercise adaptation to ROS (Steinbacher & Eckl, 2015)

Beneficial and harmful effects of exercise-induced ROS increase. When exercising, ROS are produced, and whether it is beneficial or harmful, depends on the concentration of ROS; the period of ROS exposure and the training status of a person. A single session of exercise can lead to an increase of ROS, which cannot be protected from by antioxidants, especially in untrained people. Therefore, this

leads to oxidative damage, muscle weakness and fatigue, DNA mutations, lipid peroxidation, mitochondrial dysfunction and apoptosis / necrosis. A higher level of adaptation and fewer health risks are seen in trained people. The ROS that is produced with regular exercising, increases the level of adaptation continuously. This is done through improvement of the antioxidant capacity, mitochondrial biogenesis, insulin sensitivity, cytoprotection and aerobic capacity of skeletal muscle (Steinbacher & Eckl, 2015).

Therefore, as seen in **Figure 2-7**, research shows that trained runners can have a higher adaptation level compared to untrained individuals, due to the upregulation of antioxidative mechanisms induced by regular exercise (Pingitore *et al.*, 2015; Steinbacher & Eckl, 2015).

2.6 Unaccustomed resistance exercise as a model for inducing oxidative stress and inflammation

Several studies have found that long-term exercise can reduce inflammation by reducing oxidative stress through adaptation of endogenous antioxidative systems (Pingitore *et al.*, 2015). Although regular moderate exercise appears to be beneficial for oxidative stress and health, strenuous aerobic and anaerobic exercise can induce ROS overproduction and; therefore, can induce an inflammatory response (Koenig *et al.*, 2016; Lira *et al.*, 2012; Pingitore *et al.*, 2015). Following strenuous exercise, the overproduction of ROS may not only cause cellular damage to muscles, but also enhance the risk of systemic inflammatory response (Ji *et al.*, 2009; Koenig *et al.*, 2016).

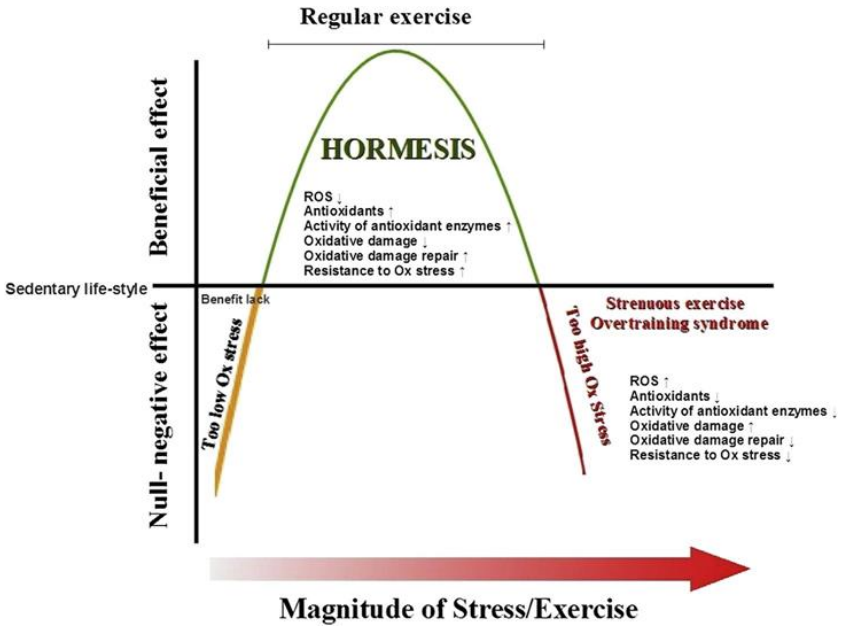

Figure 2-8: Results of regular and strenuous exercise (Pingitore et al., 2015)

Regular exercise reduces oxidative stress, causes hormesis, defends against the start of disease and development, and improves performance and quality of life. Extreme exercise and overtraining increase oxidative stress and the risk of diseases. Nevertheless, a too low oxidative stress status can cause a lack of benefits that is related to hormesis and may be harmful to a person's health. Oxidative (Ox), reactive oxygen species (ROS) (Pingitore *et al.*, 2015).

As seen in **Figure 4-8,** not only little to no exercise can have a negative effect, but also strenuous exercise, by increasing oxidative stress and the risk of related diseases (Pingitore *et al.*, 2015). Regular exercise, on the other hand, can be beneficial to health, because it reduces oxidative stress, protects against disease and improves performance and quality of life (Pingitore *et al.*, 2015).

2.7 Inflammatory (inflammation resolution) adaptation

Inflammation is an adaptive response that is caused by a harmful stimulus, such as tissue injury and infection (Markworth *et al.*, 2013; Medzhitov, 2008; Minihane *et al.*, 2015). Irrespective of the cause, inflammation will most likely evolve as an adaptive response for the restoration of homeostasis (Medzhitov, 2008). An acute inflammatory response that is successful, will result in the removal of the infectious agents, which is followed by a resolution and repair phase, mainly mediated by tissue-resident, and recruited, macrophages (Medzhitov, 2008; Serhan, 2017b; Serhan *et al.*, 2017a). The switch between lipid mediators from prostaglandins (pro-inflammatory) to lipoxins (anti-inflammatory), is very important with regards to inflammation resolution (Medzhitov, 2008). Lipoxins prevents the recruitment of neutrophils and, in its place, stimulates the recruitment of monocytes, which instigate tissue remodelling and removal of dead cells (Medzhitov, 2008). Protectins and resolvins also have important roles in the resolution of inflammation, which involve the beginning of tissue repair

(Medzhitov, 2008; Serhan, 2017b; Serhan *et al.*, 2017a). Internal environmental parameters are maintained by homeostatic control mechanisms inside a close adequate range around a certain set point (Medzhitov, 2008). Irregular conditions can trigger a change in some parameters beyond the usual homeostatic range, which either results in an acute stress response that provides a brief adaptation to the new conditions, or cause a continued adaptive change that includes a shift in the related set points (Medzhitov, 2008).

Chronic and acute inflammation are two different types of responses that happen when homeostatic mechanisms are not competent or are insufficient (Medzhitov, 2008; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). The inflammatory response is commonly triggered during severe disturbances of homeostasis, such as infection and tissue injury (Medzhitov, 2008; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). Generally, an inflammatory response is involved whenever tissue malfunctions are detected (Medzhitov, 2008). Very mild stress is mainly managed by macrophages, whereas more extensive damage or malfunctions may need additional leukocytes to be recruited and plasma proteins to be delivered locally (Medzhitov, 2008). Whatever causes the inflammatory response, its purpose is to remove the source of the disruption and allow the host to adapt to the abnormal conditions in order to eventually restore homeostasis and functionality to the tissue (Medzhitov, 2008; Serhan, 2017b; Serhan et al., 2017a; Serhan & Petasis, 2011). A successful acute inflammatory response will return the system to the basal homeostatic set points if the abnormal circumstances are temporary (Medzhitov, 2008). However, if the abnormal conditions are persistent, a continuing inflammatory state moves the system to different set points, as is the case in chronic inflammation (Medzhitov, 2008). Short term benefits can be provided by an adaptive change (Medzhitov, 2008).

In **Figure 2-9**, the initiation of acute inflammation to its resolution is demonstrated. Following injury or infection, one of the first events that occurs, is the release of PGs and LTs (Medzhitov, 2008; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). This causes a series of actions where the main purpose is to destroy the invading pathogens and repair the tissue damages (Medzhitov, 2008; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). To achieve this, LTB_4 is produced and released, and, in turn, promotes the chemotaxis of neutrophils to the inflamed sites (Medzhitov, 2008; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). At the same time the development of PGE_2 and PGD_2 further advances the inflammatory process, which leads to acute inflammation (Medzhitov, 2008; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011).

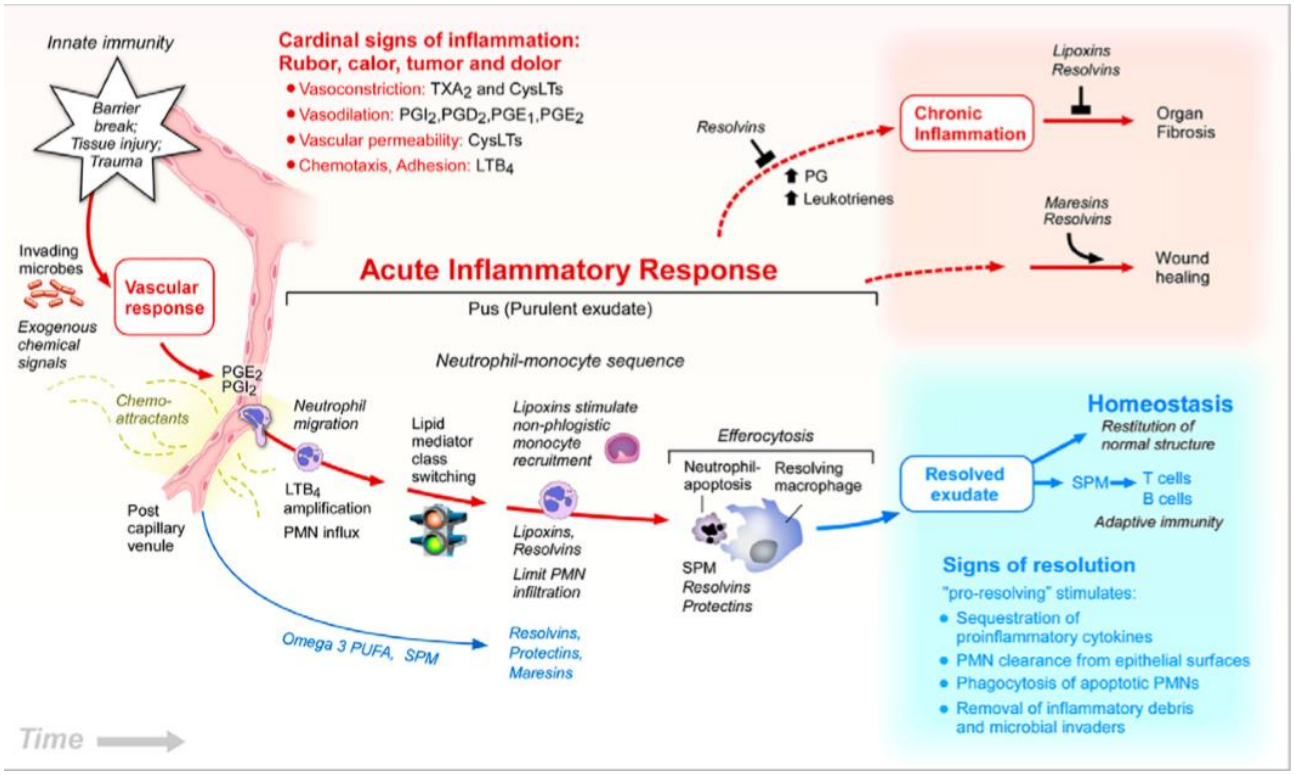

Figure 2-9: The initiation of acute inflammation to its resolution (Serhan, 2017b)

Pro-resolving mediators and eicosanoids in initiation and resolution of inflammation. Prostaglandin E_2 (PGE_2) and PGI_2 allow neutrophils to be recruited to the inflamed cite along a gradient of leukotriene B_4 (LTB_4); a process called chemotaxis. A lipid mediator class switching then occurs and lipoxins stimulate monocyte recruitment. Lipoxins, resolvins and other specialised pro-resolving mediators are generated to limit or stop further neutrophil tissue infiltration. Resolving macrophages and neutrophils also produce specialised pro-resolving mediators. Specialised pro-resolving mediators stimulate the signs of resolution. Resolvins and specialised pro-resolving mediators block chronic inflammation (Serhan, 2017b).

Consequently, a few endogenous lipid mediators act to switch the original actions of pro-inflammatory lipid mediators (LKs and PGs) to anti-inflammatory lipid mediators (pro-resolving) actions; these include: resolvins, lipoxins, protectins and maresins (Medzhitov, 2008; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). Each of these anti-inflammatory lipid mediators has their own specialised actions which include: the blocking of neutrophil recruitment, stimulating the recruitment and activation of monocytes, and activating macrophages (Medzhitov, 2008; Serhan, 2017b; Serhan et al., 2017a; Serhan & Petasis, 2011). Finally, through combining the actions of these mediators, inflammation is resolved, and homeostasis is reached (Medzhitov, 2008; Serhan, 2017b; Serhan et al., 2017a; Serhan & Petasis, 2011).

Currently uncontrolled low-grade chronic inflammation is one of the main components of many diseases such as diabetes, cardiovascular diseases, cancers, obesity, asthma, rheumatoid

arthritis and neurodegenerative diseases (Calder, 2006a; Rangel-Huerta *et al.*, 2012). It is unknown whether inflammatory adaptation takes place independently of oxidative adaptation. There is also limited data available on the lipid mediator profiles in response to unaccustomed resistance exercise. Furthermore, it is not clear if aerobic exercise training induces adaptation in terms of inflammation-resolving capacity independent of anti-oxidative adaptation, and whether the proposed adaptation is modulated by fatty acid and micronutrient intake and status. These questions are important due to the global health impact of communicable and non-communicable diseases associated with perpetuating inflammation.

CHAPTER 3 METHODOLOGY

3 METHODS

3.1 Study design

The study made use of a pre-test, post-test study design (where both the pre- and post- test components were of cross-sectional and descriptive nature). Recreational runners and untrained controls (n=40) between 18 and 35 years of age were characterised by determining their baseline ROS and endogenous antioxidant parameters, fatty acid status, antioxidant micronutrient intake and lipid mediator profiles. The participants were then challenged with an unaccustomed exercise bout after which the impact of the exercise-induced stress (inflammation) on the lipid mediators over five more time points, within three days, was assessed. This sub-study compared the exercise-induced lipid mediator inflammatory signalling in male recreational runners with untrained males, considering potential modulating factors like blood fatty acid and antioxidant status.

3.2 Setting

The study was coordinated from and partially conducted at the Metabolic Unit in building G17 on the NWU Potchefstroom Campus. The exercise, as well as the first post-exercise blood sampling, was performed in the NWU gymnasium located at the Potchefstroom Campus Indoor Sports Centre, building F14. The gymnasium is ~300m from the Metabolic Unit in building G17. The Metabolic Unit was managed by a registered nurse who drew the pre- and post-exercise blood samples (except for the immediate post-exercise samples) and oversaw the well-being of the participants in the study. The unit (G17) is equipped with a number of rooms, including changing rooms, as well as all the facilities required to draw blood, perform private anthropometrical assessments and administer the dietary assessments. The NWU gymnasium is managed by a registered biokineticist and is also equipped with changing rooms, as well as all the facilities / exercise machines needed to accommodate the respective exercise regimes. In addition, a private room adjacent to the gymnasium was available for the first post-exercise blood sampling that was performed by an additional registered nurse. This room was equipped with a first aid kit and all the facilities required to draw blood as well as to handle possible incidents were supervised by the supervising study medical doctor.

3.3 Study population and sample

The study population included apparently healthy, male, untrained individuals and recreational runners according to the inclusion and exclusion criteria below (**Table 3-1**). Moderate regular running is an aerobic, whole-body exercise, not focused on conditioning certain muscles only; therefore, to the runners, the bout of resistance exercise was also unaccustomed exercise and was intended to induce acute inflammation.

Table 3-1: Summary of inclusion and exclusion criteria at sampling

Runners	
Inclusion criteria	**Exclusion criteria**
Males between the ages of 18 and 35	Runners competing on national level and above, and / or performing more than 5 hours of structured exercise per week
18.5 > BMI < 28 kg/m^2 OR body fat percentage <20%	Runners who had performed lower body resistance training during the past 6 months
Recreational runners habitually performing between 3 and 5 sessions (~30-60 min / session) of structured running / aerobic exercise per week for the past 6 months.	Self-reported smoking and drug / alcohol abuse, self-reported steroid use
	Severe anaemia (Hb<8 g/dL)
	Any operation or medical procedure during the previous 6 months.
	Back pain or knee pain that does not go away with exercise.
	Self-reported and / or previously diagnosed chronic conditions requiring the use of NSAIDS or cortisone (systemic or inhalers). Examples of NSAIDS: active ingredient aspirin [disprin, ecotrin], ibuprofen, [brufen, nurofen] diclofenac [voltaren, panamor], celecoxib [celebrex, etoricoxib [arcoxia]
	Self-reported and / or previously diagnosed acute or chronic inflammatory disease
	Self-reported and / or previously diagnosed metabolic diseases including Diabetes Mellitus 1 and 2
	Self-reported and / or previously diagnosed hypertension or hypertension measured at the study medical screening or self-reported medical history of hypertension

	Self-reported and / or previously diagnosed cardiovascular and clotting diseases
Controls	
Inclusion criteria	**Exclusion criteria**
Males between the ages of 18 and 35	Self-reported smoking and drug / alcohol abuse, self-reported steroid use.
18.5 > BMI < 28 kg/m^2 OR body fat percentage <20%	Self-reported and / or previously diagnosed chronic conditions, requiring the use of NSAIDS or cortisone (systemic or inhalers). Examples of NSAIDS: active ingredient aspirin [disprin, ecotrin], ibuprofen, [brufen, nurofen] diclofenac [voltaren, panamor], celecoxib [celebrex, etoricoxib [arcoxia]
Untrained individuals not engaging in any regular, structured exercise activity and/or physically demanding jobs that include manual labour during the past 6 months.	Self-reported and / or previously diagnosed acute or chronic inflammatory disease
	Severe anaemia (Hb <8 g/dL)
	Any operation or medical procedure during the previous 6 months.
	Back pain or knee pain that does not go away with exercise
	Self-reported and / or previously diagnosed metabolic diseases including Diabetes Mellitus 1 and 2
	Self-reported and / or previously diagnosed hypertension or hypertension measured at the study medical screening or self-reported medical history of hypertension
	Self-reported and / or previously diagnosed cardiovascular and clotting diseases

The motivation for the exclusion criteria was mainly the fact that these factors would disproportionately influence the inflammatory response and to rule out candidates who were medically unfit to perform the exercise. These factors could confound associations with exercise or render the participant unable to do the exercise.

3.3.1 Sample size calculation

The sample size was based on two previous studies; firstly, on the basis of a bout of exercise performed and antioxidant capacity compared between amateur runners and untrained

subjects, n = 17 per group gave the study 80 % power to detect a significant difference (a = 0.05) (Falone *et al.*, 2010). Secondly, on the basis of the effect of ibuprofen treatment on the lipid mediator response of healthy male subjects, n = 10 per group gave the study 80 % power to detect a significant difference (a = 0.05) (Markworth *et al.,* 2013). The sample size needed for this study was calculated by using a 2-tailed, 2-means independent t-test. Thus, a sample size of n = 20 per group (n = 40 in total) was recruited to provide for a 20% drop-out rate and led to a final 17 participants per group. Between-group analyses were performed with lipid mediator concentrations as outcome variables, controlling for potential confounders including fatty acid and oxidative stress status, as well as antioxidant micronutrient intake.

3.3.2 Recruitment and screening

Recreational runners and controls were recruited from the Potchefstroom community by means of an advertisement in the Potchefstroom Herald that provided a description and a contact number / e-mail address for further enquiries. Posters and flyers were also handed out and put up in the greater Potchefstroom area. Additionally, the club managers of the two largest, active running clubs in Potchefstroom, Berts Bricks Running Club and the Potchefstroom Military Athletics Club, were approached. The managers of the respective running clubs were asked to send the same advertisement via e-mail to their members, and / or hand out flyers at a mid-week club training session. Furthermore, posters were put up at the notice boards of grocery shops in Potchefstroom. Individuals who were interested in participating in the study contacted the researchers and were pre-screened telephonically and / or by e-mail to ensure that they adhered to the inclusion and exclusion criteria before inviting them to an information (recruitment) session at the NWU Potchefstroom campus.

The study sample was assessed according to the main activities and timeframe as outlined in **Table 3-2**. A schematic overview of the sequence, type and duration of the study activities is presented in **Figure 3-1.** Details of the different methods employed in this study are summarised in **Table 3-3.**

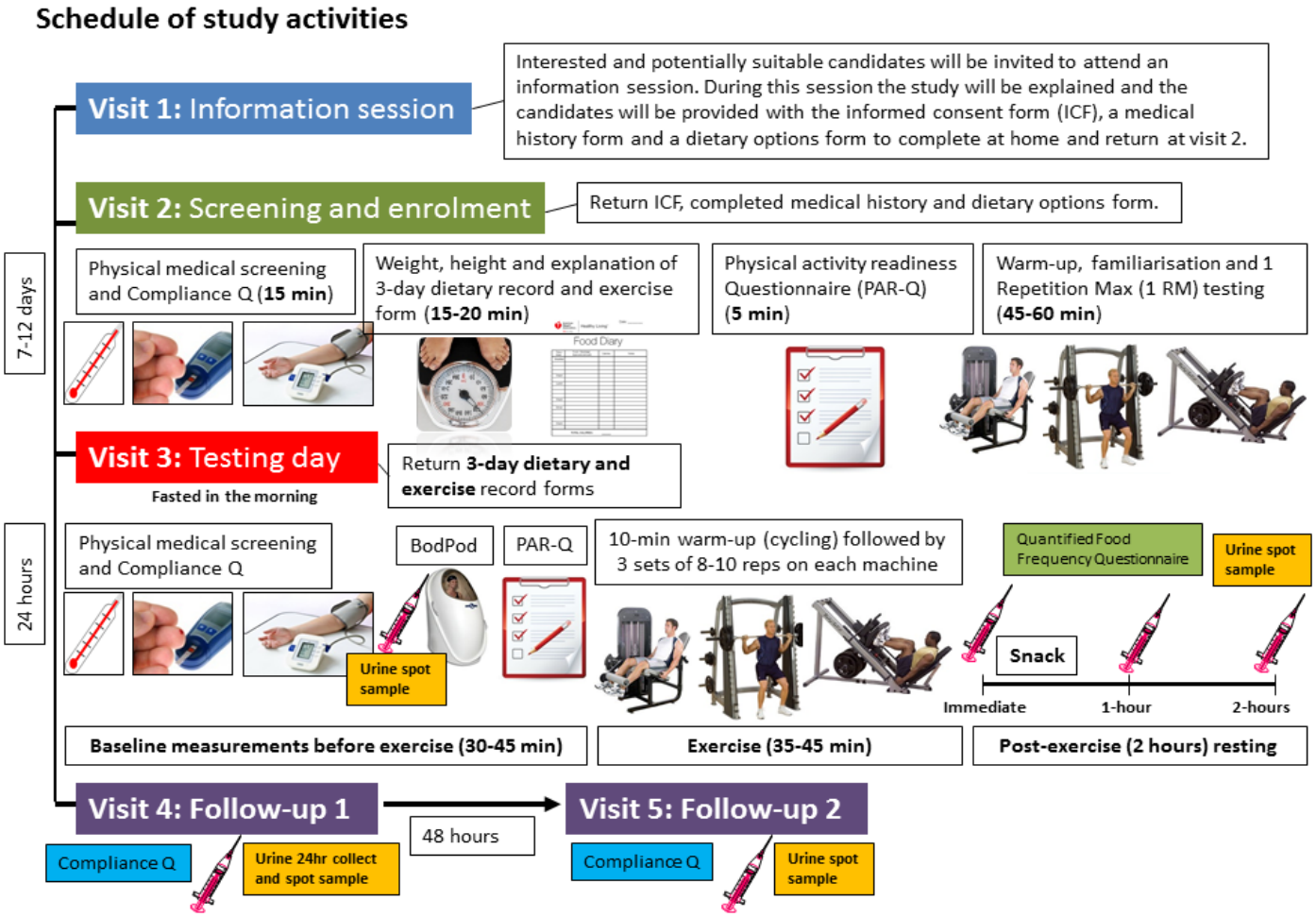

Figure 3-1: Schedule of study activities

On arrival at the information session (recruitment – visit 1), the candidates received an information sheet and written, informed consent form (ICF), outlining all the procedures of the study. The participants were also informed that they could be excluded from the study as a result of a compulsory screening visit (visit 2). Eligible participants were asked not to eat or drink anything for 2 hours prior to their screening and enrolment visit (visit 2), in order to obtain a baseline glucose value. They kept an additional ICF for their own information and also handed in a completed medical history form and discussed it with the nurse at visit 2. The candidates were informed that they had the right to decide not to take part in the study and they may withdraw from the study at any stage.

At the screening / enrolment visit (visit 2), candidates handed in their medical history forms and dietary options lists. At that point, the researcher assigned a participant number to each eligible candidate who volunteered to participate in the study, to ensure anonymity. Inclusion

and exclusion criteria were confirmed, after which the candidates underwent a physical, medical screening (blood pressure, heart rate, temperature, fingerprick haemoglobin and blood glucose). Subsequently, they received something to eat and drink. The participants' height and weight were also measured by a trained student. Additionally, they completed a physical activity readiness questionnaire, administered by a registered biokineticist. According to the exclusion criteria, participants could at each stage either be excluded or asked if we could schedule them for another day (e.g. due to acute illness such as a cold). If enrolled, familiarisation with the exercise and multiple repetition maximum strength testing to determine experimental exercise load [80% of 1 repetition maximum (1 RM)] was determined as described in **Table 3-3.** In addition, a three-day dietary record and exercise diary was handed out and explained by a registered dietician (RD) or a trained student (**Table 3-3**). Participants were instructed not to do any unusual exercise and not to take any supplements between the screening visit and the end of the study (about two weeks). They were also asked to refrain from taking NSAIDS (explained during inclusion / exclusion) for at least three days before the exercise challenge.

3.4 Research procedures and data collection

Visit 3 (exercise challenge and testing day) was scheduled to start between 07h00 and 10h00 in the morning and took about 4-5 hours. Four to six participants were invited per day, including an equal number of participants from both the athlete and control groups. Participants were instructed to arrive in a fasted state (except for water consumption), after an 8 – 10 hours overnight fast. They received a standardised meal (57% carbohydrates, 22% fat, 21% protein) and snack the previous evening to consume between 23h00 and 24h00. They underwent medical screening (blood pressure, heart rate and temperature and fingerprick blood glucose) and a physical activity readiness questionnaire was administered before the exercise challenge started. They could at this stage be asked if we should schedule them for another day if the screening and / or questionnaire had indicated that they should not exercise on that current day (e.g. with acute illness such as a cold or blood glucose < 4.0 mmol/L). A compliance questionnaire was administered to assess compliance regarding food intake, supplements, medicine use and unusual exercise.

Since participants were tested in a fasted state, the baseline blood measurements and exercise challenge needed to be completed within the 12 hours following the last meal.

Participants were required to lie down in a supine position for the insertion of a butterfly (Venofix G23) in the antecubital vein to allow venous blood sampling. A resting blood sample was then collected, followed by a BodPod measurement (**Table 3-3**). The participants were then escorted (slow walk to warm-up) to the NWU gymnasium to complete the exercise challenge under the supervision of registered biokineticists and an exercise scientist. Immediately on completion of the exercise challenge, participants were escorted to a room, adjacent to the gymnasium, where a registered nurse inserted a butterfly to draw a blood sample and provide the participants with a post-exercise snack. The participants were then transported back to the metabolic unit. The participants were further instructed not to be active (thus to lie down or sit, and to only walk when necessary) during a 2-hour recovery period, during which additional post-exercise blood samples were obtained 1 and 2 hours after cessation of the exercise.

Participants were then provided with additional standardised meals and snacks for the rest of the day. Participants were instructed not to consume any additional food or drink (apart from water) than that provided, to consume the food provided prior to 11 PM, and to return to the clinic the following morning (24 hour post-exercise) for visit 4.

On the fourth visit (24 hour post-exercise) the participants arrived (~between 8h30-10h00) at the gymnasium in a fasted state. A registered nurse drew a blood sample, after which they received a snack and drink. A compliance questionnaire was also administered to assess compliance regarding food intake, supplements, medicine use and unusual exercise. No further dietary restrictions were necessary, but participants were instructed not to perform any exercise, and not to take any supplements or NSAIDS until the end of the study (in two days' time). On the fifth visit (two days after the fourth visit) a blood sample was collected at ~9h00 (~72 hours after exercise challenge) in a non-fasted state. A compliance questionnaire was also administered again to assess compliance regarding supplements, medicine use and unusual exercise.

Table 3-2: Schedule of study activities

Activity	Recruitment	Screening and enrolment	Testing day					24 h	72 h
			Baseline	Directly After	1 hour	2 hours	> 2 hours		
Visit number	1	2	3					4	5
Informed consent	X	X							
Inclusion exclusion criteria / Compliance	X	X	X					X	X
Medical history questionnaire	X	X							
Physical activity readiness questionnaire		X	X						
Physical medical screening		X	X						
Exercise exposure									
Familiarisation and 1 RM		X							
Exercise challenge			X						
Anthropometry		X							
BodPod			X						
Dietary measurements									
3-Day dietary record		X							
Dietary control									
Provision of meals and snacks			X	X			X	X	
Blood collection for laboratory measurements									

Activity	Recruitment	Screening and enrolment	Testing day					24 h	72 h
			Baseline	Directly After	1 hour	2 hours	> 2 hours		
Visit number	1	2	3					4	5
Fatty Acid status			X						
Oxidative stress and antioxidant parameters			X						
Lipid mediators and inflammation			X	X	X	X		X	X

3.4.1 Assessments and data collected

Table 3-3: Summary of data collection and relevant methodology

Data collected	Methodology
Anthropometry (10 minutes)	Weight (kg) and height (m) were measured to the nearest decimal place with a calibrated digital scale with stadiometer (Seca 264, Hamburg, Germany) according to the standards of the International Society for the Advancement of Kinanthropometry (ISAK).
BodPod (15 minutes)	Body fat percentage was measured by means of air-displacement plethysmography (Sies & Jones) with the BodPod® body compositions system (model 2000A, Life Measurement Instruments, Concord, CA, USA) (Biaggi *et al.*, 1999).
Dietary intake and control (45 minutes)	During the enrolment visit, participants were instructed how to complete a 3-day dietary record, consisting of 2 week days and 1 weekend day, to determine self-reported energy intake and nutrient consumption. Participants were also asked to refrain from supplementing with any supplements for 1 week before the exercise challenge. Participants were also requested to refrain from drinking any alcohol or taking non-steroidal anti-inflammatory (NSAIDS) medication 2 days before the exercise challenge or during the study. Paracetamol was permissible. (Examples of NSAIDS: active ingredient aspirin [disprin, ecotrin], ibubrufen, [brufen, nurofen] diclofenac [voltaren, panamor], celecoxib [celebrex, etoricoxib [arcoxia]).
Exercise questionnaire	To assess whether the exercise activity levels of the enrolled trained and untrained groups were sufficiently different, participants completed an exercise questionnaire, administered by a trained fieldworker or student.

Data collected	Methodology
Exercise familiarisation and strength testing (1 RM) (40-60 minutes)	Participants underwent a familiarisation and strength testing session at the screening visit, at least one week prior to the exercise study trial day. Participants were instructed on correct exercise technique and underwent multiple repetition maximum strength testing to determine their experimental exercise load [80% of 1 repetition maximum (1 RM)]. The maximal weight that subjects could lift for 3–6 repetitions on the smith rack squat, 45° leg press and seated knee extension machine was determined. Subsequently, 1 RM was estimated using the Brzycki equation [1 RM = 100 X load rep/ (102.78 – 2.78 X_ reps completed)]. Participants were instructed to abstain from any unusual physical activity in the following week prior to the experimental trial day (Markworth *et al.*, 2013). A medical history and physical activity readiness questionnaire was administered and physical medical screening (blood pressure, heart rate, temperature, blood glucose and haemoglobin) was performed prior to exercise to ensure the participants could be subjected to the exercise exposure.
Exercise challenge (30-40 minutes)	Participants were required to perform a 10-min warm-up, consisting of walking from the clinic to the gymnasium, light cycling on a bicycle ergometer and one low-resistance warm-up set on each exercise machine before the official exercise challenge commenced. The exercise challenge consisted of three sets of 8–10 repetitions each on the smith rack squat, 45° leg press and seated knee extension, at 80% of predetermined 1 RM. Exercises were performed sequentially in a circuit manner with participants resting for 1 min between each exercise and 3 min between each set (Markworth *et al.*, 2013). Again, a physical activity readiness questionnaire was administered and physical medical screening (blood pressure, heart rate, temperature, blood glucose) was performed prior to exercise to ensure the participants could be subjected to the exercise exposure. Participants were also required to refrain from exercise two days prior to and during the study.
Blood to assess the following (heart rate, blood pressure, glucose, Hb and temperature)	Heart rate was measured at the radial pulse with the index and middle finger by a registered medical nurse. Blood pressure was measured with a Sphygomomameter (Tycos) by a registered medical nurse. A fingerprick was performed by a registered medical nurse to obtain blood for the medical screen. Blood glucose was measured with a OneTouch Select (Johnsons&Johnsons) by a registered medical nurse. Haemoglobin concentrations were obtained with a HemoCue® Hb 201+ System (Angelholm, Sweden), (Mathee *et al.*, 2014) and was performed by a registered medical nurse. Body temperature was measured in the ear by a registered medical nurse with a ThermoScan IRT 3020/ IRT3520 (BRAUN).
Laboratory measurements	
Fatty Acid status (red blood cells)	Fatty acid composition with gas chromatography (Baumgartner *et al.*, 2012) Please see 3.4.1.2.4 for a more detailed description.

Data collected	Methodology
Oxidative stress and antioxidant capacity	ROS, GSH and FRAP with spectrophotometric methods. Please see 3.4.1.2.3 for a more detailed description.
Lipid mediators	Lipid mediators with liquid chromatography tandem mass spectrometry (Malan, 2016). Please see 3.4.1.2.1 for a more detailed description.

3.4.1.1 Blood collection

A fingerprick was performed to obtain blood for the physical medical screen. Venous blood samples were drawn into EDTA-coated, empty (serum) and trace element-free evacuated tubes (Becton Dickinson) via antecubital venepuncture. A total volume of 60 ml, and 20 ml each was collected on visit 3, 4 and 5, respectively. The 60 ml on visit 3 was divided into 28 ml prior to exercise, 16 ml immediately post-exercise and 8 ml at 1 and 2 hours post-exercise respectively. On visit 3, a baseline blood sample was drawn prior to exercise after which a butterfly or cannula was inserted after the exercise exposure in the antecubital vein to allow multiple venous blood sampling for the rest of the visit. Blood was processed within 1 hour after blood sampling and plasma / serum was separated. Red blood cells were washed with normal saline and aliquots stored at -80°C. Blood aliquots were stored at -80°C at the CEN, NWU in the cryostorage facility.

3.4.1.2 Biochemical analyses

Gas and liquid chromatography mass spectrometry (fatty acids, lipid-derived immune modulators) were analysed at CEN and oxidative stress markers at the Biochemistry Department, NWU.

3.4.1.2.1 Lipid mediators

Lipid mediators were extracted and analysed with liquid chromatography tandem mass spectrometry (LCMSMS). Lipid mediators were extracted from plasma with solid phase extraction (SPE) using Strata-X (Phenomenex, Torrance, United States of America) in a CEN laboratory. Plasma samples (1 ml) were diluted with water and adjusted to 15% methanol (v/v), to a final volume of 4.6 ml. Internal standards, PGB2-d4 and 12-HETE-d8 (500 pg of each), were added to each sample. To remove any precipitated proteins, the samples were incubated on ice for 30 min and then centrifuged at 3000 rpm for 8 min. The resulting clear

supernatants were acidified with 1 N hydrochloric acid to pH 3.0 and immediately applied to SPE cartridges that had been preconditioned with 2 ml methanol, followed by 4 ml water. The cartridges were then washed with 10 ml 15% (v/v) methanol, and 4 ml hexane in this order. Lastly, the lipid-derived immune modulators were eluted with 6 ml methyl formate. A vacuum manifold (Phenomenex) was used to perform the SPE and the vacuum was regulated that single drops can be seen from each cartridge. The methyl format evaporated under a fine stream of nitrogen and the residue was dissolved in 20 µl acetonitrile, flushed with nitrogen and stored, at the most, for four days at –20 °C before LCMSMS analysis (Malan et al., 2016).

Samples were analysed with an Agilent Technologies 6410 MSMS, coupled to an Infinity 1260 HPLC pump (Santa Clara, United States of America). The mass spectrometer has a mass range of 15–1650 m/z, minimum multiple reaction monitoring (MRM) dwell time of 5ms with 10 MRMs and a maximum of 99 MRM transitions per time segment. The instrument was operated in negative electrospray ionisation mode. At least two transitions were monitored for each compound, using a dynamic MRM method. The collision and fragment or voltages were optimised with flow injection analysis. The gas temperature and flow were set at 350 °C and 12 l/min respectively, and the capillary voltage at 4000V. Chromatographic analysis was performed on a C18 column (Poroshell, 2.7 µ, 100x2.1mm^2, Agilent Technologies) with a flow rate of 0.4 ml/min and column temperature of 50 °C. Sample injections were performed with an Agilent G1367B auto sampler. The sample chamber temperature was set at 5 °C and the injection volume at 15 µl, subsequent to mixing 5 µl of sample (in acetonitrile) with 10 µl water in the auto sampler, just before injection. The analysis was performed using an acetonitrile-based system with a flow program, by mixing two solvents (A and B) with programmed ratio changes for optimal separation of compounds. Solvent A was water / glacial acetic acid, 99:1(v/v) and solvent B was 100% acetonitrile. Data was quantified with Masshunter B05 02, using external calibration for each compound and two internal standards to correct for losses and matrix effects during sample preparation and analysis (Malan *et al.*, 2016).

3.4.1.2.2 Analysis of oxidative stress parameters (ROS, FRAP and GSH)

ROS, FRAP and GSH were analysed with spectrophotometric assays at the Department of Biochemistry, NWU. The basis of the ROS assay is that in an acidic medium, ROS reacted with transition metals, such as iron, to form alkoxyl and peroxyl radicals. The formed radicals were then oxidised N,N-diethyl-para-phenylenediamine (DEPPD) to its cation which is then measured at 546 nm for 10 minutes each minute. The FRAP assay assesses the total ferric reducing antioxidant power of biological fluids assay. Thus, it gives an indication of how well

the antioxidants present in serum can cause reduction to take place. Glutathione (GSH) is an important endogenous antioxidant molecule. Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid, DTNB) reacts with GSH to form a spectrophotometrically detectable product at 412 nm. GSSG was determined by the reduction of GSSG to GSH, which was quantified by the reaction with Ellman's reagent. Thus, this method utilised the change in colour development during the reaction, and the reaction rate is proportional to the GSH and GSSG concentrations.

3.4.1.2.3 Fatty acids

Total phospholipid fatty acids were analysed in red blood cells samples. Phospholipids were extracted with chloroform: methanol (2:1 vol: vol, containing 0.01% butylated hydroxytoluene) by using a modification of the method.

The neutral lipids were separated from the phospholipids by using thin-layer chromatography (silica gel 60 plates, 10 x 20 cm, Merck) and eluted with diethyl ether: petroleum, ether: acetic acid (30:90:1 vol: vol: vol). The lipid band that contained phospholipids was removed from the thin-layer chromatography plate and trans- methylated with methanol: sulphuric acid (95:5 vol: vol) at 70 ºC for 2hours to yield fatty acid methyl esters (FAMEs). The resulting FAMEs were extracted with water and hexane. The organic layer was evaporated, re-dissolved in hexane, and was analysed by using quadrupole gas chromatography–electron impact–mass spectrometry on an Agilent Technologies 7890A Gas Chromatograph system, equipped with an Agilent Technologies 7000GC/MS triple quad mass selective detector. The gas chromatography separation of FAMEs was carried out on a HP88 capillary column (100 m x 0.25 mm x 0.20 µm, Agilent) by using helium as the carrier gas at a flow rate of 2.2 mL/min. The gas chromatography injector was held at a temperature of 270ºC, and the mass spectrometry source at 250ºC. The injection volume of the sample solution was 1 µL by using a split ratio of 1:20. The oven temperature was programmed from 50º C to 170 ºC at 30 ºC/min, then from 170 ºC to 215 ºC at 2 ºC/min, and it rose to 4 ºC/min to 230 ºC. After that the temperature was held isothermally at 230 ºC for 7 min. The total analysis time was 38.25 min. Mass spectrometry with 70 eV electron ionization was carried out in multiple reaction monitoring mode, with at least two transitions per compound. Quantification of FAME was done with Masshunter (B.06.00). FAME peaks were identified and calibrated against a standard reference mixture of 33 FAMEs (Nu-Check-Prep) and two single FAME standards (Larodan Fine Chemicals AB). Relative percentages of fatty acids were calculated by taking the concentration of a given fatty acid derivative as a percentage of the total concentration of all fatty acids identified in the sample (Baumgartner *et al.*, 2012; Malan, 2016).

3.5 Data quality assurance and statistical analysis

Extreme values were identified according to a 3-standard deviation rule in order to identify and correct erroneous values. Data processing and statistical analysis of data was performed using SPSS (SPSS Inc, Chicago, IL, USA) and Excel Windows XP (Microsoft, Seattle, WA, USA). The dietary records were coded, quality was checked and data were cleaned by students under the supervision of a registered dietician. The dietary data analyses were performed by the MRC FoodFinder III software (MRC, South Africa).

Data was tested for normality by means of Q-Q plots and histograms visual inspection. The Kolmogorov Smirnoff test for normality assessment was also performed. Normally distributed data was described by using means and standard deviation, non-normally distributed data were reported as medians and an interquartile range (25th percentile, 75th percentile). Non-normal data was transformed to attempt normality before analysis.

Recreational runners were compared to healthy untrained controls, considering their plasma pro- and anti-inflammatory lipid mediator profiles over the time points (baseline, directly after, 1 hour, 2 hours, 24 hours and 72 hours). To this aim, a repeated measures analysis, adjusted for possible confounders (fatty acid status, antioxidant micronutrient intake as well as oxidative stress (ROS) and endogenous antioxidant parameters) was used. Type I error rate was set at 5%.

CHAPTER 4 RESULTS

4 RESULTS

A total of 10 lipid mediators were detected in human plasma, this included mediators derived from the COX, LOX and cytochrome P450 pathways. There are no normal values of lipid mediators thus there can be no comparison if the values were in normal range or not. Significant changes were identified in both pro-inflammatory and anti-inflammatory lipid mediators after exercise. Overall the peak elevation in most of the pro-inflammatory lipid mediators after exercise was detected at 2 hours for non-runners and 24 hours for runners. The anti-inflammatory lipid mediators were mostly stable for the runners, whereas the non-runners had different peaks for the different lipid mediators, except for 17-HDHA, which reached a peak in runners, but not in non-runners. All results found, including baseline characteristics and the lipid mediator changes over time, are described below.

4.1 Baseline characteristics

A total of 38 participants were enrolled, of which 19 were runners and 19 were non-runners. Two participants, one of each group did not complete the study and thus, their data were excluded. The baseline characteristics of the participants are displayed in **Table 4.1.- 4.6**. The ages of the participants ranged between 18 and 34 years. Their height ranged from 1.62 to 1.94 m, weight was between 48.0 and 92.9 kg, while their BMI ranged from 17.4 to 28.75 kg/m^2. Overall body fat percentage ranged from 1.8% to 43.5%.

Table 4-1: Baseline characteristics of participants[1]

	Sample number (per group)	Runners	Non-Runners	P-value
Age (y)	18	23.0 (22.00, 29.00)[2]	22.0 (20.00, 27.00)	0.245
Height (m)	18	1.80 ± 0.06[3]	1.76 ± 0.07	0.018
Weight (kg)	18	80.85 ± 8.69	70.32 ± 11.22	0.004
BMI (kg/m2)	18	24.56 ± 3.43	22.64 ± 3.05	0.115
Body fat percentage (%)	18	17.98 ± 9.61	15.34 ± 10.85	0.387
Haemoglobin (g/dL)	18	16.41 ± 1.19	16.07 ± 1.93	0.793
Systolic BP (mmHg)	18	130.0 (120.0,132.5)	130.0 (120.0,137.5)	0.693
Diastolic BP (mmHg)	18	80.0 (80.00, 80.00)	80.0 (72.50, 87.50)	0.547
Heart rate (bpm)	18	64.00 ± 8.80	65.90 ± 9.70	0.583
Temperature (˚C)	17 (R) /18 (NR)	36.00 (36.00, 36.00)	36.0 (35.50, 36.00)	0.716
Glucose (mm/dL)	17 (R) /18 (NR)	4.98 ± 0.33	5.08 ± 0.31	0.379

Abbreviations: n: number of participants, R: runner, NR: non-runner, y: years, m: meter, kg: kilograms, BMI: Body mass index, kg/m^2: kilogram/cubic meter, g/dL: grams/decilitre, mmHg: millimeter/mercury, bpm: beats per minute, ˚C: degree Celsius, mm/dL: millimeter/decilitre BP: Blood pressure [1]Differences between the runners and non-runners were examined by using independent t tests (P < 0.05). [2]Median, 25th, 75th percentile (all such values).[3]Mean ± SD (all such values).

Table 4-2: One repetition maximum (1RM) weights of participants at baseline[1]

	Sample number (per group)	Runners	Non-Runners	P-value
Smith machine (kg)	18	113.28 ± 26.962	100.33 ± 16.67	0.092
Leg press (kg)	18	246.50 ± 63.71	227.33 ± 57.31	0.349
Knee extension (kg)	18	58.83 ± 5.53	50.67± 5.53	0.001

[1]Differences between the runners and non-runners were examined by using independent t tests (P < 0.05).
[2]Mean ± SD (all such values).

Significant differences between the runners and non-runners can be seen in the knee extension exercise (**Table 4-2**); the runners could lift a heavier final weight compared to the non-runners (P = 0.001). **Table 4-1** shows that the althletes were taller and heavier than the non-runners (P = 0.018 and P = 0.04, respectively) at baseline.

Table 4-3: Exercise and activity level of participants[1].

	Sample number (per group)	Runner n (%)	Non-runner n (%)	P-value
Running				
Never	16 (R)/18 (NR)	0 (0)	14 (77.7)	< 0.001
Once per month	16 (R)/18 (NR)	1 (6.2)	0 (0)	
Once every one to two weeks	16 (R)/18 (NR)	3 (18.7)	3 (16.6)	
2-3 times per week	16 (R)/18 (NR)	10 (62.5)	1 (5.5)	
More than 4 times per week	16 (R)/18 (NR)	2 (12.5)	0 (0)	
Other exercises				
Never	16 (R)/18 (NR)	0 (0)	6 (33.3)	< 0.001
Once per month	16 (R)/18 (NR)	2 (12.5)	0 (0)	
Once every one to two weeks	16 (R)/18 (NR)	2 (12.5)	0 (0)	
2-3 times per week	16 (R)/18 (NR)	10 (62.5)	2 (11.1)	
More than 4 times per week	16 (R)/18 (NR)	2 (12.5)	10 (55.5)	
Activity level				
Active	16 (R)/18 (NR)	14 (87.5)	2 (11.1)	< 0.001
Moderately active	16 (R)/18 (NR)	2 (12.5)	6 (33.3)	
Inactive	16 (R)/18 (NR)	0 (0)	10 (55.5)	

Abbreviations: n (%): Number of participants (percentage), R: runner, NR: non-runner
[1]Differences between the runners and non-runners were examined by using the chi-square test (P < 0.05).

Four non-runners participants reported that they never jogged or ran for exercise (**Table 4-4**). Three reported that they jogged or ran once every one to two weeks and the other one reported that he ran 2-3 times a week. Ten out of the sixteen runners reported to jog or run two to three times a week and two runners reported that they jogged or run more than four times a week, while only one jogged or ran once every week, and three, once every one to two weeks. Runners jogged or ran significantly more than non-runners did ($P < 0.001$). Most of the participants reported that they did other types of exercises apart from their normal daily activity ($P < 0.001$). The runners reported that they were active (n=14) and moderately active (n=2) on a daily basis apart from their regular running. Two non-runners said they were active, six said they were moderately active and ten said they were inactive on a daily basis. Apart from regular running, the runner group was significantly more active than the non-runner group ($P < 0.001$).

Table 4-4: Baseline values of endogenous oxidative stress and oxidative stress adaptation markers[1]

	Runners (n = 18)	Non-Runners (n = 18)	P-value
Glutathione (μM)	1047 ± 117 [2]	1077 ± 120	0.444
Ferric reducing ability of plasma (μM)	473 ± 119	447 ± 112[3]	0.445
Reactive oxygen species (arbitrary units)	60.2 ± 10.7[3]	63.0 ± 11.6	0.465

[1]Differences between the runners and non-runners were examined by using independent t tests ($P < 0.05$);
[2]Mean ± SD (all such values);
[3]One data point is missing, n = 17;
[4]Two data points are missing, n = 17

There were no differences at baseline between oxidative stress markers of the runner and non-runner groups (**Table 4-4**). As shown in **Table 4-5**, eicosapentaenoic acid (EPA), monounsaturated fatty acids (MUFA), n-3 polyunsaturated fatty acids (PUFA) and n-3 long chain (LC) PUFA were higher in the runner group compared to the non-runner group ($P = 0.001$, $P = 0.045$, $P = 0.040$ and $P = 0.041$). On the other hand, the n-6:n-3 PUFA and the n-6:n-3 LCPUFA ratios were higher in the non-runner group compared to the runner group ($P = 0.032$ and $P = 0.031$).

The three-day dietary intake values are presented (**Table 4-6**) as an average of three consecutive days of dietary intakes. The average energy intake was above normal for both groups, indicating a possibility of over reporting. The runners' linoleic acid intake was higher than the intake of the non-runners ($P = 0.035$). Vitamin C intake was also significantly higher for the runners compared to the non-runners ($P = 0.028$). The vitamin E intake also differed between the two groups; the runners' intake being higher than the non-runners' ($P =0.013$).

Table 4-5: Baseline red blood cell total phospholipid fatty acid composition [1]

Fatty acids (% of total fatty acids)	Runners (n = 18)	Non-Runners (n = 18)	P-value
Docosahexaenoic acid	3.97 ± 0.72[2]	3.69 ± 0.57	0.211
Eicosapentaenoic acid	0.35 (0.31, 0.47)[3]	0.27 (0.21, 0.34)	0.001
Arachidonic acid	13.79 (12.34, 14.74)	14.24 (13.86, 14.51)	0.505
Saturated fatty acids	43.45 (43.08, 43.99)	43.64 (43.15, 44.41)	0.319
Trans fatty acids	0.40 (0.36, 0.47)	0.37 (0.29, 0.43)	0.311
Monounsaturated fatty acids	16.80 (16.04, 17.51)	16.27 (14.73, 16.67)	0.045
Total PUFA	38.90 (32.39, 34.83)	39.03 (38.18, 39.59)	0.610
n-3 PUFA	6.68 ± 1.14	5.96 ± 0.86	0.040
n-6 PUFA	32.49 (31.14, 33.47)	33.21 (32.39, 34.83)	0.091
n-3 long-chain PUFA	6.61 ± 1.13	5.89 ± 0.86	0.041
n-6 long-chain PUFA	20.78 (19.13, 21.83)	20.98 (20.34, 21.87)	0.347
n-6: n-3 PUFA ratio	4.96 ± 1.03	5.68 ± 0.88	0.032
n-6: n-3 long-chain PUFA ratio	3.14 ± 0.67	3.60 ± 0.54	0.031

Abbreviations: PUFA: polyunsaturated fatty acids;
[1]Differences between the runners and non-runners were examined by using independent t tests (P < 0.05);
[2]Mean ± SD (all such values);
[3]Median, 25th, 75th percentile (all such values).

Table 4-6: Average of three-day dietary intake[1]

	Runners (n = 18)	Non-Runners (n = 18)	P-value
Total Energy (kJ)	25302.64 ± 6209.00[2]	21707.72 ± 7397.46	0.135
Protein (g)	184.96 (138.46, 260.15)[3]	188.00 (146.66, 210.70)	0.813
Carbohydrates (g)	614.87 ± 207.27	545.27 ± 169.30	0.292
Fat (g)	281.20 (186.81, 363.95)	214.26 (120.40, 252.53)	0.082
Eicosapentaenoic acid (g)	0.06 (0.03, 0.14)	0.06 (0.02, 0.08)	0.358
Docosahexaenoic acid (g)	0.09 (0.01, 0.22)	0.16 (0.01, 0.15)	0.069
Arachidonic acid (g)	0.32 (0.22, 0.46)	0.24 (0.15, 0.40)	0.091
Linoleic acid (g)	47.32 (36.48, 90.37)	38.45 (21.68, 49.96)	0.035
Alpha-linolenic acid (g)	1.25 (1.05, 1.92)	0.91 (0.78, 1.56)	0.167
Vitamin C (mcg)	123.66 (65.16, 198.33)	58.00 (29.50, 116.16)	0.028
Vitamin E (mcg)	36.68 (25.13, 61.43)	23.79 (17.83, 30.58)	0.013
Carotenoids (mcg)	3856 (437, 6192)	2206 (1162, 3729)	0.894
Zinc (mg)	22.87 (17.70, 30.81)	22.95 (19.90, 31.03)	0.587

Abbreviations: g: Grams, mcg: microgram, mg: milligram;
[1]Differences between the runners and non-runners were examined by using independent t tests (P < 0.05);
[2]Mean ± SD (all such values); [3]Median, 25th, 75th percentile (all such values).

4.2 Between- group comparisons of lipid mediator responses

As summarised in **Table 4-7**, a significant change in response over time in the runners for 5-HETE and 12-HEPE (P = 0.005 and 0.008) and a trend for 12-HETE to change over time (P = 0.091) occurs. In the non-runners, a trend for 18-HEPE to change over time (P = 0.097) is observed.

Table 4-8 summarises the unadjusted and adjusted statistical analysis between the lipid mediator concentrations of the runner and non-runner groups. Adjustments were made for 1) BMI; 2) BMI and dietary intake differences at baseline (vitamin C and LA intake, but not vitamin E, as it correlated with LA); 3) BMI and endogenous oxidative stress markers at baseline (ROS, GSH and FRAP); 4) BMI, diet and endogenous oxidative stress markers; 5) BMI, diet, endogenous oxidative stress markers and n-6/n-3 LCPUFA status ratio. The unadjusted model showed a tendency to differ between the two groups for 5-HETE and 8-HETE (specifically at time point 2 (one hour after exercise) of 8-HETE) (P = 0.055, P = 0.058, P= 0.057). There was also a trend to differ at time point 2 (one hour after exercise) of 5-HETE and time point 5 (72 hours after exercise) of 12-HETE (P = 0.097, P = 0.094).

When adjusted for BMI and diet, 5-HETE, 15-HETE and 8-HETE showed a significantly different response over time between the two groups (P = 0.012, P = 0.033, P = 0.016). There was also a significant, different response for 8-HETE, and a trend to differ for 12-HEPE and 18-HEPE when adjusted for BMI and oxidative stress (P = 0.041, P = 0.059 and P = 0.085, respectively). 15-HETE showed a trend when adjusted for BMI and oxidative stress (P = 0.087). Adjusting for BMI, diet and oxidative stress also resulted in a significant difference between groups for 5-HETE (P = 0.016) and 8-HETE (P = 0.043), where 15-HETE and 17-HDHA (P = 0.087 and P = 0.089) only showed a trend to differ.

Table 4-7: Lipid mediator concentration responses over time per group[1,2]

	Runners (n = 13-14)	Non-runners (n = 13-14)
Pro-inflammatory lipid mediators metabolised from arachidonic acid by cyclooxygenase		
PGE_2 (pg/µl)	0.333	0.234
Pro-inflammatory lipid mediators metabolised from arachidonic acid by lipoxygenase		
5-HETE (pg/µl)	0.015	0.557
12-HETE (pg/µl)	0.091	0.240
15-HETE (pg/µl)	0.268	0.374
Pro-inflammatory lipid mediators metabolised from arachidonic acid by cytochrome P450		
8-HETE (pg/µl)	0.005	0.370
11-HETE (pg/µl)	0.772	0.867
Anti-inflammatory lipid mediators metabolised from docosahexaenoic acid by lipoxygenase and cyclooxygenase		
17-HDHA (pg/µl)	0.388	0.770
Anti-inflammatory lipid mediators metabolised from eicosapentaenoic acid by lipoxygenase		
12-HEPE (pg/µl)	0.008	0.328
18-HEPE (pg/µl)	0.302	0.097
Anti-inflammatory lipid mediators metabolised from eicosapentaenoic acid by cytochrome P450		
8-HEPE (pg/µl)	0.404	0.278

Abbreviations: HDHA: hydroxydocosahexaenoic acid, HEPE: hydroxyeicosapentaenoic acid, HETE: Hydroxyeicosatetraenoic acid

[1]Responses over time in the runners and non-runners were examined by using the repeated measures general linear regression test. Sphericity was assumed when $P > 0.05$ with Mauchly's test. Greenhouse-Geisser was used when sphericity was violated $P < 0.05$.

[2]All non-parametric data log transformed before analysis.

Table 4-8: Comparisons of lipid mediator concentration responses over time after an unaccustomed exercise bout between runners and non-runners, without and with adjustments for modulating factors[1,2,3]

LIPID MEDIATOR CONCENTRATIONS (PG/µL IN 2 ML PLASMA)				**P-VALUES FOR UNADJUSTED AND ADJUSTED MODELS**					
LIPID MEDIATORS	TIME	RUNNER	NON-RUNNER	**UN ADJUSTED**	**BMI[6]**	**BMI, DIET (LA, VIT C)[7]**	**BMI, OXIDATIVE STRESS[8]**	**BMI, DIET, OXIDATIVE STRESS[9]**	**BMI, DIET, OXIDATIVE STRESS, N-6/N-3 LCPUFA[10]**
PRO-INFLAMMATORY LIPID MEDIATORS METABOLISED FROM ARACHIDONIC ACID BY CYCLOOXYGENASE									
PGE_2	T0-T5			0.700	0.635	0.625	0.754	0.684	0.794
(N: R = 14, NR = 13)	T0	3.90 (0.40, 7.78)[4]	5.46 (1.20, 7.57)	0.695					
	T1	6.00 ± 5.24[5]	5.17 ± 5.03	0.645					
	T2	5.35 ± 5.82	6.00 ± 5.06	0.729					
	T3	6.73 (1.11, 10.45)	5.63 (2.02, 8.11)	0.920					
	T4	6.09 ± 5.69	5.57 ± 5.21	0.786					
	T5	6.83 ± 7.60	5.95 ± 4.40	0.697					
PRO-INFLAMMATORY LIPID MEDIATORS METABOLISED FROM ARACHIDONIC ACID BY LIPOXYGENASE									
5-HETE	T0-T5			0.055	0.113	0.012	0.105	0.016	0.100
(N: R = 14, NR = 13)	T0	142 (107, 166)	132 (103, 168)	0.603					
	T1	148 (117, 172)	148 (104, 173)	0.853					
	T2	101 (85, 126)	110 (96, 167)	0.097					
	T3	119 (89, 147)	113 (101, 154)	0.478					
	T4	138 (104, 184)	128 (117, 172)	0.957					
	T5	153 ± 52	160 ± 64	0.729					
12-HETE	T0-T5			0.269	0.466	0.516	0.390	0.371	0.298
(N: R = 14, NR = 13)	T0	0.30 (0.00, 0.51)	0.17 (0.00, 1.30)	0.707					
	T1	0.48 (0.10, 0.85)	0.38 (0.23, 1.66)	0.554					
	T2	0.67 (0.00, 5.36)	0.70 (0.11, 3.92)	0.847					
	T3	0.84 (0.00, 2.13)	1.16 (0.00, 9.08)	0.386					
	T4	0.27 (0.00, 1.55)	0.02 (0.00, 0.77)	0.490					
	T5	0.38 (0.02, 1.04)	0.30 (0.00, 2.87)	0.094					
15-HETE	T0-T5			0.229	0.233	0.033	0.087	0.087	0.295
(N: R = 13, NR = 13)	T0	2.45 (1.95, 4.39)	2.54 (1.77, 3.49)	0.719					
	T1	3.69 (2.12, 4.48)	2.46 (1.93, 3.24)	0.826					
	T2	2.17 (1.12, 2.70)	2.25 (1.32, 3.11)	0.312					
	T3	2.38 (1.68, 3.69)	2.13 (1.43, 2.84)	0.915					
	T4	3.05 (1.68, 4.73)	2.34 (1.41, 2.70)	0.386					
	T5	3.20 ± 1.75	2.91 ± 1.65	0.704					

LIPID MEDIATOR CONCENTRATIONS (PG/µL IN 2 ML PLASMA)				**P-VALUES FOR UNADJUSTED AND ADJUSTED MODELS**					
LIPID MEDIATORS	TIME	RUNNER	NON-RUNNER	**UN ADJUSTED**	**BMI[6]**	**BMI, DIET (LA, VIT C)[7]**	**BMI, OXIDATIVE STRESS[8]**	**BMI, DIET, OXIDATIVE STRESS[9]**	**BMI, DIET, OXIDATIVE STRESS, N-6/N-3 LCPUFA[10]**
PRO-INFLAMMATORY LIPID MEDIATORS METABOLISED FROM ARACHIDONIC ACID BY CYTOCHROME P450									
8-HETE	T0-T5			0.058	0.102	0.016	0.041	0.043	0.221
(N: R = 14, NR = 13)	T0	1.29 (1.01, 1.68)	1.24 (0.95, 1.82)	0.950					
	T1	1.51 (1.24, 1.76)	1.35 (1.14, 2.24)	0.943					
	T2	0.89 (0.72, 1.32)	0.99 (0.76, 1.95)	0.057					
	T3	0.91 (0.75, 1.38)	1.01 (0.88, 1.45)	0.147					
	T4	1.33 (1.06, 2.04)	1.14 (0.92, 1.81)	0.443					
	T5	1.51 (1.13, 1.74)	1.44 (1.18, 2.65)	0.987					
11-HETE	T0-T5			0.803	0. 795	0.774	0.850	0.770	0.721
(N: R = 13, NR = 13)	T0	0.49 (0.24, 0.78)	0.45 (0.05, 1.12)	0.653					
	T1	0.57 (0.29, 0.86)	0.48 (0.11, 1.47)	0.950					
	T2	0.17 (0.00, 1.08)	0.23 (0.06, 1.34)	0.527					
	T3	0.37 (0.05, 0.91)	0.23 (0.13, 0.86)	0.508					
	T4	0.54 (0.27, 1.07)	0.38 (0.07, 0.73)	0.313					
	T5	0.57 (0.25, 0.83)	0.60 (0.38, 1.39)	0.936					
ANTI-INFLAMMATORY LIPID MEDIATORS METABOLISED FROM DOCOSAHEXAENOIC ACID BY LIPOXYGENASE AND CYCLOOXYGENASE									
17-HDHA	T0-T5			0.430	0.654	0.188	0.355	0.089	0.154
(N: R = 13, NR = 13)	T0	4.54 (3.26, 6.15)	3.83 (2.97, 6.05)	0.668					
	T1	4.37 (3.64, 6.52)	3.77 (3.04, 5.92)	0.490					
	T2	3.34 (1.65, 4.68)	4.00 (2.58, 5.16)	0.280					
	T3	4.27 (2.60, 6.15)	3.44 (2.83, 5.92)	0.631					
	T4	5.00 (3.73, 6.08)	4.08 (3.18, 6.24)	0.674					
	T5	4.32 (3.06, 6.91)	4.74 (3.57, 6.10)	0.567					
ANTI-INFLAMMATORY LIPID MEDIATORS METABOLISED FROM EICOSAPENTAENOIC ACID BY LIPOXYGENASE									
12-HEPE	T0-T5			0.134	0.145	0.194	0.059	0.117	0.487
(N: R = 14, NR = 13)	T0	0.21 (0.12, 0.29)	0.20 (0.11, 0.42)	0.856					
	T1	0.26 (0.16, 0.31)	0.18 (0.13, 0.29)	0.525					
	T2	0.17 ± 0.12	0.21 ± 0.17	0.537					
	T3	0.21 (0.09, 0.30)	0.15 (0.09, 0.32)	0.559					
	T4	0.29 (0.11, 0.44)	0.16 (0.09, 0.29)	0.160					
	T5	0.24 (0.13, 0.36)	0.19 (0.16, 0.27)	0.983					
18-HEPE	T0-T5			0.405	0.476	0.471	0.085	0.183	0.783
(N: R = 14, NR = 13)	T0	0.32 (0.21, 0.46)	0.24 (0.21, 0.33)	0.482					
	T1	0.24 (0.20, 0.36)	0.24 (0.17, 0.34)	0.802					
	T2	0.19 (0.15, 0.24)	0.20 (0.15, 0.23)	0.475					
	T3	0.21 (0.13, 0.26)	0.17 (0.14, 0.40)	0.400					
	T4	0.21 (0.18, 0.36)	0.18 (0.14, 0.28)	0.238					
	T5	0.23 (0.20, 0.36)	0.19 (0.17, 1.03)	0.995					

LIPID MEDIATOR CONCENTRATIONS (PG/µL IN 2 ML PLASMA)				**P-VALUES FOR UNADJUSTED AND ADJUSTED MODELS**					
LIPID MEDIATORS	TIME	RUNNER	NON-RUNNER	**UN ADJUSTED**	**BMI[6]**	**BMI, DIET (LA, VIT C)[7]**	**BMI, OXIDATIVE STRESS[8]**	**BMI, DIET, OXIDATIVE STRESS[9]**	**BMI, DIET, OXIDATIVE STRESS, N-6/N-3 LCPUFA[10]**
ANTI-INFLAMMATORY LIPID MEDIATORS METABOLISED FROM EICOSAPENTAENOIC ACID BY CYTOCHROME P450									
8-HEPE	T0-T5			0.638	0.666	0.753	0.767	0.847	0.493
(N: R = 13, NR = 13)	T0	0.04 (0.03, 0.07)	0.04 (0.02, 0.07)	0.760					
	T1	0.05 (0.03, 0.08)	0.04 (0.03, 0.10)	0.865					
	T2	0.02 (0.01, 0.05)	0.03 (0.01, 0.06)	0.715					
	T3	0.03 (0.01, 0.06)	0.02 (0.00, 0.09)	0.595					
	T4	0.03 (0.02, 0.08)	0.02 (0.01, 0.06)	0.650					
	T5	0.05 (0.02, 0.08)	0.04 (0.03, 0.09)	0.614					

Abbreviations: HDHA: hydroxydocosahexaenoic acid, HEPE: hydroxyeicosapentaenoic acid, HETE: Hydroxyeicosatetraenoic acid, R: runner, NR: non-runner

[1]Differencent responses over time between the runners and non-runners were examined by using the repeated measures general linear regression test with runner/non-runner group as between-subject factor. Sphericity was assumed when P > 0.05 with Mauchly's test. Greenhouse-Geisser was used for the within-subjects tests when sphericity was violated P < 0.05.

[2]Differences between groups at each individual time point were analysed with independent t-tests

[3]All non-parametric data was log transformed before analysis.

[4]Median, 25th, 75th percentile (all such values).

[5]Mean ± SD (all such values).

[6]Adjusted for BMI.

[7]Adjusted for BMI, diet (LA, vit C).

[8]Adjusted BMI and oxidative stress.

[9]Adjusted BMI, diet and oxidative stress.

[10]Adjusted BMI, diet, oxidative stress, n-6/n-3 LCPUFA ratio

Figure 4-1 illustrates the pro- and anti-inflammatory metabolism of lipid mediators. In this figure, yellow highlights shows the lipid mediator that responded significantly different between the two groups over time ($p<0.05$); green shows tendencies ($0.05<p<0.08$) and light blue highlights shows lipid mediators that showed a trend to respond differently over time between the two groups ($0.08<p<0.100$). Therefore, 5- ,15- and 8-HETE responded significantly different over time and there was a tendency for 12-HEPE, and a trend for 17-HDHA and 18-HEPE to have a different response over time between the runners and non-runners among the different models discussed below.

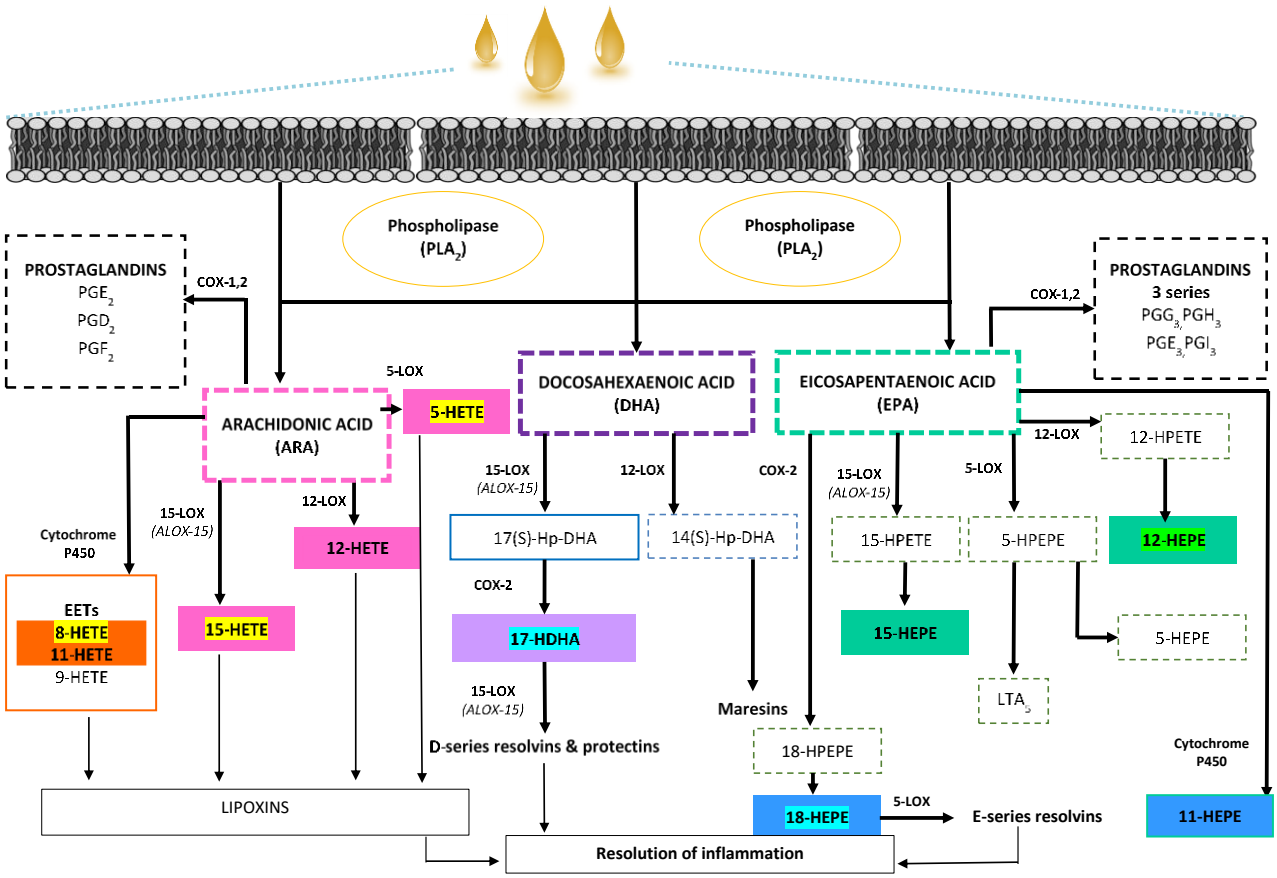

Figure 4-1: The pro- and anti-inflammatory metabolism of lipid mediators

The outline of the pathway for eicosanoid production from arachidonic acid and the pathway for production of resolvins and related mediators from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Cyclooxygenase (Sellayah *et al.*), lipoxygenase (LOX), hydroxyeicosatetraenoic acid (HETE), hydroperoxyeicosatetraenoic acid (HpETE), leukotriene (LT), lipoxin (LX), prostaglandin (PG), thromboxane (TX), leukotriene (LT), thromboxane (TX) and hydroperoxydocosahexaenoic acid (HDHA). 5-15 and 8-HETE are highlighted in yellow because they responded significantly different over time and 12-HEPE is highlighted in green because there was a tendency to have a different response over time where the runners had an earlier response than the non-runners among the different models. 17-HDHA are highlighted in blue because it showed a trend to have a different response over time where the the runners had higher concentrations than the non-runners among the different models. 18-HEPE are highlighted in

blue because it showed a trend to have a different response over time where the non-runners had higher concentrations than the runners among the different models.

Figures 4-2 to 4-7 are diagrammatic representations of the lipid mediator responses of runners and non-runners over the total response time. PGE_2 stays relatively stable between the two groups over the different time points.

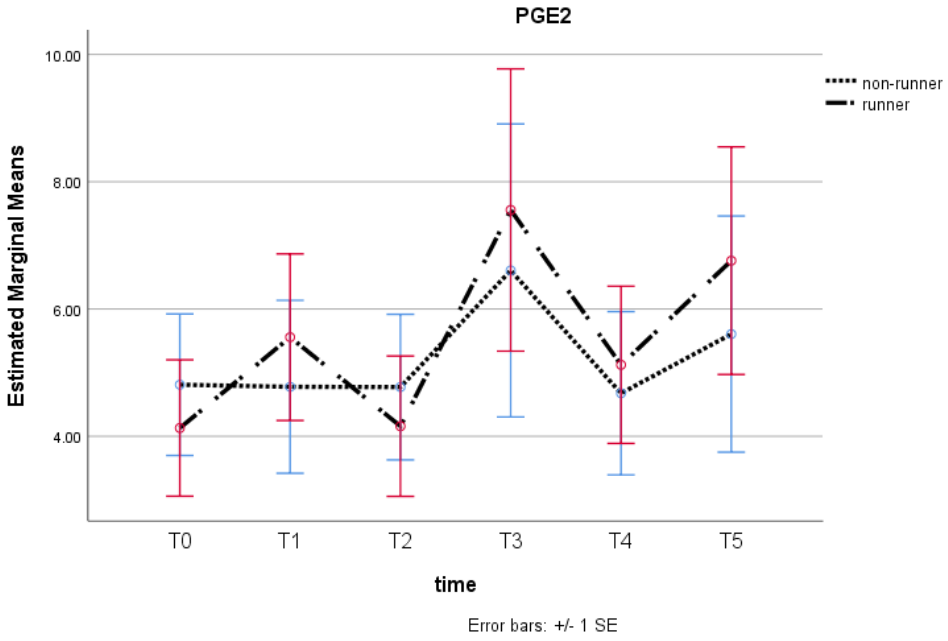

Figure 4-2: Response over time of runners versus non-runners for PGE2, a pro inflammatory lipid mediator metabolised from arachidonic acid by cyclooxygenase

Prostaglandin E2 (PGE2), Time (T), Before exercise (T0), directly after exercise (T1), 1 hour after exercise (T2), 2 hours after exercise (T3), 24 hours after exercise (T4) and 72 hours after exercise (T5).

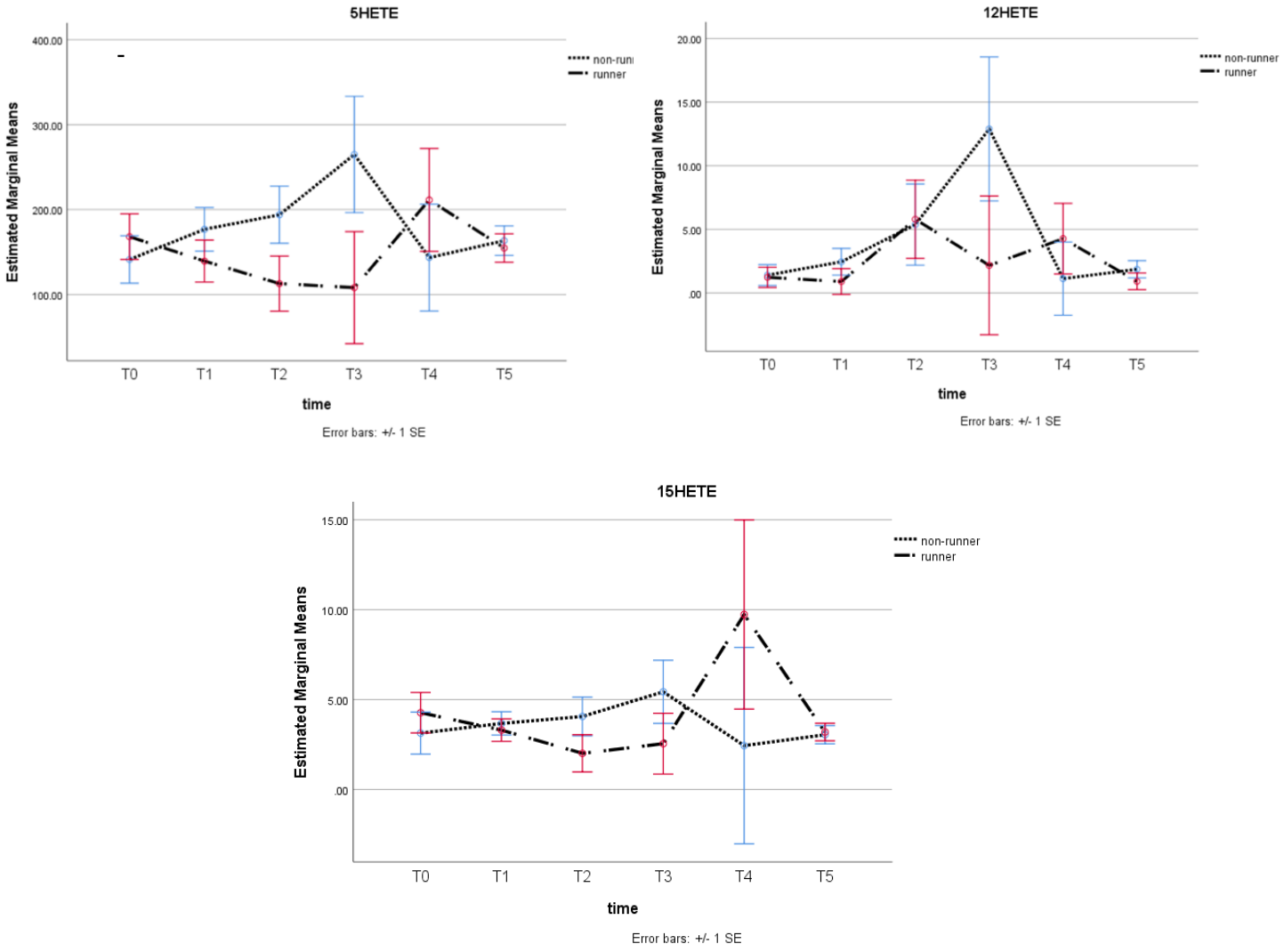

Figure 4-3: Responses over time of runners versus non-runners for pro-inflammatory lipid mediators metabolised from arachidonic acid by lipoxygenase a) 5-, b) 12- and c) 15-HETE, respectively

Hydroxyeicosatetraenoic acids (HETE), Time (T), Before exercise (T0), directly after exercise (T1), 1 hour after exercise (T2), 2 hours after exercise (T3), 24 hours after exercise (T4) and 72 hours after exercise (T5).

The 5-, 12, and 15-HETE lipid mediators start off relatively the same in both groups and then slowly rise to peak at 2 hours for non-runners and 24 hours for runners. By 72 hours both groups return to relatively the same amount as baseline.

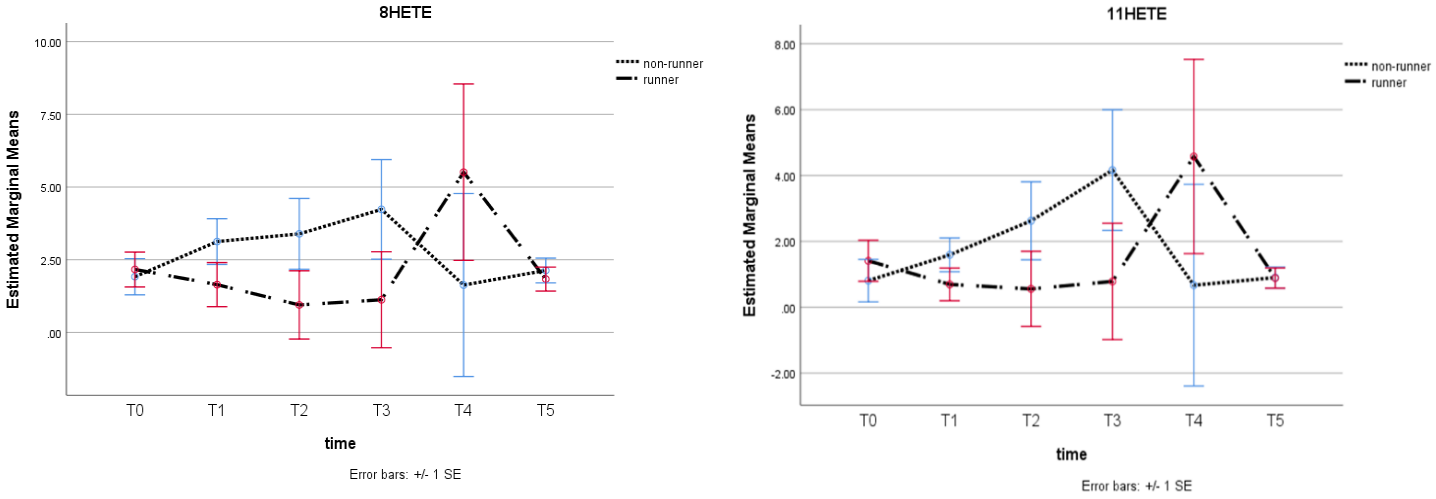

Figure 4-4: Responses over time of runners versus non-runners for pro-inflammatory lipid mediators metabolised from arachidonic acid by cytochrome P450; a) 8- and b) 11-HETE

Hydroxyeicosatetraenoic acids (HETE), Time (T), Before exercise (T0), directly after exercise (T1), 1 hour after exercise (T2), 2 hours after exercise (T3), 24 hours after exercise (T4) and 72 hours after exercise (T5).

The 8- and 12-HETE lipid mediators start off relatively similar in the groups and then slowly rise to peak at 2 hours for non-runners and 24 hours for runners. By 72 hours both groups return to relatively the same amount as baseline.

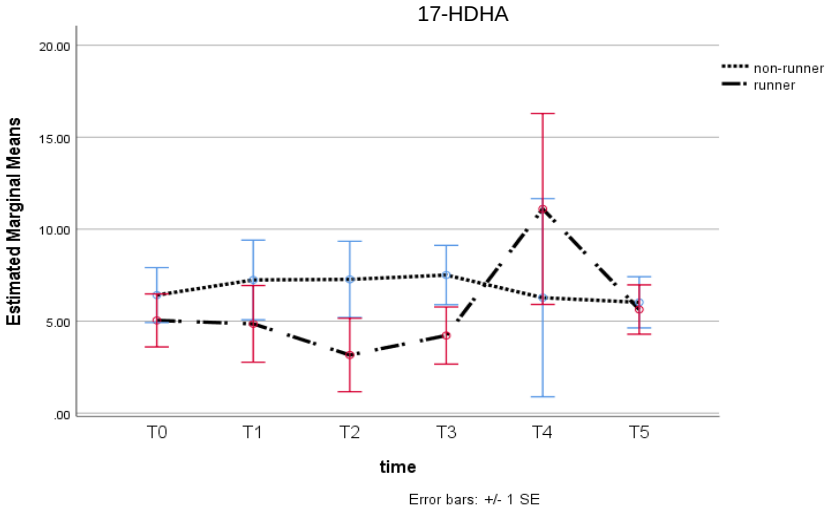

Figure 4-5: Response over time of runners versus non-runners for 17-HDHA; an anti-inflammatory lipid mediator metabolised from docosahexaenoic acid by lipoxygenase 15 and cyclooxygenase 2

Hydroxydocosahexaenoic acid (HDHA), Time (T), Before exercise (T0), directly after exercise (T1), 1 hour after exercise (T2), 2 hours after exercise (T3), 24 hours after exercise (T4) and 72 hours after exercise (T5).

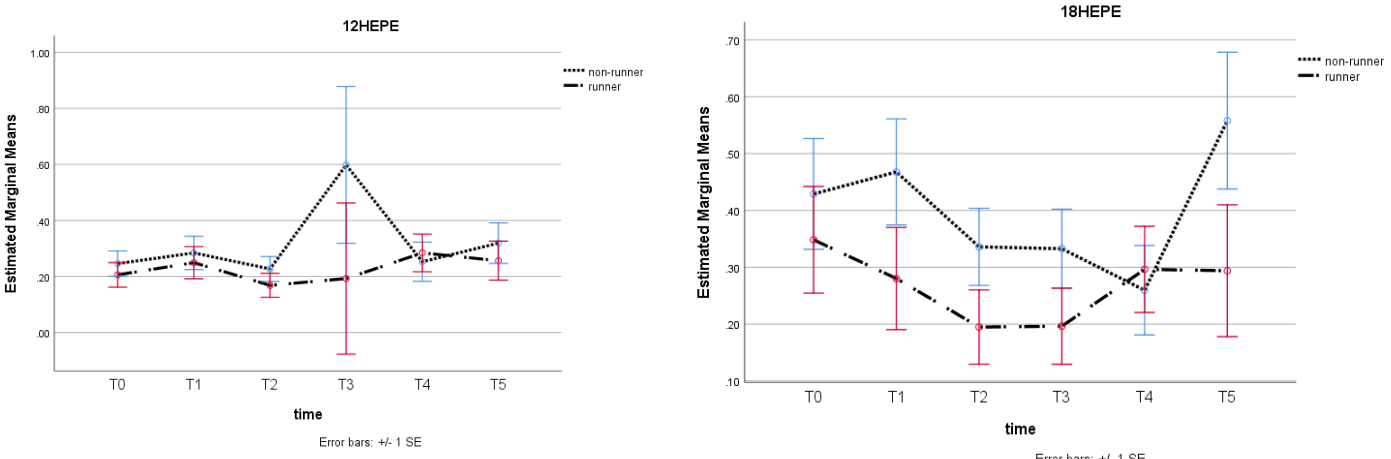

Figure 4-6: Responses over time of runners versus non-runners for a) 12-and b) 18-HEPE; anti-inflammatory lipid mediators metabolised from eicosapentaenoic acid by lipoxygenase 12 and cyclooxygenase 2, respectively.

Hydroxyeicosapentaenoic acids (HEPE), Time (T), Before exercise (T0), directly after exercise (T1), 1 hour after exercise (T2), 2 hours after exercise (T3), 24 hours after exercise (T4) and 72 hours after exercise (T5).

The 17-HDHA lipid mediator is very stable in the non-runner response, but the response in runners spikes at 24 hours. For 12-HEPE the non-runner spikes at 2 hours and for 18-HEPE it is generally higher, with a spike after 24 hours. 8-HEPE stays very stable for the runner, but for the non-runner, it peaks at 2 hours after exercise, drops at 24 hours and increases again at 72 hours after exercise.

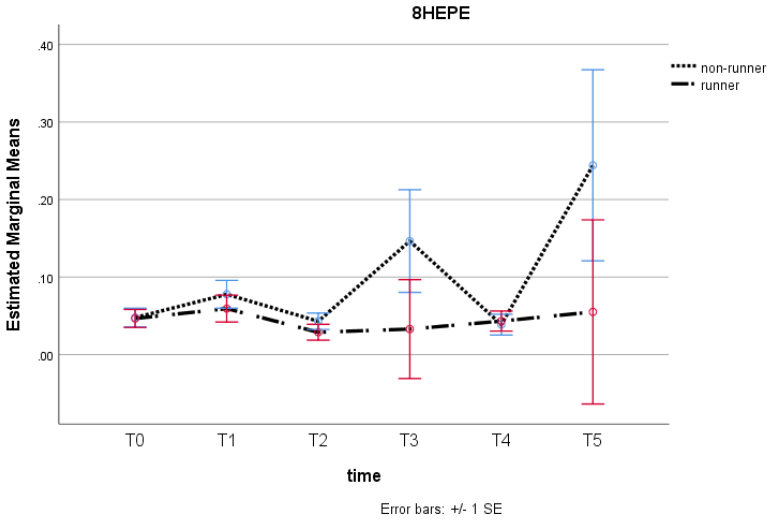

Figure 4-7: Response over time of runners versus non-runners for 8-HEPE; an anti-inflammatory lipid mediator metabolised from eicosapentaenoic acid by cytochrome P450.

Hydroxyeicosapentaenoic acids (HEPE), Time (T), Before exercise (T0), directly after exercise (T1), 1 hour after exercise (T2), 2 hours after exercise (T3), 24 hours after exercise (T4) and 72 hours after exercise (T5).

In general, the peak elevations after exercise in most of the pro-inflammatory lipid mediators, were detected at 2 hours for non-runners and 24 hours for runners. The anti-inflammatory lipid mediators were mostly stable for the runners, whereas the non-runners had different peaks at the different times, except for 17-HDHA, which spiked in the non-runners, but not in the runners. Overall, the results showed significant differences between the runners and non-runners that will be discussed in chapter 5.

CHAPTER 5 DISCUSSION

5 DISCUSSION

The present study characterised the changes in human plasma pro- and anti-inflammatory lipid mediator profiles after an exercise stress was performed. The lipid mediator response showed a significant difference between runners and non-runners post exercise. Lipid mediator profiles of human plasma were produced by LCMSMS targeted lipidomic analysis (Malan et al., 2016). A total of 10 lipid mediators were detected in human plasma. This included COX derived mediators, PGE_2 and 17-HDHA; LOX derived metabolites, 5-HETE, 12-HETE, 15-HETE, 12-HEPE and 18-HEPE; and cytochrome P450 derived mediators, 8-HETE, 11-HETE and 8-HEPE. These findings identify a complex lipid mediator response post-exercise that differs between runners and non-runners.

5.1 Participant baseline characteristics

5.1.1 Anthropometry

A healthy BMI falls between 18.5 and 24.9 kg/m^2 (Whitney & Rolfes, 2015). The BMI range of our participants ranged from 17.4 to 28.75 kg/m^2. Only one participant was under the healthy range and nine were over the healthy range. This still indicates that most of our participants had a healthy BMI. According to the American college of Sports Medicine, an acceptable body fat percentage for men falls between 10 to 22%, but an ideal body fat percentage for men is 5 to 12% (Medicine, 2013). Anything less than 5% starts to become a health risk (Medicine, 2013). Our participants' body fat % ranged from 1.8 to 43.5%, only three participants had less than 5% body fat and seven had more than 22% body fat, also indicating that most participants had a healthy body fat %.

The runners were also heavier than the non-runners group. This can be due to the fact that exercise can build muscle and reduce fat (Garber *et al.*, 2011; Thomas & Burns, 2016; Willis *et al.*, 2012). It is known that muscle has a greater density than fat does, which means that fat of the same amount as that of muscle mass, will take up more space (Deurenberg-Yap *et al.*, 2000; Leong & Yusuf, 2018). This can indicate that runners have more muscle mass which can cause their weight to be heavier. The runners were also taller than the non-runners, but there is no scientific reason for this.

Even though not significant, the runners' average fat % was still higher than that of the non-runners. According to some research, ROS are increased in adipose tissue through NADPH oxidase (Biswas, 2016; Furukawa *et al.*, 2017). It was also reported that an increase in free fatty acid levels in fat stimulates ROS production in adipose tissue by activating NADPH (Biswas, 2016; Deng *et al.*, 2016; Furukawa *et al.*, 2017). An uncontrolled amount of ROS causes oxidative stress which, in turn, causes inflammation (Biswas, 2016; Deng *et al.*, 2016; Furukawa *et al.*, 2017) . Since fat % was not significantly different between groups, and the model will be adjusted for ROS, fat % was not adjusted for.

Therefore, because weight and height can independently influence inflammation and there was a significant difference at baseline between the two groups, an adjustment was made for BMI (equals weight x height^2) in all statistical models, rather than for weight and height on their own, because height and weight correlated with each other ($r = 0.370$, $P = 0.026$).

5.1.2 One Repetition Max (1RM) weights of participants at baseline

There was only a significant difference between the two groups with the final weight at which the participants could lift in the knee extension exercise. Even though it should seem understandable that the runners should be able to lift heavier weights in all three exercises, it was not the case. Since we chose runners for our runner group, their regular exercise patterns did not include resistance exercises. Therefore, the fact that these two groups did not differ with regard to the other two exercises, one repetition max weights indicates that they had fairly equal leg strength at baseline. This finding confirms that the study design, with regard to the participants' strict inclusion and exclusion criteria, was of good quality. This significant difference in 1RM weight for knee extension was not corrected for in any model, as it correlated with BMI ($r = 0.374$, $P = 0.024$).

5.1.3 Exercise and activity level of participants

The results of the questionnaires of the participants' exercise and activity level also indicated a good study design. It is clear that there was an overall difference between the two groups. The runners ran significantly more days than the non-runners and the runners also reported to be more active than the non-runners, apart from their normal exercise programmes. Every participant also reported that they never did resistance exercises. These significant differences were not

corrected for in any model, as this result was merely used to confirm the study design and to confirm that the groups were well defined.

5.1.4 Oxidative stress and oxidative stress adaptation markers

It was found, even though not statistical significant, that glutathione (GSH) levels were lower in the runner group than in the non-runner group at baseline. This was unexpected; higher levels of GSH at baseline in the runners was expected due to the fact that regular exercise leads to the adaptation towards oxidative stress (Djordjevic *et al*., 2012; Powers & Jackson, 2008). Although regular exercise increases GSH, reduced levels of GSH correlates with the pathology of numerous diseases (Djordjevic *et al*., 2012). Glutathione can also be influenced by diet *(Choi et al.*, 2014) and genes (Perrone *et al*., 2005). Nevertheless, participants with an increased GSH status at rest upholds a more favourable redox status in responds to exercise-induced oxidative stress (Djordjevic *et al*., 2012).

While it was not significant, FRAP was higher in runners than in non-runners at baseline. This was to be expected, because FRAP measures the anti-oxidant capacity of an individual (Benzie & Strain, 1996; Marie *et al.*, 2017) and should show that the runners have a better ability to neutralise ROS (Benzie & Strain, 1996; Nikolaidis *et al.*, 2008).

Although not significant, ROS were lower in the runner group than in the non-runner group at baseline. According to Steinbacher *et al*. (2015), a single session of exercise causes oxidative stress in an untrained individual, but not in trained individuals this is not the case. This is likely due to the fact that trained individuals have a higher resistance to the oxidative stress, mediated by an adaptation response (Radak *et al.*, 2001; Steinbacher & Eckl, 2015). According to research, ROS are removed from cells under resting conditions, which prevents any subsequent damage (Kerksick & Willoughby, 2005). Nevertheless, when exercising, the body's endogenous antioxidant system cannot immediately remove the excessive ROS, which then leads to oxidative stress (Cabral-Santos *et al.*, 2015; Davis *et al.*, 2007; Kerksick & Willoughby, 2005; Malm, 2001; Markworth *et al.*, 2013).

According to research, long term exercise can reduce inflammation by reducing oxidative stress through adaptation of endogenous antioxidative systems (Lira *et al.*, 2012; Pingitore *et al.*, 2015). Although regular moderate exercise appears to be beneficial for oxidative stress and health, strenuous aerobic and anaerobic exercise can induce ROS overproduction and therefore can

induce an inflammatory response (Koenig *et al.*, 2016; Lira *et al.*, 2012; Pingitore *et al.*, 2015). Following strenuous exercise, the overproduction of ROS can cause not only cellular damage to muscles, but can also enhance the risk for systemic inflammatory response (Ji *et al.*, 2009; Koenig *et al.*, 2016). Little to no exercise, as well as strenuous exercise, can have a negative effect by leading to increased oxidative stress and the risk of related diseases (Pingitore *et al.*, 2015). Regular exercise, on the other hand, can be beneficial to health, because it reduces oxidative stress, protects against disease and improves performance and quality of life (Pingitore *et al.*, 2015).

While nothing of the oxidative stress markers was significant, an adjustment for oxidative stress markers was made in some of the models, to adjust for any possible adaptation. Adjustment for this factor was made to reveal whether changes in inflammatory response may occur independent of endogenous anti-oxidative stress adaptation.

5.1.5 Baseline red blood cell total phospholipid fatty acid composition

Eicosapentaenoic acid (EPA), n-3 PUFA and MUFA were higher in the runner group compared to the non-runner group. Phospholipid membranes of cells consist of about 30% n-6 PUFA, which is pro-inflammatory (Innes & Calder, 2018). On the other hand, protectins, maresins and resolvins are the most powerful pro-resolving mediators that are produced from n-3 LCPUFA, mostly EPA and DHA (Calder, 2015; Calder, 2017; Innes & Calder, 2018). The n-3 LCPUFA are commonly considered as anti-inflammatory, which means they promote the resolution of inflammation (Calder, 2015; Calder, 2017; Innes & Calder, 2018). Some studies have shown that when arachidonic acid, which is pro-inflammatory, was decreased, the anti-inflammatory effects of EPA and DHA was enhanced (Calder, 2015; Calder, 2017; Innes & Calder, 2018). This also implies that decreasing arachidonic acid can increase the effectiveness of EPA and DHA (Calder, 2015; Calder, 2017; Innes & Calder, 2018). This is reasonable, because n-6 and n-3 PUFA and their mediators are directly competing with one another for enzymes (Calder, 2015; Calder, 2017; Innes & Calder, 2018; Wada *et al.*, 2007).

Therefore, the n-6/n-3 LCPUFA ratio was adjusted for in the last model because they significantly differed between the two groups at baseline and included both the n-3 and n-6 PUFA moieties.

5.1.6 Three-day dietary intake

A possibility of over-reporting is indicated in the results as the average of energy intake between the three consecutive days of dietary intakes is above normal (8700 kilojoules per day) for both groups. Therefore, the dietary intake results may not be as accurate. However, since the same methodology was used for both groups, it can be assumed that the differences between the groups are relevant and useful in this analysis. The runners intakes of linoleic acid, vitamin C and vitamin E were higher than the intakes of the non-runners. Even though intake of AA, EPA and DHA contribute more to cell membrane composition than intake of their shorter chain precursors (ALA and LA); thus intake of these molecules are therefore major contributors to inflammation; there were no significant differences between the two groups' intake at baseline. Therefore, AA, EPA and DHA were not considered in the modelling. A quantified food frequency questionnaire may have given a more accurate estimation of intake and using a three day questionnaire could thus be seen as a limitation in this study.

Linoleic acid is essential as it cannot be synthesised in humans. However, it can be metabolised to other n-6 PUFAs namely gamma-linolenic acid (GLA), dihomo-gamma-linolenic acid (DGLA) and arachidonic acid. Some other n-6 PUFAs are then formed by metabolising arachidonic acid further. Linoleic acid can independently affect inflammation because its long-chain metabolite, arachidonic acid, is metabolised by the LOX pathway that produces 5-, 12- and 15-HETEs, which are pro-inflammatory lipid mediators (Innes & Calder, 2018; Malan, 2016; Masoodi *et al.*, 2008). Consequently, the higher the LA intake, the higher these pro-inflammatory lipid mediators could be expected to be, ultimately causing the inflammatory response to take place faster and difficult to resolve.

Dietary antioxidants include but are not limited to vitamin C, vitamin E, vitamin D, zinc, selenium and carotenoids (Knez *et al.*, 2006; Powers & Jackson, 2008; Steinbacher & Eckl, 2015; Urso & Clarkson, 2003). It has been shown that a low dietary intake of antioxidants can decrease an runners capability to detoxify exercise-induced ROS, leading to increased levels of oxidative stress (Slattery *et al.*, 2015). Therefore, research has shown that the intake of antioxidant supplements can reduce oxidative damage and markers of inflammation (Slattery *et al.*, 2015). Therefore, a higher vitamin C and E intake can ultimately help to reduce inflammation.

For these reasons LA and vitamin C was adjusted for in some models, but not vitamin E as it correlated with LA ($r = 0.488$, $P= 0.003$).

5.2 Lipid mediator responses over time in runners and non-runners subsequent to exercise stress

5.2.1 Overview of pro-inflammatory lipid mediator response

Lipid mediators derived from the n-6 LCPUFA, AA, include prostaglandins (PGE_2 via the COX-1 and -2); 5-,12, 15-HETE (via LOX); 8- and 11-HETE (via cytochrome P450) (Markworth *et al.*, 2013). These are well-known pro-inflammatory signalling molecules that increase vascular permeability, promote blood vessel vasodilation, encourage neutrophil activation and stimulate the signs of inflammation (Markworth *et al.*, 2013). At the beginning of the inflammatory response, phospholipase enzymes act on the phospholipids which release PUFAs; these include firstly AA and subsequently also EPA and DHA (Buckley *et al.*, 2014; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). A series of pathways, that includes the COX and LOX pathways, is then initiated which metabolises the fatty acids into the pro- and anti-inflammatory lipid mediators (**Figure 5-1**) (Buckley *et al.*, 2014; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). Arachidonic acid is known to be involved in the initiation phase of the inflammatory response by releasing pro-inflammatory prostaglandins and leukotrienes that initiate the early actions of host defence and inflammation (Buckley *et al.*, 2014; Markworth *et al.*, 2013; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). Each of these molecules causes certain inflammatory molecules to be released, which leads to an increase of inflammation (Buckley *et al.*, 2014; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). Arachidonic acid is also involved in the production of some more anti-inflammatory lipid mediators, regardless of its role in the initiation and development of inflammation, ultimately leading to resolution of inflammation (Buckley *et al.*, 2014; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). These mostly include lipoxins which are formed from pathways 15-, 12- and 5-LOX as well as Cytochrome P450 (Buckley *et al.*, 2014; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011).

5.2.1.1 Pro-inflammatory lipid mediators metabolised from arachidonic acid by cyclooxygenase

As mentioned above, PGE_2 is one of the first major lipid mediators that are released when inflammation occurs (Markworth *et al.*, 2013). The release of PGE_2 quickens the inflammatory process, eventually leading to acute inflammation (Markworth *et al.*, 2013; Serhan & Petasis, 2011). As seen in **Figure 5-2**, PGE_2 are formed within seconds or minutes after damage is caused

(Serhan & Petasis, 2011). Subsequently, upon response of the pro-inflammatory lipid mediator production, a lipid mediator class switching occurs, which initiates the production of pro-resolving lipid mediators (Fig 5-2). If these lipid mediators are not produced or are faulty, the acute inflammatory course remains unresolved and can lead to chronic inflammation (Serhan & Petasis, 2011). This can cause a wide range of diseases (Serhan & Petasis, 2011).

Even though PGE_2 was produced, there were no significant differences over time between the runners and non-runners, even in the adjusted models (Fig 4-2). However, similar to the current study, a recent study found an increase of PGE_2 2 hours post exercise (Markworth *et al.*, 2013). Other studies have found an increase of PGE_2 only 24-72-hours post exercise (Markworth *et al.*, 2013; Tartibian *et al.*, 2011; Uchida *et al.*, 2009). The reason for this is unclear, but it can be due to the exercise not being of the same intensity. Increasing the intensity of the anaerobic exercises can perhaps lead to a stronger pro-inflammatory lipid mediator response (Markworth *et al.*, 2013). PGE_2 is also of specific interest, because it is involved in all the processes that lead to the classic signs of inflammation such as swelling, redness and pain (Ricciotti & FitzGerald, 2011). PGE_2 have both anti-inflammatory and pro-inflammatory effects (Ricciotti & FitzGerald, 2011). These actions are regularly produced in relevant tissues, through the regulation of receptor gene expression (Ricciotti & FitzGerald, 2011). PGE_2 can therefore control numerous steps of inflammation in a context-dependent manner and can manage the whole process in both anti-inflammatory and pro-inflammatory directions (Ricciotti & FitzGerald, 2011). Acknowledging this, it makes sense that PGE_2 is more stable than the rest of the lipid mediators.

5.2.1.2 Pro-inflammatory lipid mediators metabolised from arachidonic acid by lipoxygenase

The production of lipid mediators from AA is demonstrated in **Figure 5-1**. Arachidonic acid is metabolised by the COX pathway and LOX pathways (Sellayah *et al*., 2014; Serhan, 2017b; Serhan *et al*., 2017a; Serhan & Petasis, 2011). The LOX pathway generates hydroperoxyeicosatetraenoic acids (HPETEs) which are metabolised to hydroxyeicosatetraenoic acids (HETEs) [5-HETE, 12-HETE, and 15-HETE] (Innes & Calder, 2018; Markworth *et al*., 2013; Sellayah *et al*., 2014; Serhan, 2017b; Serhan *et al.*, 2017a; Serhan & Petasis, 2011). The 12-LOX pathway produces 12-HETE, 15-LOX pathway produces 15-HETE and 5-LOX pathway produces 5-HETE from AA which possesses pro-inflammatory properties (Markworth *et al*., 2013). Therefore, the release of the HETE lipid mediators produced by LOX is one of the first things that happen in the inflammatory proses.

According to our results, there was a less severe response after exercise over time in the runners, indicated by the fact that these lipid mediators started off relatively the same between runners and non-runners and then peaked at around 2 hours for non-runners and 24 hours for runners. By 72 hours both groups returned to relatively the same amount as baseline (**Figures 4-3**). The runners concentration stayed relatively stable throughout the whole proses for 5- and 12 HETE. This indicates that, generally, non-runners produce more pro-inflammatory lipid mediators compared to runners and produce them earlier after the inflammation trigger (exercise) is administered. This indicates that runners cope better with inflammation. However, Markworth *et al. (2013)* found an increase of 12- and 15-HETE immediately after exercise and then again, an increase at 3 hours (12-HETE) and 24 hours (15-HETE) after exercise. According to Steinbacher *et al.* (2015), a single session of exercise causes oxidative stress and ultimately inflammation in an untrained individual, but in trained individuals, this is not the case, particularly for exercise their bodies are accustomed to. This is generally accepted to be due to the fact that trained individuals have a higher resistance to the oxidative stress (adaptation) and therefore also experience less inflammation, mediated by this adaptation response (Radak *et al.*, 2001; Steinbacher & Eckl, 2015). Oxidative stress and inflammation are closely related, as oxidative stress is one of the stimuli which trigger inflammation (Biswas, 2016; Castellani *et al.*, 2014).

The 5- and 15-HETE lipid mediators showed a difference in response over time between the two groups when adjusted for BMI and diet, indicating that, after correcting these baseline differences, there was a difference between the runners' and non-runners' responses. When adjusted for BMI, diet and oxidative stress adaptation, the 5-HETE response was different between groups and the response for 15-HETE tended to be different. However, no difference was seen when the model was adjusted for oxidative stress adaptation on its own. This indicates that both diet and oxidative stress adaptation affect the inflammatory response. However, both RBC n-6 and n-3 LCPUFA status differed between groups at baseline, and including the n-6:n-3 LCPUFA RBC status ratio in the model, resulted in the loss of the differences, indicating that n-6 and n-3 LCPUFA also play a significant role in the inflammatory response. This was expected, as LCPUFA act as the precursors of the lipid mediators.

5.2.1.3 Pro-inflammatory lipid mediators metabolised from arachidonic acid by cytochrome P450

As mentioned above, the cytochrome P450-metabolised lipid mediators are also some of the first mediators that are released in the inflammatory response. The unadjusted model showed a tendency to be different between the two groups and specifically at time point 2 (one hour after exercise). When adjusted for BMI and diet, 8-HETE showed a significantly different response over time between the two groups. There was also a significant different response when adjusted for BMI and oxidative stress. BMI, diet and oxidative stress also resulted in a significant difference between groups, with a loss of these effects when corrected for n-6:n-3 LCPUFA status. **Figures 4-4** clearly shows a pattern for these two lipid mediators. We can see that the runners' lipid mediators peak at around 24 hours after exercise and then drop to where it started before exercise. The non-runners lipid mediators peak at around 2 hours post-exercise and then drop to stabilise. This shows a clear indication that the non-runners also produce pro-inflammatory lipid mediators via the cytochrome P450 pathway faster than the runners, similar to the lipoxygenase-metabolised mediators. This can mean that they experience an inflammatory response before runners, indicating the adaptation to inflammation in runners.

The response over time, of pro-inflammatory mediators produced from arachidonic acid from both the lipoxygenase and cytochrome P450 pathways seems similar. They are both affected by the same factors, i.e. BMI, intake of LA and vitamin C, oxidative stress adaptation and n-6 and n-3 LCPUFA status.

5.2.2 Overview of the anti-inflammatory lipid mediator response

The production of lipid mediators from EPA and DHA is demonstrated in **Figure 5-1**. Eicosapentaenoic acid (EPA) is cleaved off the phospholipid of the cell membrane by the enzyme PLA_2 and is then metabolised by the COX-2, LOX or free radical catalysed pathways. The COX-2 pathway generates mediators including 18-HEPE which is further metabolised by the LOX-5 pathway to the E-series resolvins (RvE) (Fredman & Serhan, 2011; Markworth *et al.*, 2013; Masoodi *et al.*, 2008; Serhan, 2017b; Serhan & Petasis, 2011). A series of HEPEs is produced from the free radical oxidation pathway which includes 5-, 8-, 9-, 12-, 15-, and 18-HEPE. From the LOX pathway LTB_4 and 5-, 8-, 12- and 15-HEPE are formed (Fredman & Serhan, 2011; Masoodi *et al.*, 2008; Serhan, 2017b; Serhan & Petasis, 2011).

Docosahexaenoic acid is cleaved from the phospholipid in the cell membrane by the enzyme PLA_2 and is then metabolised by the LOX pathway (Fredman & Serhan, 2011; Masoodi *et al.*, 2008; Serhan, 2017b; Serhan & Petasis, 2011). This pathway produces 17S-HpDHA that is further metabolised to form the D-series resolvins (RvD) and protectins PD (Markworth *et al.*, 2013). An intermediate molecule in this pathway is 17-HDHA (Fredman & Serhan, 2011; Markworth *et al.*, 2013; Masoodi *et al.*, 2008; Serhan, 2017b; Serhan & Petasis, 2011). **Figure 5-2** shows that initially, mostly pro-inflammatory mediators are produced, while later, a lipid mediator class switching occurs which leads to the production of pro-resolving mediators that promote resolution (Serhan & Petasis, 2011).

5.2.2.1 Anti-inflammatory lipid mediators metabolised from docosahexaenoic acid by lipoxygenase and cyclooxygenase

Even though 17-HDHA was produced, there were no significant differences over time between the runners and non-runners. Another study found an increase immediately, 2 hours after and 24 hours after exercise in healthy males (Markworth *et al.*, 2013). Although not significant our runners also showed an increase 24 hour after exercise. However, when adjusted for BMI, diet and oxidative stress, 17-HDHA showed a trend to differ between runners and non-runners. This indicated that all these factors have a controlling influence on this lipid mediator. Although not significantly different between groups, it is also apparent from **Fig 4-5**, that while the non-runners responses was very stable, the responses in runners spiked at 24 hours. Anti-inflammatory lipid mediators did not respond to oxidative stress, but were triggered upon the rise of the inflammatory lipid mediators (**Fig 5-2**). This increased response of 17-HDHA in runners, who generally had a delayed and reduced inflammatory response, may therefore indicate that there may be an adaptation in runners to cope with inflammation, independent from oxidative stress adaptation.

5.2.2.2 Anti-inflammatory lipid mediators metabolised from eicosapentaenoic acid by lipoxygenase

There was a tendency toward a significant difference in response over time in the runners for 12-HEPE, and a trend for 18-HEPE, after adjusting for BMI and oxidative stress. These effects were lost when diet was added as covariate, and the effect was even less significant when corrected for n-6:n-3 LCPUFA status. In Figure 4-6 it is apparent that the responses in the non-runners

spiked at 2 hours for 12-HEPE and were generally higher for 18-HEPE, with a spike after 24 hours. Markworth *et al.* (2013) however, showed an increase of resolvin E (resolvins produced from 18-HEPE) immediately post exercise. Since the pro-inflammatory lipid mediators were generally less affected in the runners compared to the non-runners, it is expected that the runners' reactive response upon inflammation will also be less, as it was seen here. Similarly, the timing and response of the anti-inflammatory lipid mediators' response in the non-runners could be expected in following their higher and earlier inflammatory responses.

5.2.2.3 Anti-inflammatory lipid mediators metabolised from eicosapentaenoic acid by cytochrome P450

Even though 8-HEPE was produced, there were no significant differences over time between the runners and non-runners, even when adjusted for covariates. **Figure 4-7** shows that 8-HEPE stayed very stable for the runners, but for the non-runners, it peaked at 2 hours after exercise, dropped at 24 hours and increased again at 72 hours after exercise. This can indicate that the non-runners can't adapt to the exercise induced oxidative stress as well as the runners.

All these finding indicate that there were some differences between the runners and non-runners.

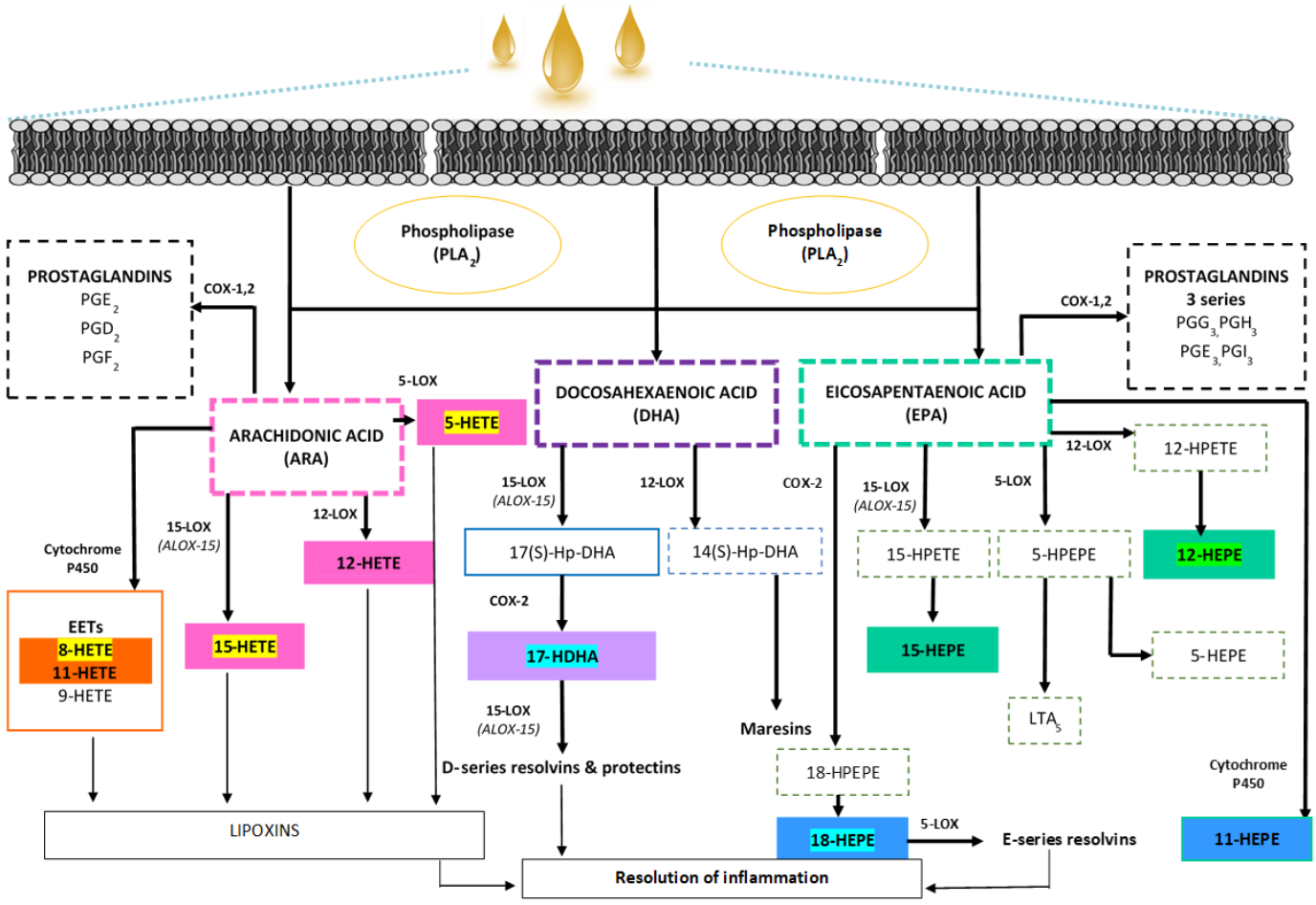

Figure 5-1: Inflammatory actions of lipid mediators derived from arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)

The outline of the pathway for eicosanoid production from arachidonic acid and the pathway for production of resolvins and related mediators from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Cyclooxygenase (Sellayah *et al.*), lipoxygenase (LOX), hydroxyeicosatetraenoic acid (HETE), hydroperoxyeicosatetraenoic acid (HpETE), leukotriene (LT), lipoxin (LX), prostaglandin (PG), thromboxane (TX), leukotriene (LT), thromboxan (TX) and hydroperoxydocosahexaenoic acid (HDHA). 5-,-15 and 8-HETE are highlighted in yellow, because they responded significantly different over time and 12-HEPE is highlighted in green, because there was a tendency to have a different response over time between the runners and non-runners among the different models. 17-HDHA and 18-HEPE are highlighted in blue, because they showed a trend to have a different response over time between the runners and non-runners among the different models.

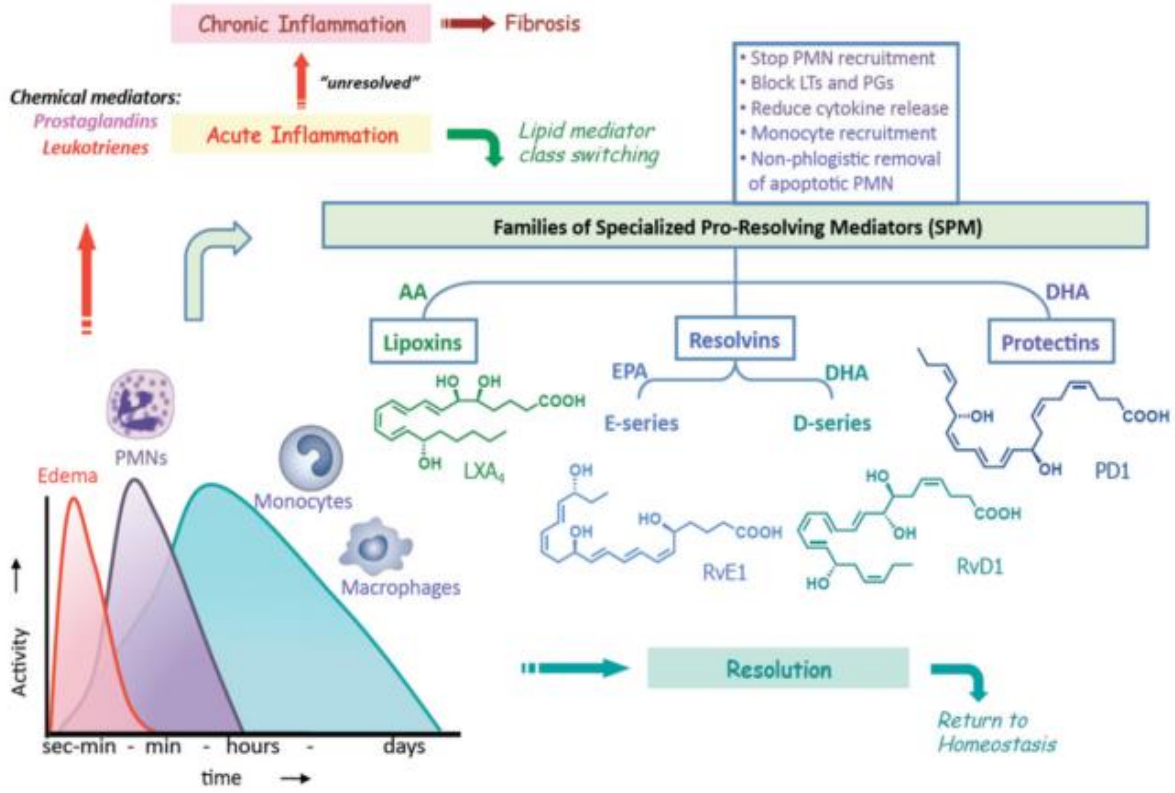

Figure 5-2: The inflammatory response and resolution time course (Serhan & Petasis, 2011)

The time course of the inflammatory response and resolution. The production of PUFA-derived lipid mediators during the inflammatory response increases with time. The initial mediators, prostaglandins and leukotrienes, are generated within seconds and minutes and regulate the recruitment of neutrophils. With time, hours up to days, there is an increasing leukocyte recruitment (monocytes and macrophages) and this leads to an increased number of mediators, involving these cells, which means pro-resolving mediators that promote resolution gets released at a later time. Leukotriens (LT), prostaglandins (PG), polymorphonuclear neutrophils (PMN), lipoxins (LX), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), Resolvins (Rv) (Serhan & Petasis, 2011).

CHAPTER 6 CONCLUSION

6 Conclusion

6.1 Summary

This study was designed to compare exercise-induced lipid mediator inflammatory signalling in male recreational runners with untrained males considering potential modulating factors such as fatty acid and antioxidant status.

Objective one was to measure the plasma lipid mediator concentrations (17-hydroxydocosahexaenoic acid (HDHA), 5- and 12-hydroxyeicosapentaenoic acid (HEPE), 5-, 8-, 11-, and 12- hydroxyeicosatetraenoic acid (HETE) and prostaglandin E_2 (PGE_2) before, and periodically after, an unaccustomed resistance exercise session. This showed that, when runners and non-runners exercised, both inflammatory and pro-resolving lipid mediators responded over time.

Objective number two was to measure markers of oxidative stress status, namely reactive oxygen species (ROS), ferric reducing antioxidant power (FRAP) and glutathione (GSH) levels before an unaccustomed resistance exercise session. There were no differences between the two groups at baseline, there might however be a difference after exercise. Therefore, a measurement of these markers after exercise will perhaps have shown that runners have less oxidative stress than non-runners after an unaccustomed exercise bout; and therefore, can also control inflammation better.

Objective number three was to assess the red blood cell fatty acid status and habitual antioxidant micronutrient intake, specifically of vitamin C, E, total carotenoids and zinc, before an unaccustomed resistance exercise session. We found that the runners' linoleic acid, vitamin C and vitamin E intake were higher in the runners than in the non-runners. From research it is also known that antioxidants and fatty acids play a substantial role in the inflammatory process. Linoleic acid (LA) can independently affect inflammation, because it is metabolised by the desaturase and elongase, and subsequently, the LOX pathway, to produce 5-, 12- and 15-HETE's, which are pro-inflammatory lipid mediators (Innes & Calder, 2018; Malan *et al*., 2016; Masoodi *et al*., 2008). Consequently, higher LA intake may ultimately promote the inflammatory response. Vitamin C and E are ant-oxidants and can reduce oxidative damage and markers of inflammation (Slattery *et al*., 2015). Therefore, a higher vitamin C and E intake can ultimately help to reduce inflammation.

Objective number four was to compare the plasma lipid mediator response to unaccustomed resistance exercise between the two groups when controlling for potential confounding factors including fatty acid and oxidative stress status, as well as antioxidant micronutrient and vitamin intake and endogenous antioxidant molecules.

This study showed that runners and non-runners have a different response to inflammation. According to our results, there is a less severe and delayed response after exercise over time in the runners compared to the non-runners. We could see that, generally, non-runners produce more pro-inflammatory lipid mediators compared to runners and produce them earlier after the inflammation trigger (exercise) was administered. It is generally accepted that this adaptation is mostly facilitated by the up-regulation of endogenous antioxidant systems, such as GSH, and as measured by FRAP, even though in the current study, there was no significant difference among groups before the exercise stress. The increased response of 17-HDHA in runners, however, indicates that runners may adapt to inflammation independently from oxidative stress adaptation. This indicates that runners are probably better equipped to handle inflammation.

It was also seen that BMI, diet and oxidative stress adaptation affect the inflammatory response. However, both RBC n-6 and n-3 LCPUFA status, including the n-6:n-3 LCPUFA ratio in the model, leads to loss of significant effects, indicating that n-6 and n-3 LCPUFA status (which was different between groups at baseline) plays a significant role in the inflammatory response. This was expected, as LCPUFA serve as the precursors of the lipid mediators.

6.2 Limitations and recommendations

Challenges experienced during this study included mostly the recruitment of participants. Posters for advertising could only have been put up in certain places around Potchefstroom; because of this, not many people were exposed to the study.

Another big challenge was to find males that did not do any resistance training, because of the large student population, most males do go to the gym to do resistance training even though they also run. Most participants that wanted to enrol also did not meet the strict inclusion criterion of being recreational runners only. People would say they are recreational runners, but upon further questioning would say they also cycle or do kickboxing. The three-day dietary data was under- or over-reported by the participants. Therefore, we would recommend that QFFQ data should be used, either with the three-day dietary data or on its own. The small sample size can also have a significant effect on all of the results; thus, a bigger sample size can contribute to better results.

The study was limited by the omission of the collection of the lean mass data. It could also be beneficial to show the lean mass records to see if there was a difference between the two groups. These data could have showed why the runners' mean weight was higher than the non-runners'. Gene expression of the COX and LOX pathways should be measured to investigate whether the adaptations in runners versus non-runners lie within this pathway.

Local production of pro-inflammatory and pro-resolving mediators within trained individuals may be metabolised or locally inactivated before reaching the blood compartment. Therefore, future studies should investigate intramuscular lipid mediator profile and would be useful in further characterization of the role of inflammatory and resolving lipid mediators during post-exercise recovery. Another limitation of the study is that plasma volume shift was not taken into consideration, and could have been different for trained and untrained individuals (Melin *et al*., 1980). Nevertheless, these results showed a different pattern of response over time, and did not only focus on absolute differences.

In conclusion, this study showed that lipid mediator responses are different among runners and non-runners and are affected by BMI, dietary intake of anti-oxidative nutrients and endogenous oxidative stress levels and adaptation. Furthermore, this study model, with a exercise stress to trigger the inflammatory response, can be useful in future to assess the effects of nutrition interventions on inflammation by means of the lipid mediator responses.

REFERENCES

Barja, G. 1999. Mitochondrial oxygen radical generation and leak: sites of production in states 4 and 3, organ specificity, and relation to aging and longevity. *Journal of bioenergetics and biomembranes*, 31(4):347-366.

Baumgartner, J., Smuts, C.M., Malan, L., Kvalsvig, J., van Stuijvenberg, M.E., Hurrell, R.F. & Zimmermann, M.B. 2012. Effects of iron and n-3 fatty acid supplementation, alone and in combination, on cognition in school children: a randomised, double-blind, placebo-controlled intervention in South Africa–. *The American journal of clinical nutrition*, 96(6):1327-1338.

Bedard, K. & Krause, K.-H. 2007. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiological reviews*, 87(1):245-313.

Benzie, I.F. & Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 239(1):70-76.

Betteridge, D.J. 2000. What is oxidative stress? *Metabolism*, 49(2):3-8.

Biaggi, R.R., Vollman, M.W., Nies, M.A., Brener, C.E., Flakoll, P.J., Levenhagen, D.K., Sun, M., Karabulut, Z. & Chen, K.Y. 1999. Comparison of air-displacement plethysmography with hydrostatic weighing and bioelectrical impedance analysis for the assessment of body composition in healthy adults–. *The American journal of clinical nutrition*, 69(5):898-903.

Biswas, S.K. 2016. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxidative medicine and cellular longevity*, 2016.

Bloomer, R.J., Larson, D.E., Fisher-Wellman, K.H., Galpin, A.J. & Schilling, B.K. 2009. Effect of eicosapentaenoic and docosahexaenoic acid on resting and exercise-induced inflammatory and oxidative stress biomarkers: a randomised, placebo controlled, cross-over study. *Lipids Health Dis*, 8:36.

Brandes, R.P., Weissmann, N. & Schröder, K. 2014. Nox family NADPH oxidases: molecular mechanisms of activation. *Free Radical Biology and Medicine*, 76:208-226.

Buckley, C.D., Gilroy, D.W. & Serhan, C.N. 2014. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. *Immunity*, 40(3):315-327.

Burdge, G.C. & Calder, P.C. 2015. Intravenous Lipid Emulsions. Karger Publishers. p. 1-16).

Cabral-Santos, C., Gerosa-Neto, J., Inoue, D.S., Panissa, V.L.G., Gobbo, L.A., Zagatto, A.M., Campos, E.Z. & Lira, F.S. 2015. Similar Anti-Inflammatory Acute Responses from Moderate-Intensity Continuous and High-Intensity Intermittent Exercise. *Journal of sports science & medicine*, 14(4):849.

Calder, P.C. 2006a. Polyunsaturated fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids*, 75(3):197-202.

Calder, P.C. 2006b. n− 3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *The American journal of clinical nutrition*, 83(6):S1505-1519S.

Calder, P.C. 2007. Immunomodulation by omega-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids*, 77(5-6):327-335.

Calder, P.C. 2010. Omega-3 fatty acids and inflammatory processes. *Nutrients*, 2(3):355-374.

Calder, P.C. 2012. Long-chain fatty acids and inflammation. *Proceedings of the Nutrition Society*, 71(2):284-289.

Calder, P.C. 2015. Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1851(4):469-484.

Calder, P.C. 2017. Omega-3 fatty acids and inflammatory processes: from molecules to man. *Biochemical Society Transactions*, 45(5):1105-1115.

Camps, J. & García-Heredia, A. 2014. Oxidative Stress and Inflammation in Non-communicable Diseases-Molecular Mechanisms and Perspectives in Therapeutics. Springer. p. 1-4).

Cárcamo, J.M., Pedraza, A., Bórquez-Ojeda, O., Zhang, B., Sanchez, R. & Golde, D.W. 2004. Vitamin C is a kinase inhibitor: dehydroascorbic acid inhibits IκBα kinase β. *Molecular and cellular biology*, 24(15):6645-6652.

Castellani, P., Balza, E. & Rubartelli, A. 2014. Inflammation, DAMPs, tumor development, and progression: a vicious circle orchestrated by redox signaling. *Antioxidants & redox signaling*, 20(7):1086-1097.

Choi, I.-Y., Lee, P., Denney, D.R., Spaeth, K., Nast, O., Ptomey, L., Roth, A.K., Lierman, J.A. & Sullivan, D.K. 2014. Dairy intake is associated with brain glutathione concentration in older adults–. *The American journal of clinical nutrition*, 101(2):287-293.

Cockbain, A., Toogood, G. & Hull, M. 2012. Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer. *Gut*, 61(1):135-149.

Davis, J.M., Murphy, E.A., Carmichael, M.D., Zielinski, M.R., Groschwitz, C.M., Brown, A.S., Gangemi, J.D., Ghaffar, A. & Mayer, E.P. 2007. Curcumin effects on inflammation and performance recovery following eccentric exercise-induced muscle damage. *Am J Physiol Regul Integr Comp Physiol*, 292(6):R2168-2173.

.

Deng, T., Lyon, C.J., Bergin, S., Caligiuri, M.A. & Hsueh, W.A. 2016. Obesity, inflammation, and cancer. *Annual Review of Pathology: Mechanisms of Disease*, 11:421-449.

Deurenberg-Yap, M., Schmidt, G., van Staveren, W.A. & Deurenberg, P. 2000. The paradox of low body mass index and high body fat percentage among Chinese, Malays and Indians in Singapore. *International journal of obesity*, 24(8):1011.

Djordjevic, D.Z., Cubrilo, D.G., Barudzic, N.S., Vuletic, M.S., Zivkovic, V.I., Nesic, M., Radovanovic, D., Djuric, D.M. & Jakovljevic, V. 2012. Comparison of blood pro/antioxidant levels before and after acute exercise in runners and non-runners. *Gen Physiol Biophys*, 31(2):211-219.

Dröge, W. 2002. Free radicals in the physiological control of cell function. *Physiological reviews*, 82(1):47-95.

Duarte, J., Appell, H.-J., Carvalho, F., Bastos, M. & Soares, J. 1993. Endothelium-derived oxidative stress may contribute to exercise-induced muscle damage. *International journal of sports medicine*, 14(08):440-443.

Durackova, Z. 2010. Some current insights into oxidative stress. *Physiological Research*, 59(4):459.

Edwards, I.J. & O'Flaherty, J.T. 2008. Omega-3 fatty acids and PPAR in cancer. *PPAR research*, 2008.

Eisen, D.P., Reid, D. & McBryde, E.S. 2012. Acetyl salicylic acid usage and mortality in critically ill patients with the systemic inflammatory response syndrome and sepsis. *Critical care medicine*, 40(6):1761-1767.

Falone, S., Mirabilio, A., Pennelli, A., Cacchio, M., Di Baldassarre, A., Gallina, S., Passerini, A. & Amicarelli, F. 2010. Differential impact of acute bout of exercise on redox-and oxidative damage-related profiles between untrained subjects and amateur runners. *Physiological research*, 59(6):953.

Fredman, G. & Serhan, C.N. 2011. Specialised proresolving mediator targets for RvE1 and RvD1 in peripheral blood and mechanisms of resolution. *Biochem J*, 437(2):185-197.

Frei, B., England, L. & Ames, B.N. 1989. Ascorbate is an outstanding antioxidant in human blood plasma. *Proceedings of the National Academy of Sciences*, 86(16):6377-6381.

Fullerton, J.N., O'Brien, A.J. & Gilroy, D.W. 2014. Lipid mediators in immune dysfunction after severe inflammation. *Trends Immunol*, 35(1):12-21.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M. & Shimomura, I. 2017. Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of clinical investigation*, 114(12):1752-1761.

Garber, C.E., Blissmer, B., Deschenes, M.R., Franklin, B.A., Lamonte, M.J., Lee, I.-M., Nieman, D.C. & Swain, D.P. 2011. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Medicine and science in sports and exercise*, 43(7):1334-1359.

Gomes, E.C., Silva, A.N. & Oliveira, M.R.d. 2012. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. *Oxidative medicine and cellular longevity*, 2012.

Harrison, R. 2002. Structure and function of xanthine oxidoreductase: where are we now? *Free Radical Biology and Medicine*, 33(6):774-797.

Hensley, K., Robinson, K.A., Gabbita, S.P., Salsman, S. & Floyd, R.A. 2000. Reactive oxygen species, cell signaling, and cell injury. *Free Radical Biology and Medicine*, 28(10):1456-1462.

Innes, J.K. & Calder, P.C. 2018. Omega-6 fatty acids and inflammation. *Prostaglandins, Leukotrienes and Essential Fatty Acids*.

Jackson, M.J., Vasilaki, A. & McArdle, A. 2016. Cellular mechanisms underlying oxidative stress in human exercise. *Free radical biology and medicine*, 98:13-17.

Ji, L., Gomez-Cabrera, M. & Vina, J. 2009. Role of antioxidants in muscle health and pathology. Infectious disorders special issue. *Infect Disord Drug Targets*, 9:428-444.

Judge, A. & Dodd, S. 2004. Xanthine oxidase and activated neutrophils cause oxidative damage to skeletal muscle after contractile claudication. *American Journal of Physiology-Heart and Circulatory Physiology*, 286(1):H252-H256.

Kang, C., Chung, E., Diffee, G. & Ji, L.L. 2013. Exercise training attenuates aging-associated mitochondrial dysfunction in rat skeletal muscle: role of PGC-1α. *Experimental gerontology*, 48(11):1343-1350.

Kerksick, C. & Willoughby, D. 2005. The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. *Journal of the International Society of Sports Nutrition*, 2(2):38.

Knez, W.L., Coombes, J.S. & Jenkins, D.G. 2006. Ultra-endurance exercise and oxidative damage. *Sports Medicine*, 36(5):429-441.

Koenig, R.T., Dickman, J.R., Kang, C.-H., Zhang, T., Chu, Y.-F. & Ji, L.L. 2016. Avenanthramide supplementation attenuates eccentric exercise-inflicted blood inflammatory markers in women. *European journal of applied physiology*, 116(1):67-76.

Leong, D.P. & Yusuf, S. 2018. Adiposity and mortality in South Asians: challenges to the existing paradigm. *The Lancet Global Health*, 6(7):e712-e713.

Li, D. 2015. Omega-3 polyunsaturated fatty acids and non-communicable diseases: Meta-analysis based systematic review. *Asia Pacific journal of clinical nutrition*, 24(1):10-15.

Lira, F.S.d., Yamashita, A.S., Rosa, J., Koyama, C., Caperuto, E., Batista Jr, M. & Seelaender, M.C.L. 2012. Exercise training decreases adipose tissue inflammation in cachectic rats. *Hormone and Metabolic Research*, 44(2):91.

Malan, L. 2016. Effects of iron and omega-3 supplementation on the immune system of iron deficient children in South Africa: a randomised controlled trial.

Malan, L., Baumgartner, J., Zandberg, L., Calder, P. & Smuts, C. 2016. Iron and a mixture of DHA and EPA supplementation, alone and in combination, affect bioactive lipid signalling and morbidity of iron deficient South African school children in a two-by-two randomised controlled trial. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, 105:15-25.

Malm, C. 2001. Exercise-induced muscle damage and inflammation: fact or fiction? *Acta Physiologica Scandinavica*, 171(3):233-239.

Marie, D., Ateba, G., Felicite, K., Fernando, K. & Chia, A. 2017. Oxidative Stress in Patients with Chronic Inflammatory Diseases in a Tertiary Health Care Setting in Africa. *J Autoimmune Disord*, 3(4):47.

Markworth, J.F., Vella, L., Lingard, B.S., Tull, D.L., Rupasinghe, T.W., Sinclair, A.J., Maddipati, K.R. & Cameron-Smith, D. 2013. Human inflammatory and resolving lipid mediator responses

to resistance exercise and ibuprofen treatment. *Am J Physiol Regul Integr Comp Physiol*, 305(11):R1281-1296.

Masoodi, M., Mir, A.A., Petasis, N.A., Serhan, C.N. & Nicolaou, A. 2008. Simultaneous lipidomic analysis of three families of bioactive lipid mediators leukotrienes, resolvins, protectins and related hydroxy-fatty acids by liquid chromatography/electrospray ionisation tandem mass spectrometry. *Rapid Commun Mass Spectrom*, 22(2):75-83.

Mathee, A., Naicker, N., Kootbodien, T., Mahuma, T., Nkomo, P., Naik, I. & De Wet, T. 2014. A cross-sectional analytical study of geophagia practices and blood metal concentrations in pregnant women in Johannesburg, South Africa. *South African Medical Journal*, 104(8):568-573.

Medicine, A.C.o.S. 2013. ACSM's health-related physical fitness assessment manual: Lippincott Williams & Wilkins.

Medzhitov, R. 2008. Origin and physiological roles of inflammation. *Nature*, 454(7203):428.

Melin, B., Eclache, J.P., Geelen, G., Annat, G., Allevard, A.M., Jarsaillon, E., Zebidi, A., Legros, J.J. and Gharib, C.L. 1980. Plasma AVP, neurophysin, renin activity, and aldosterone during submaximal exercise performed until exhaustion in trained and untrained men. *European journal of applied physiology and occupational physiology,* 44(2):141-151.

Minihane, A.M., Vinoy, S., Russell, W.R., Baka, A., Roche, H.M., Tuohy, K.M., Teeling, J.L., Blaak, E.E., Fenech, M. & Vauzour, D. 2015. Low-grade inflammation, diet composition and health: current research evidence and its translation. *British Journal of Nutrition*, 114(7):999-1012.

Moylan, J.S. & Reid, M.B. 2007. Oxidative stress, chronic disease, and muscle wasting. *Muscle & nerve*, 35(4):411-429.

Muller, F.L., Liu, Y. & Van Remmen, H. 2004. Complex III releases superoxide to both sides of the inner mitochondrial membrane. *Journal of Biological Chemistry*, 279(47):49064-49073.

Nelson, G., Kelly, D., Emken, E., Phinney, S., Kyle, D. & Ferretti, A. 1997. A human dietary arachidonic acid supplementation study conducted in a metabolic research unit: Radionale and design. *Lipids*, 32(4):415-420.

Neto, J.R., Lira, F., De Mello, M. & Santos, R.V.T. 2011. Importance of exercise immunology in health promotion. *Amino Acids*, 41(5):1165-1172.

Niess, A.M. 2007. Response and adaptation of skeletal muscle to exercise - the role of reactive oxygen species. *Frontiers in Bioscience*, 12(12):4826.

Nikolaidis, M.G., Jamurtas, A.Z., Paschalis, V., Fatouros, I.G., Koutedakis, Y. & Kouretas, D. 2008. The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress. *Sports Medicine*, 38(7):579-606.

O'Neal Jr, H.R., Koyama, T., Koehler, E.A., Siew, E., Curtis, B.R., Fremont, R.D., May, A.K., Bernard, G.R. & Ware, L.B. 2011. Prehospital statin and aspirin use and the prevalence of severe sepsis and ALI/ARDS. *Critical care medicine*, 39(6):1343.

Otto, G.P., Sossdorf, M., Boettel, J., Kabisch, B., Breuel, H., Winning, J. & Lösche, W. 2013. Effects of low-dose acetylsalicylic acid and atherosclerotic vascular diseases on the outcome in patients with severe sepsis or septic shock. *Platelets*, 24(6):480-485.
Peake, J. & Suzuki, K. 2004. Neutrophil activation, antioxidant supplements and exercise-induced oxidative stress. *Exerc Immunol Rev*, 10(2):129-141.

Pedersen, B.K. 2009. The diseasome of physical inactivity–and the role of myokines in muscle–fat cross talk. *The Journal of physiology*, 587(23):5559-5568.

Pedersen, B.K. & Febbraio, M.A. 2008. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiological reviews*, 88(4):1379-1406.

Perrone, G.G., Grant, C.M. & Dawes, I.W. 2005. Genetic and environmental factors influencing glutathione homeostasis in Saccharomyces cerevisiae. *Molecular biology of the cell*, 16(1):218-230.

Pingitore, A., Lima, G.P., Mastorci, F., Quinones, A., Iervasi, G. & Vassalle, C. 2015. Exercise and oxidative stress: potential effects of antioxidant dietary strategies in sports. *Nutrition*, 31(7-8):916-922.

Powers, S.K. & Jackson, M.J. 2008. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev*, 88(4):1243-1276.

Powers, S.K., Nelson, W.B. & Hudson, M.B. 2011. Exercise-induced oxidative stress in humans: cause and consequences. *Free Radical Biology and Medicine*, 51(5):942-950.

Pradelli, L., Mayer, K., Muscaritoli, M. & Heller, A.R. 2012. n-3 fatty acid-enriched parenteral nutrition regimens in elective surgical and ICU patients: a meta-analysis. *Crit Care*, 16(5):R184.

Prescott, S.L. 2014. Disease prevention in the age of convergence-the need for a wider, long ranging and collaborative vision. *Allergology International*, 63(1):11-20.

Radak, Z., Taylor, A.W., Ohno, H. & Goto, S. 2001. Adaptation to exercise-induced oxidative stress: from muscle to brain. *Exercise immunology review*, 7:90-107.

Radak, Z., Zhao, Z., Koltai, E., Ohno, H. & Atalay, M. 2013. Oxygen consumption and usage during physical exercise: the balance between oxidative stress and ROS-dependent adaptive signaling. *Antioxidants & redox signaling*, 18(10):1208-1246.

Rangel-Huerta, O.D., Aguilera, C.M., Mesa, M.D. & Gil, A. 2012. Omega-3 long-chain polyunsaturated fatty acids supplementation on inflammatory biomakers: a systematic review of randomised clinical trials. *Br J Nutr*, 107 Suppl 2:S159-170.

Ricciotti, E. & FitzGerald, G.A. 2011. Prostaglandins and inflammation. *Arteriosclerosis, thrombosis, and vascular biology*, 31(5):986-1000.

Sakellariou, G.K., Vasilaki, A., Palomero, J., Kayani, A., Zibrik, L., McArdle, A. & Jackson, M.J. 2013. Studies of mitochondrial and nonmitochondrial sources implicate nicotinamide adenine dinucleotide phosphate oxidase (s) in the increased skeletal muscle superoxide generation that occurs during contractile activity. *Antioxidants & redox signaling*, 18(6):603-621.

Seifi-skishahr, F., Damirchi, A., Farjaminezhad, M. & Babaei, P. 2016. Physical training status determines oxidative stress and redox changes in response to an acute aerobic exercise. *Biochemistry research international*, 2016.
Sellayah, D., Cagampang, F.R. & Cox, R.D. 2014. On the evolutionary origins of obesity: a new hypothesis. *Endocrinology*, 155(5):1573-1588.

Serhan, C.N. 2017b. Treating inflammation and infection in the 21st century: new hints from decoding resolution mediators and mechanisms. *The FASEB Journal*, 31(4):1273-1288.

Serhan, C.N., Chiang, N. & Dalli, J. 2017a. New pro-resolving n-3 mediators bridge resolution of infectious inflammation to tissue regeneration. *Molecular aspects of medicine*.

Serhan, C.N. & Petasis, N.A. 2011. Resolvins and protectins in inflammation resolution. *Chemical reviews*, 111(10):5922-5943.

Shelton, J. & Kumar, G.V.P. 2010. Sodium Bicarbonate—A Potent Ergogenic Aid? *Food and Nutrition Sciences*, 1(01):1.

Sies, H. & Jones, D. 2007. Oxidative stress.

Sjödin, B., Westing, Y.H. & Apple, F.S. 1990. Biochemical mechanisms for oxygen free radical formation during exercise. *Sports Medicine*, 10(4):236-254.

Slattery, K., Bentley, D. & Coutts, A.J. 2015. The role of oxidative, inflammatory and neuroendocrinological systems during exercise stress in runners: implications of antioxidant supplementation on physiological adaptation during intensified physical training. *Sports Medicine*, 45(4):453-471.

Smith, W.L. 1989. The eicosanoids and their biochemical mechanisms of action. *Biochemical Journal*, 259(2):315.
Sorci, G. & Faivre, B. 2009. Inflammation and oxidative stress in vertebrate host-parasite systems. *Philos Trans R Soc Lond B Biol Sci*, 364(1513):71-83.

Sossdorf, M., Otto, G.P., Boettel, J., Winning, J. & Lösche, W. 2013. Benefit of low-dose aspirin and non-steroidal anti-inflammatory drugs in septic patients. *Critical Care*, 17(1):402.

Sriram, S., Subramanian, S., Juvvuna, P.K., Ge, X., Lokireddy, S., McFarlane, C.D., Wahli, W., Kambadur, R. & Sharma, M. 2014. Myostatin augments muscle-specific ring finger protein-1 expression through an NF-kB independent mechanism in SMAD3 null muscle. *Molecular endocrinology*, 28(3):317-330.

Steinbacher, P. & Eckl, P. 2015. Impact of Oxidative Stress on Exercising Skeletal Muscle. *Biomolecules*, 5(2):356-377.

Storz, G. & Imlayt, J.A. 1999. Oxidative stress. *Current opinion in microbiology*, 2(2):188-194.

Tartibian, B., Maleki, B.H. & Abbasi, A. 2011. Omega-3 fatty acids supplementation attenuates inflammatory markers after eccentric exercise in untrained men. *Clinical Journal of Sport Medicine*, 21(2):131-137.

Thannickal, V.J. & Fanburg, B.L. 2000. Reactive oxygen species in cell signaling. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 279(6):L1005-L1028.

Thomas, M.H. & Burns, S.P. 2016. Increasing lean mass and strength: A comparison of high frequency strength training to lower frequency strength training. *International journal of exercise science*, 9(2):159.

Toft, A.D., Thorn, M., Ostrowski, K., Asp, S., Møller, K., Iversen, S., Hermann, C., Søndergaard, S.R. & Pedersen, B.K. 2000. N-3 polyunsaturated fatty acids do not affect cytokine response to strenuous exercise. *Journal of applied physiology*, 89(6):2401-2406.

Traber, M.G. & Stevens, J.F. 2011. Vitamins C and E: beneficial effects from a mechanistic perspective. *Free Radical Biology and Medicine*, 51(5):1000-1013.

Tsikas, D. 2016. Quantitative analysis of eicosanoids in biological samples by LC-MS/MS: mission accomplished? *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 1012:1013.

Turrens, J.F. 2003. Mitochondrial formation of reactive oxygen species. *J Physiol*, 552(Pt 2):335-344.

Uchida, M.C., Nosaka, K., Ugrinowitsch, C., Yamashita, A., Martins Jr, E., Moriscot, A.S. & Aoki, M.S. 2009. Effect of bench press exercise intensity on muscle soreness and inflammatory mediators. *Journal of sports sciences*, 27(5):499-507.

Urso, M.L. & Clarkson, P.M. 2003. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*, 189(1-2):41-54.

Wada, M., DeLong, C.J., Hong, Y.H., Rieke, C.J., Song, I., Sidhu, R.S., Yuan, C., Warnock, M., Schmaier, A.H. & Yokoyama, C. 2007. Enzymes and receptors of prostaglandin pathways with arachidonic acid-vs. eicosapentaenoic acid-derived substrates and products. *Journal of Biological Chemistry*.

Walsh, N.P., Gleeson, M., Shephard, R.J., Gleeson, M., Woods, J.A., Bishop, N., Fleshner, M., Green, C., Pedersen, B.K. & Hoffman-Goete, L. 2011. Position statement part one: immune function and exercise.

Whitney, E. & Rolfes, S.R. 2015. Understanding Nutrition. 14e: Canada.

Willis, L.H., Slentz, C.A., Bateman, L.A., Shields, A.T., Piner, L.W., Bales, C.W., Houmard, J.A. & Kraus, W.E. 2012. Effects of aerobic and/or resistance training on body mass and fat mass in overweight or obese adults. *Journal of applied physiology*, 113(12):1831-1837.

Winning, J., Reichel, J., Eisenhut, Y., Hamacher, J., Kohl, M., Deigner, H.P., Claus, R.A., Bauer, M. & Lösche, W. 2009. Anti-platelet drugs and outcome in severe infection: clinical impact and underlying mechanisms. *Platelets*, 20(1):50-57.

Xia, R., Webb, J.A., Gnall, L.L., Cutler, K. & Abramson, J.J. 2003. Skeletal muscle sarcoplasmic reticulum contains a NADH-dependent oxidase that generates superoxide. *American Journal of Physiology-Cell Physiology*, 285(1):C215-C221.

Zhao, X., Bey, E.A., Wientjes, F.B. & Cathcart, M.K. 2002. Cytosolic Phospholipase A2 (cPLA2) Regulation of Human Monocyte NADPH Oxidase Activity cPLA2 AFFECTS TRANSLOCATION

BUT NOT PHOSPHORYLATION OF p67 phox AND p47 phox. *Journal of Biological Chemistry*, 277(28):25385-25392.

ANNEXURES 1 ETHICAL APPROVAL

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Web: http://www.nwu.ac.za

Health Sciences Ethics Office for Research, Training and Support

Health Research Ethics Committee (HREC)
Tel: 018-285 2291
Email: Wayne.Towers@nwu.ac.za

Dr L Malan
Nutrition
CEN

22 November 2018

Dear Dr Malan

APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00061-16-A1-03

Kindly use the ethics reference number provided above in all future correspondence or documents submitted to the administrative assistant of the Health Research Ethics Committee (HREC) secretariat.

Study title: Exercise-induced lipid mediator inflammatory signalling in male recreational runners versus untrained males considering potential modulating factors

Study leader: Dr L Malan

Student: E Bezuidenhout-24185264

Application type: Sub-study

Risk level: Minimal (monitoring report required annually)

Expiry date: 30 November 2019 (monitoring report is due at the end of November annually until completion)

You are kindly informed that after review by the HREC, Faculty of Health Sciences, North-West University, your ethics approval application has been successful and was determined to fulfil all requirements for approval. Your study is approved for a year and may commence from 22/11/2018. Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation. A monitoring report should be submitted two months prior to the reporting dates as indicated i.e. annually for minimal risk studies, six-monthly for medium risk studies and three-monthly for high risk studies, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC, Faculty of Health Sciences must be notified if the study is temporarily suspended or terminated. The monitoring report template is obtainable from the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECMonitoring@nwu.ac.za. Annually, a number of studies may be randomly selected for an internal audit.

The HREC, Faculty of Health Sciences requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the proposal or other associated documentation must be submitted to the HREC, Faculty of Health Sciences prior to implementing these changes. These requests should be submitted to Ethics-HRECApply@nwu.ac.za with a cover letter with a specific subject title indicating, "Amendment request: NWU-XXXXX-XX-XX". The letter should include the title of the approved study, the names of the researchers involved, the nature of the amendment/s being made (indicating what changes have been made as well as where they have been made), which documents have been attached and any further explanation to clarify the amendment request being submitted. The amendments made should be indicated in yellow highlight in the amended documents. The *e-mail*, to which you attach the documents that you send, should have a *specific subject line* indicating that it is an amendment request e.g. "Amendment request: NWU-XXXXX-XX-XX". This e-mail should indicate the nature of the amendment. This submission will be handled via the expedited process.

ANNEXURES 2 ETHICAL APPROVAL LARGER STUDY

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Web: http://www.nwu.ac.za

Faculty of Health Sciences
Health Sciences Ethics Office for Research, Training and Support
Health Research Ethics Committee (HREC)

Tel: 018-285 2291
Email: Wayne.Towers@nwu.ac.za

16 August 2016

Dr L Malan
Nutrition

Dear Dr Malan

APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00061-16-S1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Study title: Characterisation of exercise-induced lipid-derived inflammatory signalling in male recreational athletes versus untrained males

Study leader/supervisor: Dr L Malan

Student: -

Application type: Larger study

Risk level: Medium

You are kindly informed that your application was reviewed at the meeting held on 08/06/2016 of the HREC, Faculty of Health Sciences, and was approved on 16/08/2016.

The commencement date for this study is 16/08/2016 dependent on fulfilling the conditions indicated below. Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years when extension will be facilitated during the monitoring process.

After ethical review:

Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC, Faculty of Health Sciences (if applicable).

ANNEXURES 3 CERTIFICATE OF LANGUAGE EDITING

DECLARATION OF LANGUAGE EDITING

I, Ansie Briel, hereby declare that I edited the dissertation, with the title:

Exercise-induced lipid mediator inflammatory signalling in male recreational runners versus untrained males considering potential modulating factors

for

Erika Bezuidenhout (Botes)

24185264

Research proposal for the Dissertation submitted in *partial* fulfillment of the requirements for the degree *Magister Scientiae* in Nutrition at the North-West University

The changes were affected in Track Changes and Comments. The considerations of the changes were put to the author's discretion and prerogative.

Regards
Ansie Briel
BA, HED, Hons (Translation Studies)